Journal of Molecular and Cellular Cardiology 51 (2011) 848–854

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

A polymorphic miR-155 binding site in *AGTR1* is associated with cardiac hypertrophy in Friedreich ataxia

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ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 22 June 2011
Accepted 5 July 2011
Available online 12 July 2011

Keywords:
Friedreich ataxia
Cardiac function and structure
Genes
Renin–Angiotensin–Aldosterone system
microRNA

ABSTRACT

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative condition with a heterogeneous cardiac phenotype caused primarily by an expanded GAA trinucleotide repeat in the frataxin gene (*FXN*). FXN is important in mitochondrial iron efflux, sensitivity to oxidative stress, and cell death. The number of GAA repeats on the smaller *FXN* allele (GAA1) only accounts for a portion of the observed variability in cardiac phenotype. Genetic modifying factors, such as single nucleotide polymorphisms (SNPs) in genes of the Renin–Angiotensin–Aldosterone system (RAAS), may contribute to phenotype variability. This study investigated genetic variability in the angiotensin-II type-1 receptor (*AGTR1*), angiotensin-converting enzyme (*ACE*), and *ACE2* genes as cardiac phenotype modifying factors in FRDA patients. Comprehensive review of the *AGTR1*, *ACE* and *ACE2* genes identified twelve haplotype tagging SNPs. Correlation of these SNPs with left ventricular internal diameter in diastole (LVIDd), interventricular septal wall thickness (SWT) and left ventricular mass (LVM) was examined in a large Australian FRDA cohort ($n=79$) with adjustments performed for GAA repeats, age, sex, body surface area and diastolic blood pressure. A significant inverse relationship was observed between GAA1 and LVIDd ($p=0.010$) but not with SWT or LVM after adjustment for covariates. The *AGTR1* polymorphism rs5186 was more common in FRDA patients than in a control population ($p=0.002$). Using a recessive model of inheritance, the C allele of rs5186 was associated with a significant increase in SWT ($p=0.003$) and LVM ($p=0.001$). This functional polymorphism increases expression of *AGTR1* by altering the binding site for miR-155, a regulatory microRNA. No significant associations with left ventricular structure were observed for the remaining RAAS polymorphisms. The *AGTR1* polymorphism rs5186 appears to modify the FRDA cardiac phenotype independently of GAA1. This study supports the role of RAAS polymorphisms as modifiers of cardiac phenotype in FRDA patients.

1. Introduction

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disease arising from mutations in both alleles of the frataxin gene (*FXN*), with a reported prevalence of 1 in 29000, making it the most common inherited ataxia [1,2]. In approximately 97% of cases the mutant alleles have an expansion of a GAA trinucleotide repeat in intron 1 of *FXN* that reduces the amount of frataxin available to assist with mitochondrial iron efflux and increases sensitivity to oxidative stress, resulting in cell damage and death due to excess production of free radicals [1,3–5].

As well as the neurological features of the disease, a large proportion of FRDA patients develop abnormalities of cardiac structure or function. The most common observations are increased thickness of the left ventricular wall [6–12], reduced left ventricular cavity size [10,12,13] and increased left ventricular mass index (LVMI) [10–12]. Most patients have a normal ejection fraction [7,11,13], but a proportion of patients develop left ventricular wall thinning, dilatation and a reduction in ejection fraction [14,15]. Heart failure and cardiac arrhythmias are thought to be the most important causes of death in FRDA [16,17]. The severity of cardiac involvement in patients with FRDA is highly variable [7,14], suggesting that there are factors capable of modifying the cardiac phenotype. These modifying factors could be environmental and/or genetic, and may act either by exacerbating or protecting against disease [18].

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doi:10.1016/j.yjmcc.2011.07.001

One such genetic modifying factor is the number of GAA repeats on the smaller *FXN* allele (GAA1). Previous studies have reported a positive correlation of GAA1 with the presence of cardiac structural change in FRDA [3,19,20] and increases in interventricular septal wall thickness (SWT) [6–9,11] and left ventricular mass (LVM) [7,9,13]. While most studies have reported a more important role for GAA1, associations between the number of GAA repeats on the larger *FXN* allele (GAA2) with age of disease onset [19,21,22], SWT [11] and the presence of cardiac disease [19,20] have also been reported. However, *FXN* allele GAA repeat length only accounts for a portion of the observed variability of cardiac abnormalities (up to 20%) [11,19–22], suggesting there are other contributing factors yet to be identified.

The Renin–Angiotensin–Aldosterone system (RAAS) is a well-established mediator of cardiac growth, and functions as the predominant system used by the body to maintain blood pressure homeostasis. This is mediated primarily through the activation of the angiotensin-II type 1 receptor (AT_1R) by angiotensin-II, which results in the activation of several pro-hypertrophic signaling cascades [23]. Consequently, the components of the RAAS capable of influencing the production or activity of angiotensin-II are biologically plausible candidate modifiers of cardiac hypertrophy in diseases with a cardiac phenotype, such as FRDA [24]. These include *AGTR1*, which encodes the AT_1R, as well as *ACE* and *ACE2*, which encode the converting enzymes of the RAAS, angiotensin converting enzyme (ACE) and its counter-regulatory homolog ACE2 [25]. Several studies have investigated polymorphisms in the genes encoding these key RAAS proteins as potential markers or modifiers of disease severity in other cardiovascular diseases [24,26–28].

