Thrombosis Research 135 (2015) 703–709

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

The contribution of genetic and environmental factors to changes in total and γ' fibrinogen over 5 years

Ané Jobse [a,1], Marlien Pieters [a,*,1], Cornelie Nienaber-Rousseau [a], Hendriek Boshuizen [b], Tiny Hoekstra [a,c], Moniek P.M. de Maat [d]

[a] Centre of Excellence for Nutrition, North-West University, Potchefstroom, North West, South Africa
[b] Department of Human Nutrition, Wageningen University and Research Centre, Wageningen, the Netherlands
[c] Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands
[d] Department of Hematology, Erasmus University Medical Centre, Rotterdam, the Netherlands

ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 20 November 2014
Accepted 11 January 2015
Available online 15 January 2015

Keywords:
Fibrinogen
Epidemiology
Genetics
Prospective data

ABSTRACT

Introduction: Increased fibrinogen is associated with cardiovascular disease risk. It is, however, not known to what extend environmental and genetic factors and/or their interaction influence changes in total and γ' fibrinogen over time. We aimed to determine how variation within the fibrinogen gene as well as environmental factors influence the change in total and γ' fibrinogen over time, and also whether gene-environment interactions influence total and γ' fibrinogen on a cross-sectional and prospective level in Africans.
Materials and methods: This prospective study consisted of 2010 participants at baseline and 1288 participants at follow-up (5 years).
Results: The gene–environment interactions that were associated with fibrinogen concentration on a cross-sectional level were: *FGA* 2224 G>A (rs2070011) with age ($p = 0.005$), *FGB* Arg448Lys (rs4220) with HIV status ($p < 0.0001$) and *FGB* 1038 G>A (rs1800791) with HbA1c ($p = 0.01$). The only factor that independently influenced the change in total fibrinogen levels over time, was baseline CRP ($p < 0.0001$) and *FGG* 10034 C>T (rs2066865) was the only single nucleotide polymorphism that independently influenced the change in fibrinogen γ' levels over time ($p = 0.02$). Only the *FGG* 9340 T>C (rs1049636) with HbA1c interaction was found to predict change in total fibrinogen concentrations over time ($p = 0.005$).
Conclusions: Gene-environment interactions influenced fibrinogen levels cross-sectionally and also mediated changes in levels over time.

Introduction

Increased fibrinogen concentration is associated with an increased risk of developing cardiovascular disease [1]. Fibrinogen concentration is influenced by both environmental and genetic factors [2,3]. A common splice variant of fibrinogen, fibrinogen γ', also associated with thrombotic disorders, is also influenced by both environmental and genetic factors, although only a limited number of studies have investigated these factors to date [4,5]. Genome-wide association (GWA) studies indicate that there is a difference in genetic variants that influence the total and γ' fibrinogen concentrations, suggesting that fibrinogen γ' has its own independent control mechanisms [6,7]. A better understanding of the regulation of the concentration of fibrinogen and its variants will provide valuable insight into cardiovascular disease aetiology.

Fibrinogen concentration is known to increase with age and thus elevated fibrinogen as risk factor, is particularly of concern in aging populations. Determining factors that influence the longitudinal change in fibrinogen levels can therefore, provide valuable insight into disease prevention. We hypothesize that the interaction between genetic and environmental factors determine the longitudinal changes in plasma fibrinogen levels (both total and γ' fibrinogen). Environmental factors associated with longitudinal changes in fibrinogen concentrations have been investigated in the ARIC [8] and Northwick Park Heart Study [9], and age, diabetes, smoking, hormone replacement therapy, HDL-cholesterol and triglycerides have been identified as determinants. No data are available yet regarding genetic factors or gene-environment interactions that influence changes in concentrations over time. Furthermore, no data are available on determinants of longitudinal changes in fibrinogen γ' concentration.

In African American and African populations, higher fibrinogen concentrations have been observed when compared to whites [10–12]. These inter-ethnic differences in fibrinogen concentration may be due to differences in genetic background between ethnicities as the African populations are reported to contain greater genetic variability

* Corresponding author at: Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa. Tel.: +27 18 299 2462; fax: +27 18 299 2464.
E-mail address: marlien.pieters@nwu.ac.za (M. Pieters).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.thromres.2015.01.015
0049-3848/

than non-African populations [13]. Limited data is, however, available regarding the genetic control of total and γ' fibrinogen concentrations in Africans and the interaction thereof with environmental factors. This lack of information also necessitates the cross-sectional investigation of gene-environment interactions in an African population.

Therefore, the aim of this study was to determine how known polymorphisms within the fibrinogen gene and environmental factors influence the change in total and γ' fibrinogen over a 5 year period in 1288 black South Africans enrolled in the Prospective Urban and Rural Epidemiological (PURE) study. The second aim was to determine whether gene-environment interactions influence total and γ' fibrinogen on both a cross-sectional and longitudinal (prospective) level in Africans. Details regarding the respective environmental and genetic factors which influenced total and γ' fibrinogen cross-sectionally have been published previously [14–16]

Materials and Methods

Study Population

The PURE study is a large-scale prospective study tracking changes in lifestyles, risk factors and chronic diseases in rural and urban areas of 21 countries in transition over 12 years. For the South African arm of the PURE study, participants were recruited from 6000 randomly selected households based on representativeness and feasibility for long-term follow-up as stipulated in the overarching PURE study [17, 18]. Apparently healthy black South African men and women, between the ages of 35 and 65 years were eligible to participate. Exclusion criteria were use of chronic medication, reported chronic diseases and/or any known acute diseases. The baseline data was collected in 2005 and the first follow-up data in 2010. At baseline, 2010 apparently healthy black women (n = 1260) and men (n = 750) from both rural and urban settlements in the North West Province of South Africa participated. In 2010, 1288 participants returned for follow-up, of which 435 were men and 853 were women. Reasons for the smaller number of participants were: refusal to participate again or withdrawal from the study, not being available on days of sample collection, relocation, or acute illness or death (n = 230). The study was approved by the ethics committee of the North-West University (Ethics numbers: 041M0 and NWU-00016-10-A1) and was conducted according to the Declaration of Helsinki. Participants signed informed consent before taking part in the study. All data were treated confidentially and all analyses were performed with coded data.

