International Journal of Cardiology 197 (2015) 209–215

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Renin–angiotensin–aldosterone system genotype and serum BNP in a contemporary cohort of adults late after Fontan palliation

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ARTICLE INFO

Article history:
Received 22 February 2015
Received in revised form 9 June 2015
Accepted 12 June 2015
Available online 18 June 2015

Keywords:
Angiotensin-converting enzyme
Congenital heart disease
Clinical genetics
Fontan
Heart failure
Hypertrophy

ABSTRACT

Background: Adults with single ventricle physiology palliated with a Fontan circulation experience high mortality due to circulatory failure. Renin-angiotensin-aldosterone system (RAAS) genotype contributes to adverse cardiovascular outcomes in acquired heart failure. This study evaluated associations between RAAS genotype, ventricular mass and function in a contemporary cohort of adults with a Fontan circulation.
Methods: This single-center prospective study included adults (n = 106) seen after the Fontan operation (mean age 27 ± 9 years). Patients were genotyped for 5 pro-hypertrophic RAAS gene polymorphisms. Serum BNP, ventricular mass and function, and clinical events were compared between those with ≥2 homozygous risk genotypes ("high-risk", n = 31) versus those with ≤1 homozygous risk genotypes ("low risk", n = 75).
Results: "High-risk" genotype was associated with diastolic dysfunction and higher serum BNP levels. There was no association between RAAS genotype and either ventricular mass or systolic function. During a mean follow-up duration of 9.5 ± 7.6 years, late Fontan failure occurred in 20% (n = 21) of patients, including 7 deaths. Serum BNP emerged as an independent predictor of late Fontan failure (HR 1.11 [CI 1.01–1.23] for each 50 unit increase in BNP, p = 0.04) and death alone (HR 1.25 [CI 1.07–1.47] for each 50 unit increase in BNP, p = 0.006). RAAS genotype was not associated with adverse clinical events.
Conclusions: Fontan failure is common among adults with single ventricle physiology. RAAS genotype is not associated with increased ventricular mass but does appear to influence diastolic function late after the Fontan operation. Elevated BNP is an independent predictor of Fontan failure and mortality in adulthood.

1. Introduction

The Fontan operation is a palliative surgery for children with complex congenital heart disease giving rise to single ventricle physiology. While early outcomes after surgery are excellent, late complications are common. Circulatory failure is an important cause of late mortality [1]. Understanding the pathogenesis and predictors of Fontan circulatory failure is important for selection of treatment strategies in this growing population.

The pathophysiology of the failing Fontan circulation remains poorly defined. Elevated serum brain natriuretic peptide (BNP) levels have been documented in adults post Fontan palliation [2] particularly those with older style (atriopulmonary and atrioventricular) versus more contemporary (total cavopulmonary) Fontan connections [3]. Diastolic dysfunction [4] and ventricular myocardial fibrosis [5] are common whereas systolic function is often preserved [6]. Patients with failing Fontan physiology are frequently hospitalized for heart failure (HF) [1] and referred for heart transplantation, although mortality remains high even with these interventions [7].

In acquired heart disease, the renin–angiotensin–aldosterone system (RAAS) is a key determinant of left ventricular hypertrophy (LVH) [8,9], a major independent risk factor for cardiovascular morbidity and mortality [10]. Although genetic data are limited in congenital heart

Abbreviations: ACE, angiotensin converting enzyme; BNP, B-type natriuretic peptide; CMR, cardiac magnetic resonance; CPET, cardiopulmonary exercise test; DBP, diastolic blood pressure; EDV, end-diastolic volume; ESV, end-systolic volume; HF, heart failure; IVRT, isovolumic relaxation time; LV, left ventricle; LVH, left ventricular hypertrophy; NYHA, New York Heart Association; RA, right atrium; RAAS, renin–angiotensin–aldosterone system; RER, respiratory exchange ratio; SBP, systolic blood pressure; TCPC, total cavopulmonary connection.

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[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.ijcard.2015.06.018
0167-5273/

disease (CHD), RAAS genotype has been shown to influence cardiac phenotype in simple and complex CHD. Variations in angiotensin converting enzyme (ACE) genotype are associated with increased ventricular mass after aortic coarctation repair [11]. Pro-hypertrophic RAAS polymorphisms are also associated with persistent elevation in ventricular mass and failure of reverse remodeling after volume-unloading surgery in infants with a single ventricle circulation [12]. Given the reported association between RAAS genotypes and ventricular mass in single ventricle infants, the primary objective of this study was to determine whether RAAS genotype impacts ventricular mass late after Fontan palliation. We also evaluated the relationships between RAAS genotype, ventricular mass and function, serum BNP and clinical outcomes.

2. Methods

2.1. Study design

This was a prospective study evaluating 106 adult Fontan patients (>18 years) who consented to RAAS genotyping while attending the ambulatory clinic of the Toronto Congenital Cardiac Center for Adults between 2004 and 2012. The study protocol was in accord with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the ethics research committee. Written informed consent was obtained from all participants.

