Exercise capacity after coarctation repair relates to the c.46A > G genomic polymorphism of the β2-adrenoreceptor and the c.704T > C angiotensinogen polymorphism

Alfred Hager¹, Judith Bildau², Joachim Kreuder², Christian Schreiber³, Harald Kaemmerer¹ and John Hess¹

Abstract

Background: Even after excellent repair of aortic coarctation without restenosis there are limitations in exercise capacity at long-term follow-up. This study was performed to assess the contribution of inherited genomic polymorphisms to exercise capacity in patients without restenosis.

Patients and methods: 122 patients aged 17–72 years, 46 female, 76 male, seen 2–27 years after repair of aortic coarctation with a residual brachial-ankle-gradient ≤ 20 mmHg were investigated. Genomic polymorphism of angiotensin converting enzyme (ACE I/D), angiotensinogen (AGT, c.704C > T), angiotensin II receptor type 1 (AGTR1, c.1166A > C), endothelin 1 (EDN1, EDN1/ex5-c.5665G > T), G protein (GNB3, c.825C > T), and two polymorphisms each of the β1-adrenoreceptor (ADRB1, c.145G > A and c.1165C > G), β2-adrenoreceptor (ADRB2, c.46A > G and c.79C > G), and endothelial NO synthase (NOS3, intron 4 I/D and NOS3, c.894G > T) were determined by PCR amplification and fragment length analysis. Exercise capacity was determined by an upright bicycle exercise test.

Results: Only the c.46A > G polymorphism of the ADRB2 (p = 0.024) and the c.704T > C AGT polymorphism (p = 0.042) were positively correlated with peak workload. Patients with one or especially two polymorphic alleles showed a significant higher exercise performance compared with those patients homozygous for the wild type.

Conclusions: In contrast to a previous study in heart failure patients, after coarctation repair adults had a better exercise capacity with the G allele of the β2-receptor c.46A > G polymorphism. Therefore, the exercise capacity of coarctation patients does not profit from an enhanced down regulation of their beta receptors.

Keywords
Congenital heart disease, coarctation of the aorta, exercise capacity, genomic polymorphism

Received 7 October 2010; accepted 20 January 2011

Introduction

Even after morphologically excellent repair at the aortic isthmus, many patients with aortic coarctation have a reduced exercise capacity.¹ There are at least two reasons for this limitation. Rhodes et al.² compared patients after coarctation repair with patients after surgical closure of a persistent arterial duct. They found a lower peak oxygen uptake and an earlier lactate accumulation in the coarctation patients. As they did not find a relationship with a residual gradient across the former coarctation site, they speculated on the

¹Department of Pediatric Cardiology and Congenital Heart Disease, Deutsches Herzzentrum München, Technische Universität München, Germany.
²Department of Pediatric Cardiology, Justus-Liebig-Universität Giessen, Germany.
³Department of Cardiovascular Surgery, Deutsches Herzzentrum München, Technische Universität München, Germany.

Corresponding author:
Alfred Hager, Department of Pediatric Cardiology and Congenital Heart Disease, Deutsches Herzzentrum München, Technische Universität München, Lazaretstr. 36, D-80636 München, Germany
Email: a-hager@web.de
increased vessel stiffness in coarctation patients\textsuperscript{3–5} hampering proper blood flow to the exercising muscles. Furthermore, simple deconditioning might be a reason for exercise limitations.\textsuperscript{1} Bjarnason-Wehrens et al.\textsuperscript{8} reported on motor-developmental limitations in patients with simple congenital heart defects without hemodynamic impairments. The authors speculated that overprotection of the parents and physicians led to reduced physical activity and deprivation.

On the other hand, several studies\textsuperscript{7–10} as well as the CAREGEN project\textsuperscript{11,12} showed that genomic polymorphism of blood pressure regulation has an impact on exercise capacity in patients with heart failure and coronary artery disease, and in healthy individuals.

