A promoter polymorphism -945C>G in the connective tissue growth factor in heart failure patients with mechanical circulatory support: a new marker for bridge to recovery?

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Abstract

OBJECTIVES: Mechanical circulatory support (MCS) creates improvement of cardiac function in a small portion of patients with idiopathic dilated cardiomyopathy (iDCM). Among other factors, cardiomyocyte hypertrophy seems to represent an important prerequisite for MCS-related cardiac recovery. We have previously shown that connective tissue growth factor (CTGF) leads to adaptive cardiomyocyte hypertrophy associated with a protective cardiac function in transgenic mice. To test whether a functional genetic variant in the CTGF promoter impacts MCS-related cardiac recovery, three groups of iDCM patients with and without cardiac recovery on MCS were genotyped.

METHODS: The CTGF promoter variant (c.-945C>G) was analysed in 314 patients with iDCM receiving medical treatment only (Group I). Forty-nine iDCM patients who were either weaned from MCS for more than 6 months (Group II; n = 20) or bridged to cardiac transplantation (Group III; n = 29) were also genotyped. Patients on MCS were followed up for at least 12 months. Clinical characteristics and outcome on MCS were correlated with the respective genotypes.

RESULTS: The c.-945C>G allele frequencies in 314 iDCM patients (Group I) were similar to controls deposited in the HapMap database or those published in a recent study. There were no differences in allele prevalence between patients with mild to moderate iDCM (Group I) compared with patients with severe iDCM requiring MCS (Groups II and III). Intriguingly, 50% of patients who were weaned from MCS (Group II) were homozygous for the G allele compared with only 17.2% of patients included in Group III, which is a significant difference (P = 0.03).

CONCLUSIONS: Homozygosity of the promoter-activating G allele in the CTGF_c.-945C>G variant is overrepresented in patients with cardiac recovery on MCS when compared with iDCM patients without cardiac recovery. Further studies are needed to evaluate c.-945C>G as a genetic predictor for clinical outcome on MCS.

Keywords: Ventricular assist device • Bridge-to-recovery • Connective tissue growth factor • Polymorphism

INTRODUCTION

Ventricular assist devices (VADs) have become a widely used strategy to bridge patients with idiopathic dilated cardiomyopathy (iDCM) until donor heart availability. Moreover, mechanical circulatory support (MCS) was shown to be superior to medical treatment in patients who are not eligible for heart transplantation [1] and recent improvements of device technology have further ameliorated clinical outcome of patients on MCS [2]. Intriguingly, some patients with end-stage heart failure due to iDCM experience significant improvement of cardiac function allowing a removal of the device [bridge-to-recovery (BTR)] [3]. It has been shown that young age and pulsatile devices represent beneficial preconditions for MCS-related cardiac recovery [3, 4]. Yet, molecular mechanisms underlying MCS-related myocardial recovery are incompletely understood and there are so far no stable predictors for the identification of candidates for the bridge to recovery approach before VAD implantation.

Cardiomyocyte hypertrophy is regularly observed in failing hearts as a compensatory response [5]. It has been shown that...
MCS induces a regression of cardiomyocyte hypertrophy [6]. MCS-associated atrophy of cardiomyocytes is associated with significant inactivation of myocardial kinases Akt and JNK [7, 8]. A stimulation of physiological cardiomyocyte hypertrophy by the β2 agonist clenbuterol during MCS was proposed to foster cardiac recovery of patients with iDCM [9].

Connective tissue growth factor (CTGF; also known as CCN2) is a secreted peptide that is strongly induced in human heart failure. We have previously shown that overexpression of CTGF promotes myocyte hypertrophy associated with Akt and JNK activation in transgenic mice [10]. Cardiac contractility of CTGF transgenic mice was significantly enhanced compared with wild-type mice after pressure overload and CTGF transgenic ventricles exhibited improved adaptive responses to cardiac stress [10]. In humans, CTGF expression levels are regulated by an Sp1 responsive element in the CTGF promoter, which shows a polymorphism at position c.-945C>G (Single Nucleotide Polymorphism (SNP) ID: rs6918698). It has been shown that the G allele is associated with increased transcriptional activity of the CTGF promoter, while the C-allele represses CTGF transcription [11]. The c.-945G allele was found to be significantly over-represented in patients with fibrotic heart diseases. However, the subgroup of patients with cardiac involvement was too small to allow further conclusions about a relationship of the respective genotypes with cardiac phenotypes [11].

To investigate the role of the functional c.-945C>G polymorphism in the CTGF promoter in the pathogenesis of human heart failure, we analysed the variant in 314 patients with iDCM. To further scrutinize whether or not the variant may influence clinical presentation of iDCM subjects in terms of end-stage heart failure, we also analysed 49 end-stage iDCM patients who required MCS and tested the hypothesis if CTGF c.-945G is associated with ventricular recovery during MCS.

