Transplant International

ORIGINAL ARTICLE

The recipient's heme oxygenase-1 promoter region polymorphism is associated with cardiac allograft vasculopathy

Kathrin Freystaetter[1], Martin Andreas[1], Martin Bilban[2], Thomas Perkmann[2], Alexandra Kaider[3], Marco Masetti[4], Alfred Kocher[1], Michael Wolzt[5] & Andreas Zuckermann[1]

1 Division of Cardiac Surgery, Medical University of Vienna, Vienna, Austria
2 Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
3 Section for Clinical Biometrics, Center for Medical Statistics Informatics and Intelligent Systems, Medical University of Vienna, Vienna, Austria
4 Department of Cardiology, Bologna University Hospital, Bologna, Italy
5 Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria

Correspondence
Martin Andreas, Department of Cardiac Surgery, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria.
Tel.: +43 1 40400 69660;
fax: +43 1 40400 69680;
e-mail: martin.andreas@meduniwien.ac.at

SUMMARY

Heme oxygenase-1 (HO-1) catalyses the degradation of heme to biliverdin, free iron, and carbon monoxide. The promoter region contains a highly polymorphic $(GT)_n$ repeat, where shorter $(GT)_n$ repeat sequences are linked to higher transcriptional activity, which was shown to correlate with a cytoprotective effect. Higher HO-1 levels may protect from cardiac allograft vasculopathy. Cardiac allograft recipients transplanted between 1988 and 2012 were analyzed for the HO-1 $(GT)_n$ repeat polymorphism using PCR and DNA fragment analysis with capillary electrophoresis. A relation to cardiac allograft vasculopathy (CAV) was analyzed using Cox regression including common risk factors for CAV and the occurrence of rejection episodes as explanatory variables. A total of 344 patients were analyzed, of which 127 patients were positive for CAV (36.9%). In our multivariable Cox regression analysis, the short homozygous HO-1 $(GT)_n$ genotype with <27 repeats (S/S) revealed a higher risk for CAV ($P = 0.032$). Donor age ($P = 0.001$) and donor weight ($P = 0.005$) were significant predictors for CAV. A potential risk for CAV was associated with rejection episodes ($P = 0.058$) and history of smoking ($P = 0.06$). The recipient HO-1 $(GT)_n$ genotype may contribute to CAV development. This finding has to be evaluated in larger series including studies targeting the underlying disease mechanism.

Transplant International 2017; 30: 510–518

Key words
cardiac allograft vasculopathy, cardiac transplantation, heme oxygenase-1, polymorphism

Received: 30 October 2016; Revision requested: 25 November 2016; Accepted: 31 January 2017; Published online: 15 March 2017

Introduction

Heme oxygenase (HO) catalyses the rate-limiting step in the degradation of heme to biliverdin, free iron, and carbon monoxide [1]. Three isoforms of HO have been described; the inducible isoform HO-1 is found mainly in the liver and spleen, but it is also located in numerous other tissues including the heart, lung, brain, vascular smooth muscle cells, and endothelial cells [2,3]. Stimuli for induction include its substrate heme, heat shock proteins, and cellular stress, such as oxidative stress and exposure to ultraviolet light [4]. The region upstream of the transcription initiation site of the HO-1 gene, including the promoter region, contains a highly polymorphic poly[d(T-G)] element [5,6]. This $(GT)_n$ length polymorphism has been shown to be linked to

doi:10.1111/tri.12935

the transcriptional activity of the gene, where longer $(GT)_n$ repeat sequences resulted in lower transcriptional activity, and shorter $(GT)_n$ repeat sequences resulted in higher transcriptional activity [7,8]. Higher transcriptional activity was shown to correlate with a protective effect regarding the occurrence of cardiovascular disease, but the relevance of the HO-1 $(GT)_n$ length polymorphism has also been questioned by studies that were not able to determine a statistically significant association between coronary artery disease and the HO-1 $(GT)_n$ repeat allele status [7,9–13].

