Atherosclerosis 237 (2014) 38–44

Contents lists available at ScienceDirect

Atherosclerosis

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

Relationship between dimethylarginine dimethylaminohydrolase gene variants and asymmetric dimethylarginine in patients with rheumatoid arthritis☆

Theodoros Dimitroulas [a, *], Aamer Sandoo [a, b], James Hodson [c], Jacqueline Smith [a], Vasileios F. Panoulas [d], George D. Kitas [a, e]

[a] Department of Rheumatology, Dudley Group of Hospitals NHS Trust, Russells Hall Hospital, Dudley DY1 2HQ, UK
[b] School of Sport, Health and Exercise Sciences, Bangor University, George Building, Bangor, Gwynedd, Wales LL57 2PZ, UK
[c] Wolfson Computer Laboratory, University Hospital Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Birmingham B15 2WB, UK
[d] National Heart and Lung Institute, Imperial College London, UK
[e] Arthritis Research UK Epidemiology Unit, University of Manchester, Oxford Road, Manchester M13 9PT, UK

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form
6 July 2014
Accepted 29 July 2014
Available online 27 August 2014

Keywords:
Rheumatoid arthritis
ADMA
DDAH
CV disease

ABSTRACT

Objective: The aim of our study was to determine whether Dimethylarginine Dimethylaminohydrolase (DDAH) 1 and 2 gene polymorphisms – the main enzyme involved in ADMA degradation – are associated with high Asymmetric Dimethylarginine (ADMA) levels in Rheumatoid Arthritis (RA).
Methods: Serum ADMA levels were measured in 201 individuals with RA [155 females median age 67 (59–73)]. Four tag SNPs in DDAH1 gene and 2 in the DDAH2 gene were genotyped by using the LightCycler™ System. ADMA was initially compared across the genetic variables using one-way ANOVA and then multivariate analysis examined each of the genes after adjustment for parameters of systemic inflammation and insulin resistance, namely erythrocyte sedimentation rate (ESR) and homeostatic model assessment (HOMA), which we have previously shown affect ADMA levels in RA.
Results: No significant relationship between DDAH genetic variables and ADMA levels was established in ANOVA analysis. Multivariate model adjusted for age, HOMA and ESR did not demonstrate any significant association between DDAH variants and ADMA.
Conclusion: The results of our study give no evidence to suggest that increased ADMA levels in RA relate to DDAH genetic polymorphisms. Better understanding of disease-related factors and their interactions with traditional CV risk factors may represent mechanisms responsible for ADMA accumulation in this population.

1. Introduction

In recent years there has been increasing interest in the cardiovascular (CV) complications of chronic systemic inflammatory conditions such as rheumatoid arthritis (RA). It is now well recognised that RA is closely associated with the development of premature atherosclerosis resulting in high CV mortality and morbidity [1,2]. Pathophysiological and epidemiological data suggest a clinically important relationship between RA and accelerated atherosclerosis as both conditions appear to be provoked by initial synovial/endothelial cell injury respectively [3]. However the mechanisms of vascular changes in RA remain partially understood with systemic inflammatory burden as well as classical cardiovascular disease (CVD) risk factors appearing to be pivotal in the initiation and progression of endothelial dysfunction [4,5]. Over the last years the implication of genetic factors in the CVD risk has been studied with several studies reporting associations between gene variants such as tumour necrosis factor promoter polymorphism and atherosclerosis in RA patients [6–8].

Endothelium-derived nitric oxide (NO) is a vasodilatory mediator with atheroprotective and anti-proliferative effects on the

☆ DDAH variants and ADMA in RA.

* Corresponding author. Department of Rheumatology, Dudley Group of Hospitals NHS Trust, Russells Hall Hospital, Pensnett Road, Dudley, West Midlands DY1 2HQ, United Kingdom. Tel.: +44 1384 456111x3717.
E-mail address: dimitroul@hotmail.com (T. Dimitroulas).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.07.033
0021-9150/

vascular wall. Constitutive production of NO is essential for the regulation of blood flow, the maintenance of vasorelaxation and the prevention of oxidative injury to the vascular endothelium [9]. Pro-inflammatory mediators and cytokines which are abundantly produced in RA exert numerous deleterious effects on the endothelial cells including a reduction in NO production, activation of endothelial cells and platelets, and derangement of fibrinolysis, all of which promote thrombosis and atherosclerosis.

Impairment of NO synthesis is multifactorial but a growing body of evidence suggests that circulating inhibitors of NO synthase play a crucial role [10]. Asymmetric dimethylarginine (ADMA) is an endogenous guanido-substituted analogue of L-arginine and decreases the bioavailability of NO by competing with L-Arginine at the active site of all the three isoforms of NO synthase (NOS). ADMA is generated during proteolysis of various proteins containing methylated arginine residues, a procedure catalysed by a group of enzymes referred to as protein-arginine methyl transferase's (PRMT's) [11]. Over the last decade ADMA has emerged as a novel biochemical marker of endothelial dysfunction and CV risk in various disease settings associated with atherosclerosis such as peripheral and coronal artery disease, lipid disorders, diabetes mellitus, insulin resistance, hypertension, chronic heart and renal failure [12,13] as well as rheumatic diseases [14]. Several studies have demonstrated that plasma ADMA is an independent predictor for adverse cardiovascular events and death in patients with coronary artery disease [15] and in the general population [16]. Elevated ADMA levels have been reported in RA patients irrespective of the disease stage, the initiation and the type of treatment, the presence of clinical CVD or the detection of subclinical atherosclerosis in non-invasive assessments of endothelial function [17–20]. In addition a handful of studies have assessed the impact of biologic disease modifying drugs on ADMA levels [21,22].

