Meta Gene 9 (2016) 76–89

Contents lists available at ScienceDirect

Meta Gene

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

Research Paper

Association of *TLR* and *TREM-1* gene polymorphisms with atherosclerosis severity in a Russian population

Anton G. Kutikhin [a,*], Anastasia V. Ponasenko [a], Maria V. Khutornaya [a], Arseniy E. Yuzhalin [b], Irina I. Zhidkova [a], Ramil R. Salakhov [a], Alexey S. Golovkin [a], Olga L. Barbarash [a], Leonid S. Barbarash [a]

[a] Research Institute for Complex Issues of Cardiovascular Diseases, Sosnovy Boulevard 6, 650002, Kemerovo, Russian Federation
[b] Department of Oncology, Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, OX3 7DQ, Oxford, United Kingdom

ARTICLE INFO

Article history:
Received 25 January 2016
Revised 12 April 2016
Accepted 14 April 2016
Available online 19 April 2016

Keywords:
Atherosclerosis
Coronary artery disease
Toll-like receptors
Triggering receptor expressed on myeloid cells-1
Gene polymorphisms

ABSTRACT

Local vascular immune response is primarily initiated via Toll-like receptors (TLRs) and triggering receptor expressed on myeloid cells-1 (TREM-1). We previously showed that certain *TLR* and *TREM-1* gene polymorphisms are associated with coronary artery disease (CAD). Therefore, we hypothesized that these gene polymorphisms are associated with atherosclerosis severity. This study included 292 consecutive patients with CAD who were admitted to the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation) during 2011–2012. Sample genotyping was performed in 96-well format using the TaqMan SNP genotyping assay. We found that C/C genotype of the rs3804099 polymorphism within *TLR2* gene and T/T genotype of the rs4711668 polymorphism within *TREM-1* gene were significantly associated with severe coronary atherosclerosis while C allele of the rs5743551 polymorphism within *TLR1* gene, A/G genotype of the rs4986790 polymorphism and C/T genotype of the rs4986791 polymorphism within *TLR4* gene, and C allele of the rs3775073 polymorphism within *TLR6* gene were significantly associated with severe noncoronary atherosclerosis. However, A/A genotype of the rs5743810 polymorphism within *TLR6* gene was significantly associated with mild noncoronary atherosclerosis. We conclude that certain *TLR* and *TREM-1* gene polymorphisms are significantly associated with atherosclerosis severity in a Russian population.

1. Introduction

Atherosclerosis, manifesting itself as acute coronary syndrome, stroke, and peripheral artery disease (Bentzon et al., 2014), is a chronic progressive inflammatory disease characterized by the accumulation of lipid and fibrous elements in arterial walls, which is driven by innate and adaptive immune response (Shah et al., 2014). The underlying mechanism of the chronic inflammatory process in atherosclerosis is still unknown in a significant extent (Ammirati et al., 2015). However, it is known that local vascular immune response is primarily initiated through the pattern recognition receptors, particularly Toll-like receptors (TLRs) (Pelham and Agrawal, 2014), and via triggering receptor expressed on myeloid cells-1 (TREM-1) (Eguchi and Manabe, 2014). Moreover, it was recently demonstrated that TREM-1 has complex signal integration with certain TLRs; in particular, it was observed that TREM-1 is able to enhance TLR-induced inflammatory response (Eguchi and Manabe, 2014).

Widespread distribution of genotyping technologies resulted in the emergence of studies examining the association of gene polymorphisms with various diseases (Yuzhalin and Kutikhin, 2012). Gene polymorphisms can result in various effects according to their location in the genome (Bakhtiar et al., 2014). For instance, gene polymorphisms within noncoding regions may influence transcription initiation or mRNA splicing (Bakhtiar et al., 2014). Nonsynonymous (i.e. those causing amino acid change) gene polymorphisms are able to alter protein expression, stability, and folding, or affect post-translational modifications (Bakhtiar et al., 2014).