This study sought to investigate the association between genetic variability of the *AGTR1*, *ACE* and *ACE2* genes and cardiac phenotype in FRDA patients by examining the correlation between several polymorphisms in these genes and three echocardiographic measures of cardiac structure previously shown to be abnormal in FRDA — left ventricular internal diastolic diameter (LVIDd), SWT and LVM.

2. Materials and methods

2.1. Study populations

Patients were recruited from a multidisciplinary FRDA clinic which sees all individuals with FRDA who request to be seen. Approximately two thirds of attendees are from the states of Victoria and New South Wales, Australia with the other third being from other states or overseas. The clinic sees individuals with the full range of the phenotypic spectrum. Clinical evaluation included a detailed physical examination, ECG and echocardiography. All participants were homozygous for an expanded GAA repeat within intron 1 of *FXN*; patients with point mutations were excluded. Two patients were excluded, one due to the presence of aortic valvular disease and one due to previous myocardial infarction. The study protocol was approved by the Southern Health Human Research and Ethics Committee and all patients gave informed consent. Echocardiographic findings on a subgroup (41/79) of the current study cohort have been previously reported [12]. The control population used in this study consisted of 170 predominantly Caucasian individuals from Sydney, Australia who did not suffer from any cardiac-related health problems at the time of blood collection.

2.2. Echocardiography

Transthoracic echocardiography was performed using a Sonos 5500 ultrasound machine (Philips, Amsterdam, The Netherlands) and measurements were performed off-line using Xcelera V1.2 L4 SP2 (Philips, Amsterdam, The Netherlands). Nearly all studies were performed by the one echocardiographer (LD). M-mode images of the left ventricle were obtained in the parasternal long axis view just distal to the mitral valve leaflet tips after alignment of the cursor perpendicular to the left ventricular wall. 2-D images were used to facilitate identification of the endocardium and standard M-mode measurements of LVIDd and SWT were performed [29,30]. LVM was calculated using the modified formula of Devereux et al.[31] and corrected for body surface area (BSA) as LVM index (LVMI). Relative wall thickness (RWT) was calculated as two times the posterior wall thickness (PWT) divided by LVIDd. Four- and two-chamber 2-dimensional loops of left ventricular contraction were recorded and used for measurement of left ventricular end-diastolic volume (LVEDV) and ejection fraction using the biplane method of discs. Left ventricular length (LVL) was measured as the distance between the plane of the mitral annulus and the apical endocardium in the two and four-chamber views at end-diastole, using the longest dimension observed from these two views.

2.3. Genetic analysis

Genomic DNA was extracted [32] and the number of GAA repeats in the *FXN* gene was measured [33] as described previously, with GAA1 representing the shorter allele and GAA2 representing the longer allele. Analysis of the linkage disequilibrium (LD) structure of the *AGTR1*, *ACE* and *ACE2* genes was performed using the HapMap Phase II project data from CEPH Caucasian trios [34]. The haplotype tagging SNPs that defined the underlying haplotype structures were identified using pairwise tagging with r^2 thresholds of 0.8 and minor allele frequency thresholds of 0.05 using the Haploview 4.2 software (version 12.2.0) [35,36]. The *AGTR1, ACE* and *ACE2* genes had five, two and three tag SNPs selected, respectively, which captured the majority of the common haplotype variation within these genes (Fig. 1). The *AGTR1* SNP rs5186 was also examined based on multiple previously reported associations with several cardiovascular diseases [37–42].

Genotyping of SNPs was performed using the TaqMan 5′ nuclease activity assay (Applied Biosystems, USA), according to the manufacturer's recommendations. Briefly, the reaction mixture consisted of 10 ng DNA, 1× Express qPCR Supermix with premixed ROX (Invitrogen), and 1× SNP-specific TaqMan probe, made up to a final volume of 5 μL with triple distilled water. PCR amplification was performed at 95 °C for 10 min, followed by 40 thermal cycles of 95 °C for 15 s, and 60 °C for 1 min. Fluorescence was measured for FAM (excitation wavelength 488 nm, emission wavelength 518 nm) and VIC (excitation wavelength 488 nm, emission wavelength 552 nm) using a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA). Data were gathered and analyzed using SDS version 2.4 software (Applied Biosystems, USA).

In addition to the selected tag SNPs, an insertion/deletion (I/D) polymorphism in the *ACE* gene (*ACE* I/D) was genotyped by PCR as described previously, whereby the insertion (I) and deletion (D) alleles correspond to a 480 bp and 193 bp amplicon, respectively [43].

2.4. Statistical analysis

Statistical analyses were performed using PASW Statistics 17.0 for Macintosh (SPSS Inc. Chicago, IL, USA). Deviation from Hardy–Weinberg equilibrium was assessed using the χ^2 goodness of fit test with one degree of freedom. All data are expressed as mean ± standard deviation, unless noted otherwise. Differences in categorical and continuous variables between cohorts were compared using the χ^2-test and independent samples *t*-test, respectively. The relationship of GAA1 and GAA2 with LVIDd, SWT and LVM was evaluated using multiple linear regression, with adjustment performed for age and factors known to influence left ventricular structure (sex, BSA and diastolic blood pressure). The relations of GAA1 with LVEDV and LVL were also analyzed to assess for consistency with findings obtained for LVIDd.

The association of individual polymorphisms with LVIDd, SWT and LVM was assessed by univariate general linear model analysis with