The influence of genetic polymorphisms of fibrinogen genes on changes in total fibrinogen and fibrinogen gamma prime concentrations over time in black South Africans

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It all starts here ™

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"And whatever you do, do it heartily, as to the Lord and not to men, knowing that from the Lord you will receive the reward of the inheritance; for you serve the Lord Christ." Col 3:23-24 NKJV

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ABSTRACT

INTRODUCTION AND AIM

Cardiovascular disease is globally a major risk factor for morbidity and mortality. It is caused by various factors, one of which is an abnormal haemostatic process. Fibrinogen is a haemostatic factor that is considered to be an independent risk factor for cardiovascular disease. Elevated fibrinogen can be caused by environmental and genetic factors which increase the risk of the occurrence of thrombosis. The fibrinogen γ chain, which is one of the three chains of fibrinogen, has two different variants, the γA and γ'. The presence of the fibrinogen γ' chain has been associated with thrombotic disorders. Many studies have investigated the fibrinogen variables in Caucasian individuals, but only a few such studies have been conducted on non-Caucasian individuals. The genetic diversity of ethnic groups differs and could cause differences in the fibrinogen variables between these groups. Fibrinogen is known to increase with age; therefore to explain changes over time in fibrinogen concentrations it was also important to investigate whether genetic determinants and possible gene–environment interactions influenced fibrinogen over time. In this study the main aim was to determine the change in the fibrinogen variables over a five-year period within a black South African cohort subdivided according to genotypes associated with fibrinogen variables, and to determine whether the observed changes were modulated by environmental factors.

PARTICIPANTS AND METHODS

Data [baseline (n=2010) and follow-up (n=1288)] were collected in the Prospective Urban and Rural Epidemiology (PURE) study during 2005 and 2010 from apparently healthy black men and women aged between 35 and 65 years and residing in rural or urban settlements. Experimental methods included analysis of fibrinogen and fibrinogen γ' concentrations, single nucleotide polymorphisms (SNPs) and determination of environmental factors associated with the fibrinogen variables.

RESULTS

The fibrinogen variables increased significantly from 2005 to 2010 in both the rural and urban participants, as well as in both men and women. The major environmental factors that affected the fibrinogen variables were C-reactive protein (CRP), interleukin-6 (IL-6), body mass index (BMI), glycated haemoglobin (HbA1c), age, blood lipids, human immunodeficiency virus (HIV) and tobacco use. Fibrinogen increased consistently from 2005 to 2010 in the respective genotypes of all SNPs analysed, except in the FGG 9340 T>C homozygous mutant carriers. Fibrinogen γ' also increased in general in most genotypes from 2005 to 2010, except in the FGG 10034 C>T mutant allele carriers, where a decrease was observed. It was determined that CRP was the only environmental factor that influenced the change in fibrinogen over time and that FGG 10034 C>T was the only SNP that influenced the change in fibrinogen γ' over the five years. Four gene–environment interactions also influenced fibrinogen on a cross-sectional level, *i.e.* FGA 2224 G>A with age, FGB Arg448Lys with HIV status, FGB 1643 C>T with urbanisation and FGB 1038 G>A with HbA1c. Only the FGG 9340 T>C with HbA1c interaction was found to predict change in fibrinogen concentrations over the five years.

CONCLUSION

Both environmental and genetic factors significantly influenced the fibrinogen variables cross-sectionally as well as prospectively. It was clear that the influence of the environmental factors was mediated by genetic polymorphisms and *vice versa*, as can be seen by the gene–environment interactions found in this study. An important finding of this study was that the interaction of HbA1c with two SNPs on fibrinogen variables may explain the known inconsistent relationship found between fibrinogen concentrations and diabetes.