Cardiac allograft vasculopathy is characterized by diffuse luminal narrowing, affecting epicardial and intramyocardial arteries and veins, and presenting an accelerated progression compared to traditional atherosclerosis [14]. One year after heart transplantation, 10% of recipients are diagnosed with CAV, the incidence rising to more than 50% of recipients 10 years after transplant [15]. Although the incidence of CAV has decreased in patients receiving an allograft between 2003 and 2010 when compared to patients receiving an allograft between 1994 and 2003 (37% and 42%, respectively), CAV remains one of the leading causes of death in this patient population, particularly more than 3 years after transplant [15]. The traditional risk factors for CAV include male gender, hyperlipidemia, hypertension, obesity, diabetes, and metabolic syndrome, and are thus similar to the risk factors for traditional atherosclerosis [14,16,17]. Transplant-associated risk factors for CAV include cytomegalovirus (CMV) infection, immunological factors such as HLA antigen mismatch and cellular and antibody-mediated rejection episodes, and donor factors such as donor age, explosive brain death and intracranial hemorrhage [15,16]. The early diagnosis of CAV is critical to ensure appropriate treatment, but the denervated allograft often prevents typical signs of ischemia such as chest pain [16,18]. Coronary angiography is currently the standard diagnostic tool, but may underestimate the extent of CAV due to the characteristic diffuse luminal narrowing [16,18]. Intravascular ultrasound (IVUS) has proven to be a sensitive surrogate marker for the diagnosis of CAV, allowing the exact definition of the lumen diameter as well as the morphology of the intima [16,18,19]. As a noninvasive test, dobutamine stress echocardiography is available for screening purposes. Biomarkers such as C-reactive protein (CRP), brain natriuretic peptide (BNP), troponin I, and von Willebrand factor can be used to complete the clinical information on a patient, but are currently not used as predictors of CAV [16,18]. It is, therefore, highly desirable to develop a new noninvasive test to serve as a predictor of CAV.

We aimed to determine whether the HO-1 $(GT)_n$ length polymorphism in heart transplant recipients can be linked to the occurrence of CAV. This genetic polymorphism could serve as a potential predictive biomarker for CAV, possibly providing a new noninvasive method for risk stratification of heart transplant patients for CAV.

Materials and methods

Patient population

Patients were consented to participate in the Medical University of Vienna Heart Transplant Biobank project to provide blood samples in the course of their scheduled clinic visits at the Vienna General Hospital between 2009 and 2012. The patients' demographic and clinical data were collected in a prospective institutional transplant database and analyzed for this project. Coronary angiographies are performed at standard intervals after cardiac transplantation, namely 1, 5, and 10 years after transplantation, or whenever clinically indicated. All available coronary angiographies within follow-up were used to evaluate the patients for CAV, defined according to the standardized nomenclature of the International Society for Heart and Lung Transplantation (ISHLT): "ISHLT CAV_0 (not significant) is defined as no detectable angiographic lesion; ISHLT CAV_1 (mild) is defined as angiographic left main <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction; ISHLT CAV_2 (moderate) is defined as angiographic left main <50%, a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of two systems, without allograft dysfunction; ISHLT CAV_3 is defined as angiographic left main ≥50%, or two primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all three systems, or ISHLT CAV_1 or CAV_2 with allograft dysfunction (defined as left ventricular ejection fraction, LVEF, ≤45% usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology" [19]. Cardiac allograft recipients were further evaluated for common risk factors such as a previous history of smoking, diabetes mellitus type 2, alcohol abuse, ischemic time, the underlying disease and CMV risk. Rejection episodes were classified as defined by the ISHLT standardized nomenclature [20].

HO-1 $(GT)_n$ genotype assessment

DNA extraction from whole blood samples was performed according to standard guidelines, using the E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA). For the HO-1 $(GT)_n$ length polymorphism assessment, DNA was amplified by PCR followed by DNA fragment analysis with capillary electrophoresis, according to the protocol described by Doberer *et al.* [21]. The samples were grouped into genotype classes according to the number of $(GT)_n$ repeats, where samples with fewer than 27 repeats were included in "class S" [short (S), GT <27] and samples with 27 or more repeats were included in "class L" [long (L), GT ≥27], resulting in the three genotype classes homozygous S/S, homozygous L/L and heterozygous S/L, as previously described [21].

Statistical analysis

Descriptive statistical methods were applied to depict the study population regarding preoperative risk factors. Continuous variables were presented as mean and standard deviation, total numbers, and proportions were reported for categorical variables. The inverse Kaplan-Meier method was used to calculate the median follow-up time [22]. The time to first positive CAV evaluation after heart transplantation, irrespective of CAV severity as categorized according to the ISHLT standardized nomenclature [19], was considered as the primary outcome variable. Patients without angiographically positive CAV evaluation were classified as negative for CAV and censored at the last follow-up date. To estimate the probability of developing CAV, the cumulative incidence function (CIF) was calculated accounting for death as a competing risk event. Gray's test was used to test for differences between groups of patients. Univariate and multivariable Cox regression analyses were performed, evaluating the correlation of the HO-1 $(GT)_n$ genotype and other common risk factors for CAV with the incidence of CAV. Patients, who died without previous CAV, were considered as censored observations. Within these Cox regression models, the prognostic factor rejection was considered as a time-dependent variable. Furthermore, possible time-varying effects of the considered risk factors were tested and included in the multivariable regression model in case of statistical significance. A *P*-value ≤0.05 was considered as indicating statistical significance. SAS version 9.4 (2002–2012; SAS Institute Inc., Cary, NC, USA) was applied for all analyses.