Previously, we demonstrated that certain *TLR* and *TREM-1* gene polymorphisms are associated with coronary artery disease (CAD) in a Russian population (Golovkin et al., 2014). In this study we asked whether these polymorphisms are associated with atherosclerosis severity in patients with CAD.

2. Material and methods

2.1. Study population

The criteria of inclusion into the study were Russian ethnicity, inhabitance in Kemerovo Region during at least two generations, angiographically proved coronary artery stenosis, and written informed

* Corresponding author at: Sosnovy Boulevard 6, 650002 Kemerovo, Russian Federation.
E-mail address: antonkutikhin@gmail.com (A.G. Kutikhin).

http://dx.doi.org/10.1016/j.mgene.2016.04.001

consent to participate in the study after a full explanation of its aims and design. The criteria of exclusion included past medical history of malignant tumors, concomitant autoimmune disorders, chronic infectious diseases, and mental disorders. The study was approved by the local ethical committee.

A total of 946 consecutive patients who were admitted to the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation) during 2011–2012 and underwent coronary artery bypass graft (CABG) surgery due to CAD were involved in the study. 244 patients were excluded from the study in accordance with the above-mentioned criteria. Clinical data sufficient for the statistical analysis were obtained for 292 out of remaining 702 patients (239 males, 53 females) between 40 and 70 years of age (mean age 57.75 years, 95% confidence interval (CI) for the mean 57.04–58.45 years, standard deviation 6.14 years).

The diagnosis of CAD was based on the Russian Society of Cardiology (RSC) National Guidelines on Stable Angina and was further revisited according to 2014 American College of Cardiology/American Heart Association/American Association for Thoracic Surgery/Preventive Cardiovascular Nurses Association/Society for Cardiovascular Angiography and Interventions/Society of Thoracic Surgeons guideline for the diagnosis and management of patients with stable ischemic heart disease (Fihn et al., 2014). Coronary angiography was performed using GE Healthcare Innova 3100 Cardiac Angiography System (General Electric Healthcare, USA). Luminal stenosis ≥50% was defined as hemodynamically significant coronary stenosis. For the further assessment of coronary stenosis severity, we used widely accepted SYNTAX Score (Sianos et al., 2005). Median SYNTAX score was 19.50 (95%CI for the median 19.00–21.50, interquartile range 14.00–26.50). Color duplex screening of the extracranial arteries (ECA) and lower extremity arteries (LEA) was performed at the 5th–7th day of hospitalization in all patients using the cardiovascular ultrasound system Vivid 7 Dimension (General Electric Healthcare, USA) with a 5.7 MHz linear array transducer (for ECA), a 2.5-3 MHz curved array transducer, and a 5 MHz linear array transducer (for LEA). The extent of arterial stenosis was assessed in B regimen and by dopplerography (visualizing the local hemodynamics in the stenosis zone). Common and internal carotid arteries, vertebral, and subclavial arteries were visualized from both sides during the ECA screening; common and deep femoral arteries, popliteal, anterior and posterior tibial arteries were visualized from both sides during the LEA screening. The intima-media thickness (IMT) of the common carotid artery was measured in automatic mode (the value up to 1 mm was considered normal). Polyvascular disease (PVD) was defined as IMT increase ≥1 mm or ECA and/or LEA stenosis. The clinicopathological features of the patients are represented in Table 1. Fig. 1 demonstrates the study pipeline.

Table 1
Clinicopathological features of the patients who underwent CABG surgery.