KEY TERMS: Fibrinogen; fibrinogen γ'; fibrinogen polymorphisms; black South African population; change over time; gene–environment interactions

OPSOMMING

INLEIDING EN DOEL

Kardiovaskulêre siekte is wêreldwyd 'n groot risikofaktor vir morbiditeit en sterftes. Dit word veroorsaak deur verskeie faktore, waarvan een 'n abnormale hemostatiese proses is. Fibrinogeen is 'n hemostatiese faktor wat beskou word as 'n onafhanklike risikofaktor vir kardiovaskulêre siekte. Verhoogde fibrinogeen kan veroorsaak word deur omgewings- en genetiese faktore wat die risiko verhoog om trombose te ontwikkel. Die fibrinogeen-γ-ketting, wat een van die drie kettings van fibrinogeen is, het twee verskillende variante, naamlik γA en γ'. Die teenwoordigheid van die fibrinogeen-γ'-ketting word geassosieer met trombotiese siektes. Fibrinogeen-veranderlikes is al in baie studies op blanke individue ondersoek, maar slegs 'n paar sulke studies is gedoen op Afrikaan populasiegroepe. Die genetiese diversiteit van etniese groepe verskil van mekaar en kan verskille in die fibrinogeen-veranderlikes tussen hierdie groepe veroorsaak. Fibrinogeen-konsentrasies verhoog met ouderdom. Om die oorsaak van hierdie verandering te bepaal, is dit belangrik om die invloed van genetiese faktore en geen-omgewing-interaksies oor 'n tydperk te evalueer. Die hoofdoel van hierdie studie was om die verandering in fibrinogeen-veranderlikes oor 'n tydperk van vyf jaar, in 'n swart Suid-Afrikaanse bevolking te bepaal, onderverdeel volgens die genotipes wat verband hou met fibrinogeen veranderlikes, asook om te bepaal of die waargenome veranderinge deur omgewingsfaktore gemoduleer is.

STUDIEPOPULASIE EN METODES

Data [basislyn (n = 2010) en opvolg (n = 1288)] is versamel in die *Prospective Urban and Rural Epidemiology* (PURE) studie gedurende 2005 en 2010, van skynbaar gesonde swart mans en vrouens, tussen die ouderdom van 35 en 65 jaar oud en wat in landelike of stedelike nedersettings woon. Eksperimentele metodes het die ontleding van fibrinogeen- en fibrinogeen-γ'-konsentrasies, enkelnukleotied polimorfismes (SNPs) en die bepaling van omgewingsfaktore, wat verband hou met die fibrinogeen-veranderlikes, ingesluit.

RESULTATE

Die fibrinogeen veranderlikes het aansienlik toegeneem van 2005 tot 2010 in beide die landelike en stedelike proefpersone, sowel as in beide mans en vrouens. Die hoof omgewingsfaktore wat bygedra het tot hierdie verhoging was C-reaktiewe proteïen (CRP), interleukin-6 (IL-6), liggaamsmassa-indeks (LMI), geglikosileerde hemoglobien (HbA1c), ouderdom, bloedlipiede, menslike immuniteitsgebreksvirus (MIV) en tabak-gebruik. Fibrinogeen het konsekwent verhoog van 2005 tot 2010 tussen die onderskeie genotipes van al die SNPs ontleed, behalwe in FGG 9340 T>C homosigotiese mutante draers. Fibrinogeen-γ' het ook in die algemeen toegeneem in die meeste genotipes van 2005 tot 2010, behalwe vir FGG 10034 C>T mutante alleel-draers, waar 'n afname waargeneem is. Daar is vasgestel dat CRP die enigste omgewingsfaktor was wat die verandering in fibrinogeen oor tyd beïnvloed het en dat FGG 10034 C>T die enigste SNP was, wat die verandering in fibrinogeen-γ' beïnvloed het, oor die vyf jaar. Vier geen-omgewing-interaksies het ook fibrinogeen beïnvloed op 'n dwarsdeursnitvlak, naamlik FGA 2224 G>A met ouderdom, FGB Arg448Lys met MIV-status, FGB 1643 C>T met verstedeliking en FGB 1038 G>A met HbA1c. Slegs die FGG 9340 T>C met HbA1c interaksie het verandering in fibrinogeen-konsentrasies voorspel oor die vyf jaar.