Results

Patient characteristics

A total of 344 heart transplant recipients were analyzed, including 272 male patients and 72 female patients (79% and 21%, respectively, see Table 1). The median age of the cardiac allograft donors was 34 years, ranging from 10 to 70 years, while the median age of the cardiac allograft recipients was 54 years, ranging from 9 to 73 years. The median follow-up time was 187 months. The evaluation of the patients' coronary angiographies classified 127 patients as positive for CAV (36.9%), of which 76 patients showed mild CAV (ISHLT CAV_1, 22.1%), 30 patients showed moderate CAV (ISHLT CAV_2, 8.7%), and 21 patients showed severe CAV (ISHLT CAV_3, 6.1%). 217 patients were classified as negative for CAV (ISHLT CAV_0, 63.1%). The occurrence of a moderate (grade 2R) or severe rejection (grade 3R) episode was observed in 45 patients (13%).

HO-1 repeat polymorphism

The analysis of the HO-1 repeat polymorphism showed a total of 21 repeat alleles, ranging from 17 repeats to 39 repeats, with 30 being the most common repeat allele. The alleles were grouped into genotype classes as described above [short (S), GT <27; long (L), GT ≥27; S/S, L/L, S/L]. Within HO-1 genotype class S/S, 21 patients were negative for CAV (ISHLT CAV_0, 50.0%), 13 patients showed mild CAV (ISHLT CAV_1, 31.0%), five patients showed moderate CAV (ISHLT CAV_2, 11.9%), and three patients showed severe CAV (ISHLT CAV_3, 7.1%). Within HO-1 genotype class S/L, 107 patients were negative for CAV (ISHLT CAV_0, 66.0%), 32 patients showed mild CAV (ISHLT CAV_1, 19.8%), 11 patients showed moderate CAV (ISHLT CAV_2, 6.8%), and 12 patients showed severe CAV (ISHLT CAV_3, 7.4%). Within HO-1 genotype class L/L, 89 patients were negative for CAV (ISHLT CAV_0, 63.6%), 31 patients showed mild CAV (ISHLT CAV_1, 22.1%), 14 patients showed moderate CAV (ISHLT CAV_2, 10.0%), and six patients showed severe CAV (ISHLT CAV_3, 4.3%, see Table 2). In the cumulative incidence analysis, the likelihood of developing CAV 10 years after transplantation was 26.2% (95% CI 18.7–34.4%) in the L/L genotype class, 20.2% (95% CI 14.1–27.2%) in the S/L genotype class, and 36.3% (95% CI 20.2–52.5%) in

Table 1. Characteristics of the study population.

Factor	Frequency	Percent	Median	Range
Genotype class				
S/S	42	12.2		
S/L	162	47.1		
L/L	140	40.7		
CAV				
ISHLT 0	217	63.1		
ISHLT 1	76	22.1		
ISHLT 2	30	8.7		
ISHLT 3	21	6.1		
Recipient age (years)			54	9–73
Recipient sex (f versus m)	72/272	20.9/79.1		
Recipient height (cm)			174	129–197
Recipient weight (kg)			74	23–127
Donor age (years)			34	10–70
Donor sex (f versus m)	93/248	27.3/72.7		
Donor height (cm)			176	110–195
Donor weight (kg)			75	25–130
Ischemia time minutes			174	50–356
Rejection episodes	45	13.1		
CMV risk				
Low risk (D−/R−)	63	18.3		
Medium risk (D+/R+, D−/R+)	216	62.8		
High risk (D+/R−)	65	18.9		
Diabetes mellitus type 2 (yes versus no)	68/276	19.8/80.2		
History of smoking (yes versus no)	115/222	34.1/65.9		

S/S, short/short; S/L, short/long; L/L, long/long; CAV, cardiac allograft vasculopathy; ISHLT, International Society of Heart and Lung Transplantation; f, female; m, male; CMV, cytomegalovirus; D, donor; R, recipient.

Table 2. Prevalence of CAV by HO-1 genotype class.