Dimethylarginine dimethylaminohydrolase (DDAH) is the key enzyme for the degradation of ADMA into citrulline and dimethylamine [23]. Over 90% of endogenous ADMA is hydrolysed by DDAH with the remainder renally extracted. DDAH exists in two isoforms (DDAH1, DDAH2) encoded by different genes, with DDAH1 being primarily an enzyme of epithelial cells whereas DDAH2 is present in the vasculature [24]. Recent insights indicate that reduced DDAH activity occurs in several pathological conditions accompanied by excess CV morbidity and it is considered one of the crucial mechanisms responsible for ADMA accumulation and endothelial dysfunction. Both deleting the DDAH-1 gene in mice and inhibiting its activity through DDAH-specific inhibitors resulted in structural and functional endothelial changes, increased systemic vascular resistance and abnormal systemic blood pressure via ADMA mediated dysregulation of NO production [25,26]. On the other hand overexpression of DDAH in transgenic mice attenuates ADMA production and restores NO synthesis with favourable vascular outcomes such as reduced arterial stiffness, stabilisation of endothelial function and enhanced insulin sensitivity [27]. Functional variant of DDAH-2 gene is associated with chronic kidney disease and insulin sensitivity conditions linked with endothelial dysfunction and increased CVD risk [28,29]. These observations underlie the important role of DDAH in the regulation of vascular homoeostasis. Despite experimental data suggesting that DDAH1 is primarily responsible for the degradation of methylarginines [30], the relative role of these isoforms in ADMA metabolism in humans remains unknown. Derangement of DDAH/ADMA pathway has recently been described to participate in the pathogenesis of RA in a collagen-induced arthritis animal model [31].

The aim of the present study was to determine whether DDAH1 and DDAH2 gene polymorphisms are associated with circulating ADMA in individuals with established RA.

2. Methods

2.1. Study population

Two-hundred and one consecutive RA patients were recruited from the rheumatology outpatient clinics of the Dudley Group NHS Foundation Trust, UK, between March 2011–March 2013. All patients met the retrospective application of the 1987 revised RA criteria of the American College of Rheumatology [32]. The study received local Research Ethics Committee approval and all participants gave their written informed consent according to the Declaration of Helsinki.

All participants underwent a thorough assessment including a detailed review of their medical history, hospital records, physical examination, and contemporary assessments of height, weight and body mass index. All medications were recorded, including disease modifying anti-rheumatic drug (DMARD) use, oral prednisolone and anti-platelet agents. In addition, demographic information was collected by questionnaire. Insulin resistance was evaluated from fasting glucose and insulin using the Homeostasis Model Assessment of Insulin Resistance (HOMA) [33].

Blood was collected from the patient's antecubital vein using a 23G butterfly needle (Greiner Bio One GmbH, Austria). All tests were carried out in the routine and research laboratories of Russells Hall Hospital, Dudley Group NHS Foundation Trust, UK and were analysed for routine laboratory biochemistry, lipids, haematology, Westergren erythrocyte sedimentation rate (ESR), and C-reactive protein. ADMA levels were measured in serum samples by using a commercial enzyme immunoassay ELISA kit (Immundiagnostik, Bensheim, Germany) as previously described [17]. The intra-assay ($n = 26$) standard deviation was 0.031% and the inter-assay ($n = 6$) standard deviation was 0.037%.

2.2. DDAH single nucleotide polymorphism (SNP) genotyping

2.2.1. DNA extraction

DNA was extracted from whole blood using the QuickGene-810 system. Blood was collected in EDTA-containing tubes, placed in an isolation vessel and the red cells were lysed. The white blood cells were then captured in a filter matrix and lysed so that the DNA was physically entrapped around the fibres. Isolated DNA was released from the matrix and eluted into a collection vessel in the enclosed environment of the QuickGene-810 system. Resulting DNA samples were stored at 4 °C until analysis. Quality control with each batch of extractions was performed by running a blank tube through the whole process, omitting only the addition of blood. If any DNA was found in the blank, the whole DNA batch was rejected.

2.2.2. Polymorphisms

DDAH1/2 Polymorphisms were assessed in all subjects by using the LightCycler™ 480 System (Idaho Technology Inc. Salt Lake City, Utah, USA), as previously described [34]. Rs2474123 (DDAH1), rs669173 (DDAH1), rs13373844 (DDAH1), rs7521189 (DDAH1) and rs3131383 (DDAH2) were identified using LightSNip probes and primers (TIB Molbiol GmbH, Berlin, Germany). Cycle conditions were as follows: denaturation of the template DNA for 1 cycle of 95 °C for 10 min, programmed transition rate of 4.4 °C/s; amplification of the target DNA for 45 cycles of 95 °C for 10 s, 60 °C for 10 s and 72 °C for 15 s, each with a temperature transition rate of 4.4/2.2/4.4 °C/s; melting curve analysis for 1 cycle of 95 °C for 30 s and 40 °C for 2 min, each with a transition temperature rate of 4.4/1.5 °C/s, and then ramping to 75 °C continuous.

The temperatures for DDAH2 (rs3131383) melting peaks were 63.5 °C for the CC and 69.5 °C for the AA genotype and heterozygous (CA) there was a peak at 63.5 °C and another at 69.5 °C.