Therefore, we reanalyzed the data from our study on the impact of genomic polymorphism on arterial hypertension after coarctation repair\textsuperscript{13} with the hypothesis that genomic polymorphism of blood pressure regulation has an impact on exercise capacity.

**Patients and methods**

**Study subjects**

From April 1974 to July 1999, 404 patients born before 1985 underwent surgical repair of isolated aortic coarctation in our institution; 273 of them (71\% of those alive) were prospectively investigated in the COALA study performing a symptom-limited exercise test according to the recently published protocol and results.\textsuperscript{1,14}

In order to investigate the impact of genomic polymorphism on blood pressure,\textsuperscript{13} we excluded all patients with a brachial ankle blood pressure difference of more than 20 mmHg. In total, 122 patients agreed to participate in the study and were analyzed. They had a median age of 31 years (range 17–72 years); 46 were female and 76 male. The median follow-up was 20 years (range 2–27 years) after coarctation repair.

Primary surgical repair was performed with resection and end-to-end anastomosis in 85 patients, resection and tube graft interposition in 33 and other surgical techniques in 4 patients (Dacron patch aortoplasty, PTFE onlay-patch, subclavian flap, aorto-aortic bypass in one each). At the time of the COALA study, 34 (28\%) patients were on antihypertensive medication, 20 of them on \( \beta \)-blockers, 18 on ACE inhibitors/AT-blockers, three on diuretics, and three on calcium channel blocker.

The study was in accordance with the declaration of Helsinki (revision 2008)\textsuperscript{15} and approved by the ethical board of the Medical Faculty of our institution (project numbers 335/00 and 757/02). Written informed consent was obtained from every patient.

**Exercise testing**

The follow-up examination with a standardized protocol also for exercise testing has been previously published.\textsuperscript{14} In short, a symptom-limited exercise test was performed on a bicycle in a sitting position, starting with 25 W and increasing workload by 25 W every 2 minutes. Exercise capacity for this study was expressed as the highest workload in Watts being achieved at the end of the test.

**Genomic polymorphism**

The details of the genetic methods have been reported previously.\textsuperscript{13} As the Hardy-Weinberg equilibrium was not fulfilled in two polymorphisms, we again excluded the CYP11B2 and the GRK4 polymorphism for the current analysis. An overview of the currently investigated genetic polymorphisms is given in Table 1.

The staff of the laboratory was blinded to the clinical data.

**Statistical analysis**

Data were analyzed with PASW Statistics 18.0.2 software (IBM Corporation, Somer, NY, USA). A multiple regression model was established predicting exercise capacity by age, sex, body mass and length, as well as all genomic polymorphisms. Afterward, the non-significant \(( p > 0.05)\) polymorphisms were removed until only significant polymorphisms were left in the final model. The stepwise backward exclusion model was also chosen to find interactions of more than one polymorphism and to encounter linkage disequilibrium.

In an additional regression analysis we tested whether drugs, age at surgery, time since surgery, or surgical technique had an impact on the final model.

**Results**

After correcting peak workload for sex, age, body mass, and body height all investigated genomic polymorphism were included in the regression model (Table 2). In the stepwise removal of the non-significant variables all but two genomic polymorphisms were withdrawn. In the c.46A \( \rightarrow \) G polymorphism of the \( \beta \)-adrenoreceptor \(( p = 0.024)\), patients with more G alleles in the c.46 position had a better exercise capacity (Figure 1). Independently, the angiotensinogen
c.704T > C genomic polymorphism (p = 0.042) was related to exercise capacity with patients showing the T/C and especially the C/C genotype having a better exercise capacity (Figure 2).

Age at surgery and surgical technique, as well as time since surgery and current medication had no significant impact on the final regression model.

**Discussion**

This study showed that the c.46A > G polymorphism of the β2-adrenoceptor and the angiotensinogen c.704T > C polymorphism play a genetic role on the exercise capacity in the long-term course after coarctation repair. Patients with the heterozygous
**Figure 1.** Association of a β2-adrenoreceptor c.46A>G genomic polymorphism with exercise capacity expressed as peak workload (W) in 122 patients at long-term follow-up after surgical coarctation repair showing that patients with the A/G and especially the G/G genotype have a better exercise capacity.