METHODOLOGY

PATIENT COHORTS

All patients included in the study (Groups I–III) were at least 18 years of age and gave their written informed consent. Clinical data were evaluated prospectively or retrospectively by chart review. The study was approved by the Institutional Review Board. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Group I. Three hundred and fourteen patients with iDCM were attending the outpatient clinic at the Charité University Hospital Berlin between 1998 and 2006 and were recruited after their written informed consent. The mean left ventricular diameter and the ejection fraction were 67.4 mm (±9.2) and 33.4% (±10.4), respectively. Detailed demographic and clinical data of the iDCM cohort have been described previously [12]. All patients included in group I were on medical treatment only and did not require MCS or cardiac transplantation.

Group II. Twenty iDCM patients who could be successfully weaned off MCS between 1995 and 2009 were included in group II. These patients were attending the Department for Cardiothoracic and Vascular Surgery at the German Heart Institute Berlin (Deutsches Herzzentrum Berlin (DHZB)), Germany. All subjects showed sustained cardiac recovery lasting at least 3 months after VAD removal. Coronary artery disease was excluded as a cause for heart failure by coronary angiography. Myocardial samples and serological analyses obtained before VAD implantation did not show histological signs of myocarditis or evidence for infection with cardiotropic viruses. The majority of patients matched for Group II had the Novacor LVAD (n = 12). Three patients had Berlin Heart Excor (2 LVAD and 1 BVAD) or Incor LVADs, respectively. Two subjects were weaned from Thoratec Heartmate I LVADs.

Group III. Twenty-nine iDCM patients who did not reveal significant improvement of cardiac function during MCS were included in Group III. Diagnosis of iDCM was established as described for Group II. All patients in Group III underwent eventual cardiac transplantation. Implanted VADs were Berlin Heart Excor in 13 patients (9 BVAD and 4 LVAD), eleven Berlin Heart Incor, three Novacor and two Thoratec Heartmate I LVADs.

GENETIC ANALYSES

The CTGF c.-945C>G polymorphism (dbSNP ID rs6918698) is located in the promoter region upstream of the gene encoding CTGF. The G-allele causes an increased affinity of the transcription factor Sp1 and is associated with enhanced expression of CTGF protein [11]. DNA was isolated from blood lymphocytes by an Autopure LS robot (Qiagen) as described [12]. Polymerase Chain Reaction (PCR) fragments were amplified using the following primers F: GAGAACAAAGACGGTGTGA; R: TCTCTAGGTGAA CCCCCCTT. The c.-945C>G variant creates an Mnl1 restriction site in the PCR amplicon, leading to fragments of 120 and 93 bp. Twelve samples were randomly selected for evaluation of the genotype by direct sequencing, which confirmed the results in all samples.

STATISTICAL ANALYSIS

Discrete variables are expressed as counts or percentages and compared using the χ² test. Continuous variables are expressed as mean value ± standard deviation (SD) and compared by using the Mann–Whitney test. Statistical analyses were performed using the SPSS 21 software (SPSS, Inc., Chicago, IL, USA).

| Table 1: Clinical features of study cohort at baseline (time of device implantation) |
|---------------------------------|-----------------|----------------|-----------|
|                                | Group II (n = 20) | Group III (n = 29) | P-value   |
| BSA (m²)                       | 1.93 (±0.2)      | 2.01 (±0.2)      | 0.20      |
| Gender (male/female)           | 19/1             | 25/4             | 0.32      |
| Age (years)                    | 43.1 (±11.1)     | 45.9 (±11.9)     | 0.39      |
| LVEDD (%)                      | 153 (±14)        | 160 (±27)        | 0.35      |
| EF (%)                         | 15.2 (±4.0)      | 15.9 (±3.8)      | 0.92      |
| CI                             | 1.53 (±0.38)     | 1.70 (±0.41)     | 0.45      |
| Pulsatile (MCS)                | 16 (80%)         | 16 (55%)         | 0.07      |
| Biventricular support          | 2 (10%)          | 9 (31%)          | 0.08      |
| Days on MCS                    | 130.2 (±84.5)    | 345.9 (±214.9)   | 0.001     |

*At time of VAD implantation.

*Calculated according to the formula of Henry et al [13].

BSA: body surface area; LVEDD: left ventricular end-diastolic diameter; EF: ejection fraction; CI: cardiac index.
RESULTS

Clinical data

Demographic data of iDCM patients who were weaned off the VAD (Group II) did not differ compared with patients with bridge to cardiac transplantation (Group III) at the time of VAD implantation (as given in Table 1).