Severity of CAV	S/S	S/L	L/L	Total
ISHLT CAV_0	21	107	89	217
ISHLT CAV_1	13	32	31	76
ISHLT CAV_2	5	11	14	30
ISHLT CAV_3	3	12	6	21
Total	42	162	140	344

CAV, cardiac allograft vasculopathy; S/S, short/short; S/L, short/long; L/L, long/long; ISHLT, International Society of Heart and Lung Transplantation.

the S/S genotype class (Fig. 1). The difference was not statistically significant ($P = 0.105$).

In the univariate Cox regression analysis, no statistical significance could be defined for the overall effect of the three genotype classes on the development of CAV ($P = 0.088$, see Table 3). However, in our multivariable model, the homozygous S/S $(GT)_n$ repeat genotype class revealed a higher risk for CAV ($P = 0.0316$), where the L/L class compared to the S/S class showed a hazard ratio of 0.520 (95% CI 0.302–0.895) and the S/L class compared to the S/S class showed a hazard ratio of 0.509 (95% CI 0.300–0.865, see Table 4).

Common risk factors for CAV

Calculating the cumulative incidence function, the likelihood of developing CAV after transplantation was shown to be 2.3% after 1 year (95% CI 1.1–4.4%), 24.5% after 10 years (95% CI 19.8–29.6%), 51.5% after 20 years (95% CI 43.8–58.7%), and 62.5% after 30 years (95% CI 52.2–71.1%). Comparing the cumulative incidence functions with respect to preoperative patient characteristics, only diabetes mellitus type 2 ($P = 0.031$), donor age (categorized at the median age: $P = 0.022$), and recipient weight (categorized at the median weight: $P = 0.005$) could be shown to have a statistically significant effect on the incidence of CAV.

In the univariate Cox regression analysis, a time-dependent effect could be seen of donor weight on the incidence of CAV ($P = 0.0003$, see Table 3). The statistically significant effect of

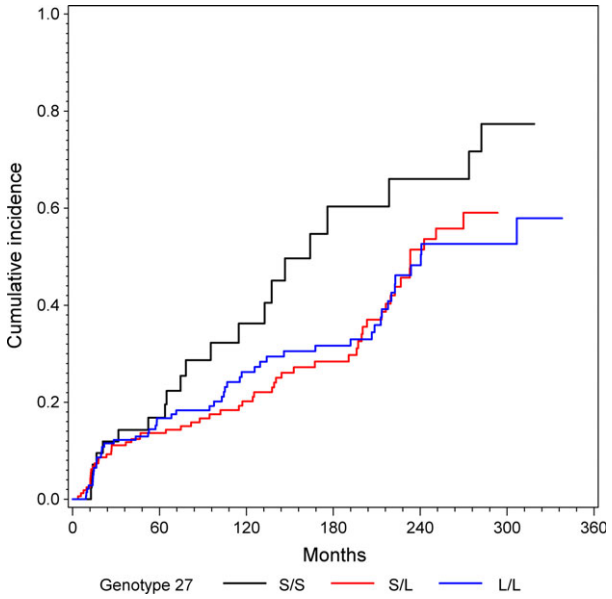

Figure 1 Kaplan-Meier cumulative incidence plot with the incidence of cardiac allograft vasculopathy (CAV) and months of follow-up grouped by heme oxygenase-1 (HO-1) genotype class. S/S, short/short; S/L, short/long; L/L, long/long.

diabetes mellitus ($P = 0.039$), donor age ($P < 0.001$), and recipient weight ($P = 0.016$) on the development of CAV was confirmed in the univariate model. The occurrence of a moderate (grade 2R) or severe rejection (grade 3R) episode showed a potential risk for CAV ($P = 0.064$).

In the multivariable Cox regression model, donor age showed a statistically significant effect on the development of CAV ($P = 0.001$, see Table 4). A time-dependent effect of donor weight on the development of CAV was seen, where the effect diminished over time ($P = 0.005$). History of rejection episodes again revealed a potential risk for CAV ($P = 0.058$), as well as a history of smoking ($P = 0.060$). No statistically significant independent association was found concerning the incidence of CAV and recipient sex, donor sex, diabetes, recipient weight, donor height, recipient height, left ventricular ejection fraction, CMV risk, or ischemia time.

Discussion

Previous studies were not able to show an association between the HO-1 $(GT)_n$ genotype and the manifestation of cardiac allograft vasculopathy [23,24]. In our multivariable Cox model, we could show a statistically significant effect of the HO-1 genotype on CAV development when corrected for common risk factors, where the homozygous short $(GT)_n$ length polymorphism group (S/S) revealed a higher risk for CAV

Table 3. Univariate Cox regression analysis of study population.