**Figure 2.** Association of an angiotensinogen c.704T>C genomic polymorphism with exercise capacity expressed as peak workload (W) in 122 patients at long-term follow-up after surgical coarctation repair showing that patients with the T/C and especially the C/C genotype have a better exercise capacity.
genotype and especially those with the homozygous polymorphic genotype showed a significant higher exercise performance as those homozygous for the wild type for both genetic polymorphisms.

The β2-adrenoreceptor c.46A > G polymorphism is a single nucleotide polymorphism substituting the 46th base pair of the intron-less ADRB2 gene on chromosome 5q31-32 from adenine to guanine. This results in a substitution of the amino acid arginine to glycine at position 16 in the protein. This change in the β2-adrenoreceptor leads in vitro to an increased agonist promoted down regulation of the receptor expression.17 However, in vivo studies showed just the opposite effect. Patients with A/A genotype (Arg16 protein type) had a profound down regulation of the receptor on chronic adrenergic stimulation.18 Liggett19 proposed that A/A genotype receptors subjects are more prone to down regulation and even down regulated by endogenous catecholamines, whereas G/G genotype receptors are down regulated only by high levels or exogenous catecholamines.

There is no direct effect of the c.46A > G polymorphism on the cardiac receptor function.16 On the vascular site, the studies are contradictive and it still remains unclear whether the β2-adrenoreceptor c.46A > G polymorphism has an effect on the β2-mediated vasodilatation.16

In clinical studies the c.46A > G polymorphism has shown an effect on exercise capacity depending on the patient group. In healthy humans Snider et al.20 reported a reduced cardiac output at rest and at exercise for the A/A genotype. For patients with heart failure from ischemic or idiopathic dilated cardiomyopathy, Wagoner et al.10 found an increased exercise capacity measured as peak VO₂ in subjects with the A/A genotype. In contrast to healthy subjects, patients with heart failure profit from the enhanced agonist promoted down regulation.

Our study was in line with that on healthy subjects. They did not profit from the enhanced agonist promoted down regulation. These findings in our study provoke the hypothesis that the reduced exercise capacity in coarctation patients is not the result of processes similar to myocardial heart failure. It is rather the result of peripheral dysregulation,21,22 or vessel stiffness,3–5 or simply deconditioning, often seen in congenital heart defects, even when they are without impact on cardiovascular function.6

The angiotensinogen c.704T > C polymorphism is a single nucleotide polymorphism substituting the 704th base pair in the 2nd exon of the AGT gene on chromosome 1q from thymine to cytosine. This results in a substitution of the amino acid methionine to threonine at position 235 in the protein. However, this change in the protein is not responsible for the increased AGT plasma levels measured in patients with the polymorphic allele. There is a complete cross-linkage to the c.-6G > A polymorphism located in the promoter region that leads to an enhanced transcription of the AGT gene.23 The polymorphic allele is associated with hypertension, left ventricular hypertrophy in hypertensive patients and athletes, as well as with an improved aerobic capacity after exercise training.24 The latter could be confirmed in our study despite the association with hypertension or exercise-induced blood pressure response failing to be significant in our previous study.13

Limitations

For a linkage disequilibrium study between a polymorphic marker genotype and a multifactorial phenotype, the number of patients and, thereby, the power of the study to detect significant correlations is rather low. Therefore, the failure to find other significant correlations of exercise capacity to genomic polymorphisms in our patient group should not be interpreted as if there were no relationship. However, this lack of power does not curtail the reported significant correlations and the conclusions drawn from them.

Acknowledgements

Parts of this paper have been previously presented at the 29th Congress of the European Society of Cardiology, 30.8.-3.9.2008 in Munich/DE.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interests.

References