Feature	Value, N (%)
Male gender	239 (81.85%)
Age >55 years	185 (63.36%)
SYNTAX score >22	112 (38.36%)
Number of coronary arteries affected by atherosclerosis >1	223 (76.37%)
Polyvascular disease	253 (86.64%)
Hemodynamically significant (≥50%) stenosis of extracranial or/and lower extremity arteries	83 (28.42%)
New York Heart Association functional class III–IV symptoms	139 (47.60%)
Past medical history of myocardial infarction	224 (76.71%)
Past medical history of stroke	31 (10.62%)
Arterial hypertension	262 (89.73%)
Carbohydrate metabolism disorders	87 (29.79%)
Dyslipidemia	228 (78.08%)
Smoking	196 (67.12%)
Overweight and obesity (body mass index >25)	220 (75.34%)

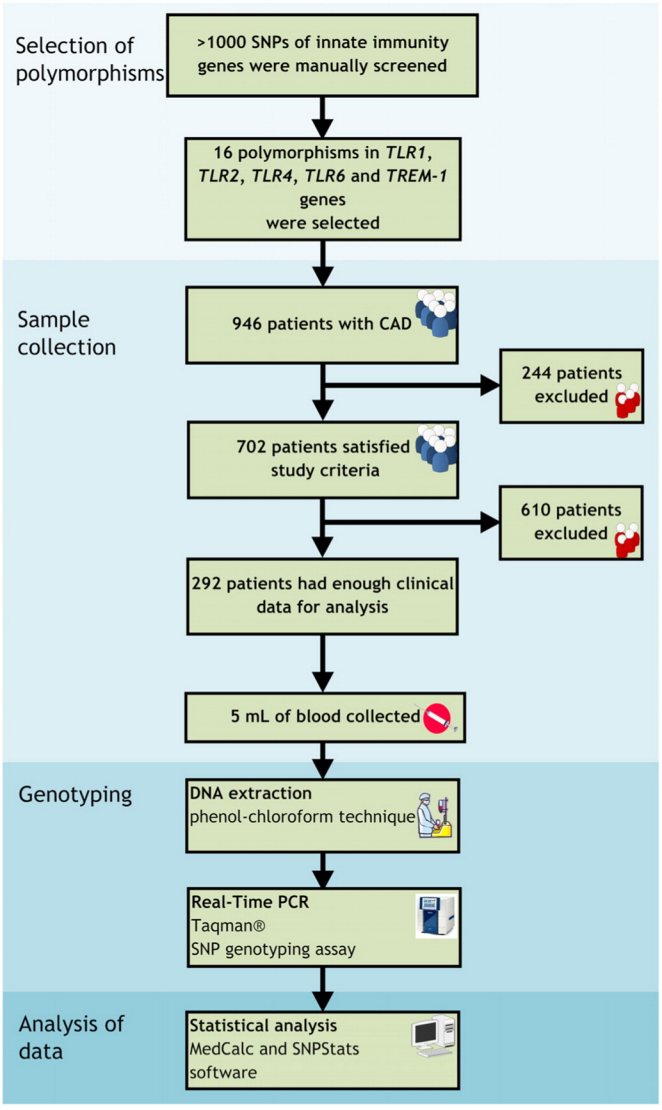

Fig. 1. Study pipeline.

2.2. Gene polymorphism selection and genotyping

Three selection criteria for the gene polymorphisms were: (1) high prevalence in a population (minor allele frequency ≥5% for Russian population according to HapMap), (2) suggested or proven functional consequence on a molecular level, and (3) few or no studies investigating the role of the gene polymorphism with respect to atherosclerosis severity. The National Center for Biotechnology Information dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP), SNPinfo (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) (Xu and Taylor, 2009), and SNPnexus (http://www.snp-nexus.org/) (Dayem Ullah et al., 2012) databases were used for the selection of the gene polymorphisms for the study. A total of 16 polymorphisms in 5 genes were investigated: *TLR1* (rs5743551 and rs5743611), *TLR2* (rs3804099 and rs5743708), *TLR4* (rs4986790 and rs4986791), *TLR6* (rs3775073 and rs5743810), and *TREM-1* (rs1817537, rs3804277, rs6910730, rs7768162, rs2234246, rs4711668, rs9471535, and rs2234237). The data on investigated gene polymorphisms are represented in Table 2.

All study participants provided 5 mL of peripheral venous blood which was collected into a tube containing ethylenediaminetetraacetic acid. Then, 0.5 mL of blood was immediately transferred into a fresh