Factor	HR	95% CI	*P*-value
Genotype class			
L/L versus S/S	0.610	0.366–1.016	0.088
S/L versus S/S	0.575	0.347–0.953	
Recipient age (years)	1.008	0.994–1.022	0.260
Recipient sex (f versus m)	1.018	0.656–1.581	0.936
Recipient height (cm)	1.000	0.982–1.019	0.967
Recipient weight (kg)	1.016	1.003–1.029	0.016
Donor age (years)	1.024	1.010–1.038	<0.001
Donor sex (f versus m)	0.826	0.547–1.246	0.362
Donor height (cm)	1.017	0.998–1.037	0.083
Donor weight (kg)			
1 year post-transplant	1.039	1.020–1.060	0.0003
5 years post-transplant	1.018	1.006–1.030	
10 years post-transplant	1.009	0.995–1.023	
20 years post-transplant	1.000	0.982–1.018	
Ischemia time minutes	1.001	0.998–1.005	0.460
Rejection episodes	1.648	0.971–2.798	0.064
CMV risk			
Low risk versus high risk	1.236	0.700–2.182	0.322
Medium risk versus high risk	0.894	0.545–1.467	
Diabetes mellitus type 2	1.538	1.023–2.312	0.039
History of smoking	0.836	0.574–1.218	0.351

L/L, long/long; S/S, short/short; S/L, short/long; f, female; m, male; CMV, cytomegalovirus.

Data expressed as hazard ratio (HR) and 95% confidence interval (CI).

Table 4. Multivariable Cox regression analysis of study population.

Factor	HR	95% CI	*P*-value
Genotype class			
L/L versus S/S	0.520	0.302–0.895	0.0316
S/L versus S/S	0.509	0.300–0.865	
Recipient age (years)	0.995	0.979–1.012	0.589
Recipient sex (f versus m)	1.603	0.892–2.879	0.114
Recipient weight (kg)	1.012	0.995–1.030	0.179
Donor age (years)	1.027	1.011–1.044	0.001
Donor sex (f versus m)	0.809	0.483–1.355	0.421
Donor weight (kg)			
1 year post-transplant	1.036	1.014–1.058	0.005
5 years post-transplant	1.013	0.997–1.029	
10 years post-transplant	1.003	0.985–1.022	
20 years post-transplant	0.994	0.970–1.017	
Rejection episodes	1.710	0.981–2.980	0.058
Diabetes mellitus type 2	1.413	0.893–2.234	0.140
History of smoking	0.680	0.455–1.017	0.060
CMV risk			
Low risk versus high risk	1.302	0.713–2.379	0.088
Medium risk versus high risk	0.791	0.464–1.348	

L/L, long/long; S/S, short/short; S/L, short/long; f, female; m, male; CMV, cytomegalovirus. Data expressed as hazard ratio (HR) and 95% confidence interval (CI).

development. We were able to show an opposite effect compared to prior results describing the S/S polymorphism as a protective variant in several settings.

It has been shown that the overexpression of HO-1 in rat aorta chronic rejection models resulted in the inhibition of chronic rejection and the prevention of graft arteriosclerosis, implying a beneficial effect of HO-1 on the long-term function of cardiac allografts, raising the question whether the HO-1 $(GT)_n$ length polymorphism and the related transcriptional activity may have an impact on long-term survival [25,26]. Unfortunately, these findings could not be verified in humans, where the analysis of the HO-1 $(GT)_n$ length polymorphism in endomyocardial biopsies of allografts did not show a significant difference in the frequency of CAV or mortality [24]. Holweg *et al.* [23], who analyzed both donor and recipient DNA, were not able to show a statistically significant difference in the frequency of CAV, defined as abnormalities in a coronary angiogram 1 year after transplantation. Considering this, we analyzed the allele status of allograft recipients and used the standardized nomenclature as defined by the ISHLT to describe the clinical onset of CAV, which takes into account both angiographic lesions and allograft dysfunction as defined by a LVEF ≤45% [19]. Furthermore, we were able to analyze a larger patient population with a longer follow-up time after transplantation.