2.2. Genotype

Genotyping was performed at The Centre for Applied Genomics (TCAG), The Hospital for Sick Children, Toronto, Canada, according to previously published methods [13]. Patients were genotyped for polymorphisms in 5 RAAS genes: (1) angiotensinogen (*AGT*; rs11568053) (2) angiotensin-converting enzyme (*ACE*; rs4340) (3) angiotensinogen receptor type 1 (*AGTR1*; rs5186) (4) aldosterone synthase (*CYP11B2*; rs1799998) and (5) cardiac chymase A (*CMA1*; rs1800875) (primer sequences and primers available upon request) [14]. RAAS polymorphisms were selected on the basis of prior association studies, functional effects, and population allele frequencies. Alleles associated with RAAS upregulation were classified as risk alleles and high-risk was defined as homozygosity for ≥2 risk alleles [12,15,16].

2.3. Clinical phenotype

Details pertaining to age, cardiac anatomy, Fontan type, and current medications were recorded at study enrolment. Patient diagnoses were divided into tricuspid atresia, double inlet left ventricle or complex [17]. The complex group comprised those with anatomy unsuitable for biventricular repair and who did not have tricuspid atresia or double-inlet left ventricle. Types of Fontan were classified on the basis of operative reports into 5 categories: (1) right atrium to pulmonary artery anastomosis, (2) right atrium to right ventricle anastomosis, (3) extracardiac conduit, (4) intra-atrial lateral tunnel and (5) other. Serum BNP >100 pg/ml was classified as abnormal on the basis that a level <100 pg/ml has a high negative predictive value for the diagnosis of HF [18,19] and mortality in ACHD [20]. Peak oxygen uptake (VO_2) was obtained with cardiopulmonary exercise testing (CPET) [21,22].

Late Fontan failure was defined as HF hospitalization, heart transplantation or death (only the highest order event was entered into analysis of adverse clinical outcomes). HF hospitalization was defined a priori as hospital admission for clinical evidence of HF, leading to initiation/up-titration of diuretic therapy or inotropic support [23], a definition previously applied to the adult Fontan population [24]. Adverse events were adjudicated by two ACHD cardiologists.

2.4. Ventricular mass and function

2.4.1. Echocardiography

All subjects underwent 2-dimensional echocardiography to assess systolic function of the dominant ventricle. Ventricular systolic function by echocardiogram graded as normal (LVEF ≥55%), mildly reduced (45–54%), moderately reduced (30–44%) or severely reduced (<30%) [25]. Standard measures of diastolic function were available in a subset of patients (n = 52) [26]. All measurements were performed by a dedicated sonographer with expertise in CHD imaging and according to a standardized Fontan imaging protocol. All echocardiograms were read by congenital cardiologists blinded to patient genotype.

2.4.2. Cardiac magnetic resonance imaging (CMR)

Short axis steady-state free-precession (SSFP) cine imaging was performed and endo/epicardial contours were manually defined on end-diastolic and end-systolic phases on a slice-by-slice basis, as previously described [27]. Late enhancement imaging was performed in multiple planes, at least 10 min after gadolinium injection for evaluation of myocardial fibrosis. The presence of myocardial fibrosis, as evidenced by bright signal in the myocardium late after gadolinium enhancement, was noted when present.

Offline analysis was completed on a dedicated workstation using commercially available software (CMR 42, Circle Cardiovascular Imaging Inc, Calgary, Alberta) by a single experienced observer. Trabeculations were excluded to the blood pool during measurement of end-diastolic and end-systolic volumes (ESV) for calculation of ejection fraction (EF) but were manually contoured for inclusion in total mass measurements [27,28]. Total indexed mass (dominant and accessory [hypoplastic] ventricle) and indexed mass for the dominant ventricle alone were recorded separately. Intra/interobserver measurements were repeated in a random subset of patients, in a blinded fashion, >6 months after the initial analysis was completed; inter-class correlation coefficients within and between observers for mass measurements were 0.92 (95% CI 0.42–0.99) and 0.78 (95% CI 0.03–0.97), respectively.

2.5. Statistical analysis

Data are described as frequencies, means ± SD, or the median and range, as appropriate. Continuous variables were analyzed using either the student *t*-test or the Wilcoxon rank sum test. Categorical data were compared using the Chi square test or the Fisher exact test, as applicable. Because the BNP values were highly skewed, these were analyzed following logarithmic transformation (logBNP).

RAAS genotype frequencies were confirmed to be in Hardy–Weinberg equilibrium using the Pearson chi-squared test. Multivariate regression analyses were performed to evaluate whether ventricular mass was associated with gene subgroup after adjustment for potential confounding factors including RAAS blockers, age, gender, blood pressure, and LV morphology [12,14,16]. Cox proportional hazard models were created to evaluate the impact of pre-specified variables (RAAS genotype, BNP, ventricular mass, mass/volume ratio) on the composite endpoint of heart failure, death or transplant occurring after transfer to adult care (age >18 years). Time to event was determined as the time from the first adult clinic review to adverse outcome. A p value < 0.05 was regarded as statistically significant. All analyses were performed using the Statistical Package Social Sciences, version 17.0, software program (SPSS, Chicago, Illinois).

3. Results

3.1. Clinical characteristics

Clinical features of the study population are listed in Table 1. The mean age at the time of enrollment was 30 ± 10 years and study