**Group II.** The mean age at the time of VAD implantation was 43.1 (22–64 years). Left ventricular end-diastolic diameters (LVEDDs) and left ventricular ejection fraction (LVEF) at the time of VAD implantation ranged 66–93 mm (mean 72.4 mm) and 10–20% (mean 15.5%), respectively. LVEDD enlargement normalized for body surface area according to the formula of Henry et al. [13] was 153% in Group II and 160% for Group III and did not differ significantly (P = 0.54). Eighteen patients were on left ventricular mechanical support. Two patients had biventricular assist devices. During MCS (duration 37–243 days), cardiac parameters significantly improved. LVEDD and LVEF as assessed by the final off-pump echocardiography before VAD removal were 52.2 ± 6.5 mm and 51.1 ± 8.5%, respectively. The majority of patients (70%) showed long-term improvement of cardiac function for more than 24 months. In 6 patients a relapse of heart failure occurred, necessitating cardiac transplantation 215, 269, 1413, 2317, 2563 and 3408 days after VAD removal. All patients with heart failure relapse were on left ventricular devices with pulsatile after VAD removal. All patients with heart failure relapse were on medical treatment only (Group I) compared with patients who were weaned off MCS (Group II and Group III). It was found that 24.5% of patients with MCS harboured C/C, 44.9% C/G and 30.6% G/G genotypes (Table 2). No differences were seen between patients requiring MCS compared with iDCM patients who were on medical treatment only (P = 0.83; see Table 2). To assess whether the variant modifies the clinical course of MCS, genotypes of Groups II and III were compared. Allele frequencies did not differ significantly (Table 3). However, 50% of patients who were weaned off the VAD were harbouring G/G genotypes compared with 17.2% of patients who were bridged to heart transplantation.

**Group III.** Twenty-nine iDCM patients had ongoing cardiac dysfunction while on MCS, necessitating cardiac transplantation and were included in Group III. Age at the time of VAD implantation ranged from 7 to 823 days (mean 345.9 ± 241.9). As given in Table 1, age, gender and echocardiographic data at the time of device implantation did not differ significantly between the groups. Naturally, due to limited supply of appropriate donor organs, the time on the VAD was significantly longer in bridge-to-transplant (BTX) patients.

**Genetic analysis**

To investigate the prevalence of CTGF_c.-945C>G in subjects with non-ischaemic heart failure, we genotyped 314 patients with iDCM (Group I) for c.-945C >G by the use of MnlI digestion. We found that 22.6, 46.5 and 30.9% iDCM patients harboured either C/C, C/G or G/G alleles, respectively (Table 2). The prevalence of the G/G genotype in European ancestry ranges among 19.3, 43.9 and 36.8% on the basis of the HapMap (International HapMap Project home page [14]), with more than 2400 control individuals who were reported previously [15]. Hence, the c.-945C>G variant is similarly represented among iDCM patients compared with healthy subjects (p: 0.41). To further examine whether the polymorphism may modify the severity of iDCM, we tested 49 iDCM patients on MCS for c.-945C>G (Groups II and III). It was found that 24.5% of patients with MCS harboured C/C, 44.9% C/G and 30.6% G/G genotypes (Table 2). No differences were seen between patients requiring MCS compared with iDCM patients who were on medical treatment only (P = 0.83; see Table 2). To assess whether the variant modifies the clinical course of MCS, genotypes of Groups II and III were compared. Allele frequencies did not differ significantly (Table 3). However, 50% of patients who were weaned off the VAD were harbouring G/G genotypes compared with 17.2% of patients who were bridged to heart transplantation. When comparing subjects with either C/C or C/G genotypes to those who harbour G/G alleles (recessive model), genotypes between Groups II and III differ significantly (P = 0.03; Table 3).

**Table 2:** Distribution of genotypes among iDCM patients with medical treatment only (Group 1) compared with iDCM patients with MCS (Group II and Group III)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Group I (n = 314)</th>
<th>Group II + Group III (n = 49)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>71 (22.6%)</td>
<td>12 (24.5%)</td>
<td>0.83</td>
</tr>
<tr>
<td>C/G</td>
<td>146 (46.5%)</td>
<td>22 (44.9%)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>97 (30.9%)</td>
<td>15 (30.6%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Distribution of genotypes among patients with significant cardiac recovery weaned off MCS (Group II) compared with patients without recovery (Group III)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Group II (n = 20)</th>
<th>Group III (n = 29)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>15</td>
<td>31</td>
<td>0.12</td>
</tr>
<tr>
<td>C/G</td>
<td>25</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Cardiac recovery upon MCS is a well-documented phenomenon in patients with end-stage iDCM [3]. Studies from our centre have shown that echocardiography facilitates—at least in part—a prediction of those patients who are most likely to gain long-term recovery after explantation of the VAD [16, 17]. However, the selection of appropriate weaning candidates remains a challenging task and a prospective identification of subjects suitable for MCS weaning is hardly possible. Clinical predictors that may also include genetically predisposing factors may facilitate difficult decisions regarding device implantation and removal as well as hospital discharge and listing for transplantation.
In the present study we chose the CTGF_c.-945 polymorphism as a candidate to investigate the hypothesis of an association between genotype and clinical outcome for the following reasons: The CTGF_c.-945G allele leads to a significantly increased transcriptional activation of the CTGF promoter [11]. Its prevalence among healthy volunteers is high enough to allow a case–control study among a relatively small number of patients on MCS. Moreover, as we could show previously, an increased cardiac expression of CTGF is correlated with adaptive responses of the heart to myocardial stress [10].