Chen *et al.* [27] evaluated more than 2000 patients with coronary heart disease and their matched controls for the HO-1 $(GT)_n$ genotype, showing that subjects carrying a S/S $(GT)_n$ genotype were less likely to have coronary heart disease, especially in subjects with high levels of oxidative stress as seen by elevated levels of plasma malonaldehyde. Additionally, lower transcriptional activity of the HO-1 gene was observed more frequently in type 2 diabetic patients with coronary artery disease, suggesting that elevated oxidative stress levels and decreased HO-1 levels may contribute to the development of coronary artery disease [7,28]. However, high levels of oxidative stress were required for high levels of HO-1 expression in subjects carrying the S/S $(GT)_n$ genotype, and lower levels of oxidative stress did not affect HO-1 levels regardless of the genotype class [27]. In 2013, White *et al.* [29] analyzed cardiac transplantation recipients for plasma biomarkers of oxidative stress using enzyme-linked immunosorbent assay (ELISA), showing that peak levels were reached early on in the post-transplant phase (weeks 2–4), thereafter decreasing to reach a plateau. This characteristic oxidative stress pattern after cardiac transplantation may indicate chronic dysregulation in the development of CAV rather than acute ischemic damage, a process in which HO-1 activity may not be implicated.

Recently, the analysis of HO-1 mRNA and protein levels in serum, liver and adipose biopsies from obese insulin-resistant and obese insulin-sensitive individuals showed that obese insulin-resistant individuals were exposed to higher HO-1 expression levels than insulin-sensitive individuals, and the HO-1 expression levels served as positive predictors of metabolic dysregulation [30]. As these findings were contradictory to expectancies, the same group used mouse HO-1 deletion models to verify these results, which showed that the deletion of HO-1 in hepatocytes led to normal metabolic regulation as determined by glucose and insulin tolerance, indicating a driving role of HO-1 in the development of metabolic inflammation and metabolic disease [30]. These results indicate an effect similar to the results we were able to show in our model, clearly indicating the necessity of further research regarding the interplay of HO-1 and inflammatory pathways, potentially with the notion that the loss of HO-1 actually has an anti-inflammatory effect.

The multitude of effects associated with HO-1 and its by-products, particularly free iron, bilirubin, biliverdin, and carbon monoxide, observed *in vitro* and *in vivo* in animal studies, results in an array of consequences that have all been shown to affect the pathogenesis of human diseases [4,31–34]. The protective effects observed in relation to HO-1 and the heme degradation pathway have been linked to the collaboration of various immune factors including macrophage migration inhibitory factor, TNF-alpha, TGF-beta 1, IL-4, IL-10, IL-17 and the complement system [33–38]. Additional research is required to further define the interplay among these factors and thus elicit the precise role of HO-1.

In addition to the HO-1 $(GT)_n$ repeat polymorphism, it has been shown that a −413A>T single nucleotide polymorphism in the promoter region of the gene may also affect the development of coronary artery disease [39]. The HO-1 $(GT)_n$ repeat polymorphism that we analyzed for this study may, therefore, not be the only genetic polymorphism responsible for the regulation of the transcriptional activity of HO-1, along with other yet undefined genetic or epigenetic factors. Interestingly, Lüblinghoff *et al.* [40] analyzed both the HO-1 $(GT)_n$ repeat polymorphism and the −413A>T single nucleotide polymorphism, but were not able to demonstrate a correlation with the development of coronary artery disease. Thus, it is possible that the HO-1 $(GT)_n$ genotype alone is not sufficient as a risk factor for coronary artery disease.

It is established that CAV is correlated with a combination of traditional vascular risk factors and transplant-associated risk factors. In our study, we took into account a number of common risk factors for the development of CAV, including donor age, donor weight, donor height, recipient age, recipient weight, recipient height, left ventricular ejection fraction prior to transplantation, time from transplant to diagnosis, ischemia time, rejection episodes, diabetes, and smoking. The univariate Cox regression analysis showed a statistically significant effect of donor age, donor weight, recipient weight, and diabetes mellitus on the development of CAV, with a borderline significant effect of the occurrence of rejection episodes. Additionally, donor weight and donor age were statistically significant predictors of CAV in our multivariable Cox model, with borderline significant effects of rejection episodes and history of smoking. The multitude of risk factors implicated in the development of CAV presents a confounding factor that may influence the results of studies performed, as well as the medication regimen including immunosuppressive drugs, and the effects of these aspects needs to be evaluated in a larger series to determine their position in the development of CAV [16].

At our institution, coronary angiography is routinely performed 1, 5 and 10 years after cardiac transplantation, or whenever clinically indicated. Therefore, important data on the timeline of CAV development may be missed, patients may be classified as CAV negative because the diagnosis has not yet been made if the patient is asymptomatic, and patients with severe CAV may die prior to diagnosis. Additionally, evidence has been presented that coronary angiography may underestimate the extent of CAV and thus provide false negative results in the diagnosis of CAV, suggesting the requirement of more sensitive methods such as IVUS [16,18,41].