Data Collection

Fasting blood samples were collected from the antecubital vein between 07:00 and 11:00. For the analysis of lipids, interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations, serum samples were prepared by collecting blood in tubes without additives. For the analysis of glucose and glycated haemoglobin (HbA1C), samples were collected in fluoride and ethylenediamine tetra acetic acid (EDTA) tubes, respectively. For the analysis of total and γ' fibrinogen, plasma samples were prepared by collecting blood in citrate tubes. Samples were centrifuged at 2000 x g for 15 minutes at 10 °C within 30 minutes of collection. Aliquots were frozen on dry ice, stored in the field at -18 °C and then after 2-4 days at -82 °C until analysis. Following centrifugation, the leucocyte layer was transferred into a separate aliquot from which DNA was isolated using the FlexiGene™ DNA extraction kit (QIAGEN® Valencia, CA). Details regarding the data collection of anthropometry, alcohol consumption and HIV status are provided elsewhere [19].

Laboratory Analysis

Methods used to analyse serum lipids, high sensitivity CRP (hs-CRP), IL-6, HbA1C and plasma glucose have been described previously [14]. Total fibrinogen concentration was measured by the use of a modified Clauss method on an automated coagulation analyser (Multifibrin U-test, BCS analyser, Dade Behring, Deerfield, IL, USA for the 2005 samples and ACL-200, Instrumentation Laboratories, Milan, Italy for the 2010 samples). One hundred and forty of the 2005 samples were re-run with the 2010 samples to exclude the possibility that batch differences were artefacts resulting from the use of the different analysers. Fibrinogen γ' was measured by ELISA, using the 2.G.H9 mouse monoclonal coating antibody against the human γ' sequence from Santa Cruz Biotechnology (Santa Cruz, USA) for antigen capture and a goat polyclonal HRP-conjugated antibody against human fibrinogen from Abcam (Cambridge, USA) for development, according to the method of Uitte de Willige et al. [20]. Since fibrinogen γ' correlates to some degree with total fibrinogen, it is not only reported as absolute concentration (g/l), but also as relative concentration by expressing it as percentage (%) of total fibrinogen concentration, reported as γ' ratio [15]. The coefficient of variance for all assays was <10%.

Genotyping

Genotyping of selected single nucleotide polymorphisms (SNPs) was performed by using Illumina's VeraCode technology and determined by the BeadXpress™ Reader (Illumina®, San Diego, CA, USA). Based on their observed effects on total and γ' fibrinogen concentration, the following SNPs in the fibrinogen genes were selected from our previous research [16] and the literature [4,20–25] for genotyping: *FGA:* Reference SNP cluster ID (rs) 2070011 and rs6050; *FGB:* rs1800791, rs4220, and rs1800787; *FGG:* rs2066865 and rs1049636. For more details regarding the quality control of the BeadXpress genotyping please refer to Van Zyl et al. [26]. The following additional SNPs were genotyped using allelic discrimination with TaqMan-based assays (Thermo Fisher Scientific, Walthem, MA, USA) employing the MylQ Bio-Rad real-time polymerase chain reaction (rtPCR) machine (Bio-Rad Laboratories Inc. CA, USA): *FGB:* rs1800788, rs2227385 and rs2227388.

Statistical Analysis

The computer software packages IBM® SPSS® Statistics 21 version 21 (Statistical Package for Social Sciences, IBM, New York, USA) and Statistica® version 11 (Statsoft Inc., Tulsa Oklahoma, USA) were used for statistical analysis. Significance was set at $p < 0.05$. Non-parametric data was logarithmically transformed to improve normality to allow parametric statistics, but still reported as median (25th-75th percentiles). In order to determine if gene–environment interactions affected total and γ' fibrinogen cross-sectionally and also the change in concentrations over the five-year period, a mixed-models approach was used, incorporating both 2005 and 2010 data. Missing data for environmental factors were imputed using multiple-imputation methodology (10x). Models were created for total fibrinogen concentration as well as for the γ' ratio as this is considered to be a true reflection of the relative fibrinogen γ' concentration in plasma [15]. Different models were created based on the different interaction terms entered in the models *i.e.* a model for gene–environment interaction (to determine whether gene–environment interactions influenced the fibrinogen variables cross-sectionally); environment–time interaction (to determine whether environmental factors influenced the change over time); gene–time interaction (to determine whether genetic factors influenced the change over time); and gene-environment–time interactions (to determine whether there were gene-environment interactions that influenced the change over time). Time was entered as a categorical variable indicating the two time points at which data was collected (2005 and 2010). Gender, tobacco history, human immunodeficiency virus (HIV) status, BMI category, urbanisation status, alcohol category, systolic blood pressure, age, LDL-cholesterol, HDL-cholesterol, HbA1c, CRP, IL-6 and weighted physical activity index