No significant allele frequency was found in patients with mild to moderate iDCM (Group 1) when compared with previously published genotypes among more than 1000 healthy subjects [15]. Therefore, the variant does not seem to confer a predisposition to iDCM itself. Moreover, the polymorphism was similarly represented among 49 patients with end-stage heart failure (Groups I and III), which makes the variant unlikely to represent a significant modifier allele. Yet, in contrast to overall allele frequency, we could observe a difference in the distribution of the G/G genotype between the BTX and BTR groups.

A growing number of studies highlight the role of CTGF as a favourable factor in heart failure and cardiac remodelling [10, 18]. As shown by Gravning et al. [18], CTGF causes smaller areas of scar tissue and substantially reduces mortality after myocardial infarction in vivo. Patients with ST-elevation myocardial infarction (STEMI) had a reduced infarct size and improved ejection fraction after one year post STEMI when CTGF serum levels were elevated at the time of infarction [18]. These findings and our own data [10] support the idea that CTGF may act as a protective factor under certain conditions. A recent study investigated myocardial CTGF expression at the time of VAD implantation and consecutive VAD removal at the time of cardiac transplantation and found that MCS leads to a significant reduction of CTGF within the myocardium [19]. These results further indicate a connection between CTGF and reverse remodelling processes that take place in unloaded ventricles. One may speculate that homozygous G alleles may cause sustained expression of CTGF which in turn may harbour a beneficial effect on cardiac recovery during MCS.

Unfortunately, in the present study we were unable to evaluate levels of myocardial CTGF expression to associate mRNA levels with the respective genotypes. Though the G allele may enhance expression of CTGF mRNA in vitro [11], it remains unclear whether myocardial protein expression is also enhanced under in vivo conditions. Pantou et al. [20] did not find an association with the respective genotype and cardiac gene expression among patients after cardiac transplantation. Yet, the same study showed a functional effect of CTGF_c.-945GG on allograft vessels.

Several factors (in particular young age at the time of VAD implantation) harbour beneficial effects on myocardial recovery [3]. To avoid a selection bias, the patients with BTX were stratified to the BTR cohort with regard to demographic data, height, weight, age, gender, echocardiographic parameters and the type of ventricular support (Table 1). Yet, patients who showed no recovery of cardiac function were more often on devices with axial flow VADs or had biventricular support. It has been proposed previously that cardiac recovery is observed more frequently in patients with pulsatile mechanical support than with continuous flow support [4], a fact that is also reflected in the subgroup of patients included in the present study (Table 1). Intriguingly, among a total of four patients who showed ventricular recovery on continuous-flow devices, three were homozygous carriers of the G allele. One may speculate that a G/G genotype may compensate for the lower ventricular unloading properties of continuous-flow devices compared with pulsatile-flow devices by enhancing myocardial CTGF expression. Another interesting finding in our study is that the mean age of G/G carriers in the BTR group was 59.6 years which is significantly above the average age of the BTR cohort. Of note, the three eldest patients aged 56, 59 and 64 at the time of implantation were harbouring G/G alleles. Thus, homozygosity of CTGF_c.-945G might alleviate cardiac recovery even in elder patients.

We are aware that the major limitation of the present study is the small number of patients included in the subgroups, in particular Group III. However, the worldwide number of iDCM patients weaned from MCS does not exceed one hundred subjects, which constitutes the main challenge to thoroughly investigate a genetic predisposition to BTR. Replication studies are needed to confirm an association of the CTGF_c.-945 variant with cardiac recovery.

To our knowledge, we describe herein a first approach to unravel genetic markers for cardiac recovery in DCM. However, due to the small sample size our findings should be interpreted with caution and are only a first step in the quest for better prediction of cardiac recovery and may open the search for further variants. It seems likely that several genetic variations distributed over different genes may act in interaction. Especially in DCM with its significant genetic aetiology, the last years have provided a great deal of data concerning the landscape of genetic variation, as recently shown by Pugh et al. [21]. To evaluate these variants, especially those with a functional impact, more studies are necessary to unravel genetic mechanisms involved in MCS-related cardiac recovery and identify genetic predictors for weaning candidates.

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**Conflict of interest:** none declared.

**REFERENCES**