Taken together, evidence suggest that it is highly probable that there are several factors influencing the development of cardiac allograft vasculopathy after heart transplantation, which are not likely be traced to the HO-1 $(GT)_n$ genotype alone. Further studies need to be conducted to increase the understanding of the underlying mechanism of the development of CAV. The impact of the various genetic polymorphisms found in the *HMOX1* gene on the transcriptional activity of HO-1 and thus the actual levels of HO-1 found in the bloodstream, and finally the interaction between HO-1, oxidative stress, and the development of CAV remains to be determined.

Conclusion

In our patient population, the multivariable Cox regression analysis revealed a statistically significant effect of the HO-1 $(GT)_n$ length polymorphism on the development of cardiac allograft vasculopathy, where the homozygous short group was associated with a higher risk for CAV ($P = 0.032$). These results are contradictory to results presented in previous studies, which either showed evidence for a protective effect of the short $(GT)_n$ length polymorphism group on the development of CAV, or no statistically significant effect of the $(GT)_n$ length genotype on the development of CAV. This finding has to be evaluated in a larger series including studies targeting the underlying disease mechanism, to provide further evidence whether the HO-1 $(GT)_n$ genotype could be used as a reliable marker for cardiac allograft vasculopathy.

Authorship

KF: data collection and analysis, drafting and review of the manuscript. MA: study design, interpretation of results and manuscript review. MB and TP: study design and contribution of important laboratory examinations. AK: analysis and interpretation of data. MM: data collection and analysis. AK: protocol writing, study design, interpretation of results. MW and AZ: interpretation of results and critical review of manuscript for publication.

Funding

The study was funded by the Department of Cardiac Surgery, Medical University of Vienna.

Conflicts of interest

The authors have no conflicts of interest to disclose.

REFERENCES

1. Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 1996; **2**: 87.
2. Exner M, Minar E, Wagner O, Schillinger M. The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med* 2004; **37**: 1097.
3. Linnenbaum M, Busker M, Kraehling JR, Behrends S. Heme oxygenase isoforms differ in their subcellular trafficking during hypoxia and are differentially modulated by cytochrome P450 reductase. *PLoS One* 2012; **7**: e35483.
4. Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev* 2008; **60**: 79.
5. Kimpara T, Takeda A, Watanabe K, *et al.* Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease. *Hum Genet* 1997; **100**: 145.
6. Shibahara S, Sato M, Muller RM, Yoshida T. Structural organization of the human heme oxygenase gene and the function of its promoter. *Eur J Biochem* 1989; **179**: 557.
7. Chen YH, Lin SJ, Lin MW, *et al.* Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet* 2002; **111**: 1.
8. Yamada N, Yamaya M, Okinaga S, *et al.* Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 2000; **66**: 187.
9. Endler G, Exner M, Schillinger M, *et al.* A microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with increased bilirubin and HDL levels but not with coronary artery disease. *Thromb Haemost* 2004; **91**: 155.
10. Han SW, Song W, Kim HS, *et al.* HMOX1 gene promoter polymorphism is not associated with coronary artery disease in Koreans. *Ann Lab Med* 2014; **34**: 337.
11. Tiroch K, Koch W, von Beckerath N, Kastrati A, Schomig A. Heme oxygenase-1 gene promoter polymorphism and restenosis following coronary stenting. *Eur Heart J* 2007; **28**: 968.
12. Kaneda H, Ohno M, Taguchi J, *et al.* Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1680.
13. Schillinger M, Exner M, Minar E, *et al.* Heme oxygenase-1 genotype and restenosis after balloon angioplasty: a novel vascular protective factor. *J Am Coll Cardiol* 2004; **43**: 950.
14. Rahmani M, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. *Circ Res* 2006; **99**: 801.
15. Stehlik J, Edwards LB, Kucheryavaya AY, *et al.* The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report – 2012. *J Heart Lung Transplant* 2012; **31**: 1052.
16. Colvin-Adams M, Agnihotri A. Cardiac allograft vasculopathy: current knowledge and future direction. *Clin Transplant* 2011; **25**: 175.
17. Pollack A, Nazif T, Mancini D, Weisz G. Detection and imaging of cardiac allograft vasculopathy. *JACC Cardiovasc Imaging* 2013; **6**: 613.
18. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008; **117**: 2131.
19. Mehra MR, Crespo-Leiro MG, Dipchand A, *et al.* International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010; **29**: 717.
20. Stewart S, Winters GL, Fishbein MC, *et al.* Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart

rejection. *J Heart Lung Transplant* 2005; **24**: 1710.
21. Doberer D, Haschemi A, Andreas M, *et al.* Haem arginate infusion stimulates haem oxygenase-1 expression in healthy subjects. *Br J Pharmacol* 2010; **161**: 1751.
22. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996; **17**: 343.
23. Holweg CT, Balk AH, Uitterlinden AG, *et al.* Functional heme oxygenase-1 promoter polymorphism in relation to heart failure and cardiac transplantation. *J Heart Lung Transplant* 2005; **24**: 493.
24. Ullrich R, Exner M, Schillinger M, *et al.* Microsatellite polymorphism in the heme oxygenase-1 gene promoter and cardiac allograft vasculopathy. *J Heart Lung Transplant* 2005; **24**: 1600.
25. Bouche D, Chauveau C, Roussel JC, *et al.* Inhibition of graft arteriosclerosis development in rat aortas following heme oxygenase-1 gene transfer. *Transpl Immunol* 2002; **9**: 235.
26. Du D, Chang S, Chen B, Zhou H, Chen ZK. Adenovirus-mediated heme oxygenase transfer inhibits graft arteriosclerosis in rat aortic transplants. *Transplant Proc* 2007; **39**: 3446.
27. Chen M, Zhou L, Ding H, *et al.* Short (GT) (n) repeats in heme oxygenase-1 gene promoter are associated with lower risk of coronary heart disease in subjects with high levels of oxidative stress. *Cell Stress Chaperones* 2012; **17**: 329.
28. Chen YH, Chau LY, Chen JW, Lin SJ. Serum bilirubin and ferritin levels link heme oxygenase-1 gene promoter polymorphism and susceptibility to coronary artery disease in diabetic patients. *Diabetes Care* 2008; **31**: 1615.
29. White M, Cantin B, Haddad H, *et al.* Cardiac signaling molecules and plasma biomarkers after cardiac transplantation: impact of tacrolimus versus cyclosporine. *J Heart Lung Transplant* 2013; **32**: 1222.
30. Jais A, Einwallner E, Sharif O, *et al.* Heme oxygenase-1 drives metaflammation and insulin resistance in mouse and man. *Cell* 2014; **158**: 25.
31. Araujo JA, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front Pharmacol* 2012; **3**: 119.
32. Durante W. Targeting heme oxygenase-1 in vascular disease. *Curr Drug Targets* 2010; **11**: 1504.
33. Li W, Iyer S, Lu L, *et al.* Attenuation of aortic graft arteriosclerosis by systemic administration of Allotrap peptide RDP58. *Transpl Int* 2003; **16**: 849.
34. Soares MP, Lin Y, Anrather J, *et al.* Expression of heme oxygenase-1 can determine cardiac xenograft survival. *Nat Med* 1998; **4**: 1073.
35. Braudeau C, Bouchet D, Tesson L, *et al.* Induction of long-term cardiac allograft survival by heme oxygenase-1 gene transfer. *Gene Ther* 2004; **11**: 701.
36. Evans JM, Navarro S, Doki T, Stewart JM, Mitsuhashi N, Kearns-Jonker M. Gene transfer of heme oxygenase-1 using an adeno-associated virus serotype 6 vector prolongs cardiac allograft survival. *J Transplant* 2012; **2012**: 740653.
37. Tsui TY, Wu X, Lau CK, *et al.* Prevention of chronic deterioration of heart allograft by recombinant adeno-associated virus-mediated heme oxygenase-1 gene transfer. *Circulation* 2003; **107**: 2623.
38. Woo J, Iyer S, Cornejo MC, *et al.* Stress protein-induced immunosuppression: inhibition of cellular immune effector functions following overexpression of haem oxygenase (HSP 32). *Transpl Immunol* 1998; **6**: 84.
39. Ono K, Goto Y, Takagi S, *et al.* A promoter variant of the heme oxygenase-1 gene may reduce the incidence of ischemic heart disease in Japanese. *Atherosclerosis* 2004; **173**: 315.
40. Lublinghoff N, Winkler K, Winkelmann BR, *et al.* Genetic variants of the promoter of the heme oxygenase-1 gene and their influence on cardiovascular disease (the Ludwigshafen Risk and Cardiovascular Health study). *BMC Med Genet* 2009; **10**: 36.
41. Weis M, von Scheidt W. Cardiac allograft vasculopathy: a review. *Circulation* 1997; **96**: 2069.