SAMEVATTING

Beide omgewings- en genetiese faktore het 'n beduidende invloed op die fibrinogeen-veranderlikes gehad op 'n dwarsdeursnitvlak sowel as prospektief. Dit was duidelik dat die invloed van die omgewingsfaktore bemiddel is deur genetiese polimorfismes en omgekeerd, soos gesien kan word deur die geen-omgewing-interaksies in hierdie studie. 'n Belangrike bevinding van hierdie studie was dat die interaksie van HbA1c met twee SNPs op fibrinogeen-veranderlikes, die bekende teenstrydige verhouding, tussen fibrinogeen-konsentrasies en diabetes, kan verduidelik.

SLEUTELTERME: Fibrinogeen; fibrinogeen-γ'; fibrinogeen-polimorfismes; swart Suid-Afrikaanse bevolking, verandering oor tyd; geen-omgewing-interaksies

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LIST OF ABBREVIATIONS

A	Adenine
α	Alpha
Å	Angstrom
Aα	A alpha
αC	Alpha C
ACL	Automated Coagulation Laboratory
AIDS	Acquired immunodeficiency syndrome
Ala	Alanine
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
ApoB	Apolipoprotein B
Arg	Arginine
ARV	Anti-retroviral
AxSYM	Abbott automated immunoassay analyser
β	Beta
Bβ	B beta
BMI	Body mass index
BSA	Bovine serum albumin
^{o}C	Degrees Celsius
C	Cytosine
CD4	Cluster of differentiation 4
C_{HDL}	Concentration of high-density lipoprotein cholesterol
CI	Confidence interval
C_{LDL}	Concentration of low-density lipoprotein cholesterol

cm	Centimetre
C_{plasma}	Concentration of plasma
CRP	C-reactive protein
C_{TG}	Concentration of triglycerides
CV	Coefficient of variance
CVD	Cardiovascular disease
D	Distal regions
D’	Standardised disequilibrium
DBP	Diastolic blood pressure
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
DNG	Dienogest
DVT	Deep vein thrombosis
EDTA	Ethylenediamine tetra acetic acid
EE	Ethinylestradiol
ELISA	Enzyme-linked immunosorbent assay
EV	Estradiol valerate
f	Frequency
F	Forward
FGA	Fibrinogen alpha
FGB	Fibrinogen beta
FGG	Fibrinogen gamma
γ	Gamma
γA	Gamma A
γ’	Gamma prime
g	Gram

g/L	Gram per litre
G	Guanine
GWA	Genome-wide association
H_2SO_4	Sulphuric acid
HART	Hypertension in Africa Research Team
HbA1C	Glycated haemoglobin
HDL	High-density lipoprotein
Hez	Heterozygote
HIV	Human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
Hoz	Homozygote
HRT	Hormone replacement therapy
HW	Hardy-Weinberg
IL	Instrumentation Laboratory
IL-1RN	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IRF1	Interferon regulatory factor 1
kb	Kilo base
kDA	Kilodalton
kg/m^2	Weight by height squared
km	Kilometre
L	Litre
LD	Linkage disequilibrium
LDL	Low-density lipoprotein
LNG	Levonorgestrel
Lys	Lysine

µl	Micro litre
M	Molar mass
MAF	Minor allele frequency
mg/L	Milligram per litre
mg/ml	Milligram per millilitre
MI	Myocardial infarction
ml	Millilitre
mmHg	Millimetre of mercury
mmol/L	Millimoles per litre
mRNA	Messenger ribonucleic acid
MT	Mutant
n	Population size
N	Amino
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NLRP3	Nucleotide-binding leucine rich family pyrin domain containing 3 isoforms
nm	nanometre
NNRTI	Nonnucleoside reverse transcriptase inhibitor
NPHS-II	Second Northwick Park Heart study
NRTI	Nucleoside reverse transcriptase inhibitor
p-value	Statistical significance test
PAI	Plasminogen activator inhibitor
PCCB	Propionyl coenzyme A carboxylase
PE	Pulmonary embolism

pg/ml	Picograms per millilitre
PI	Protease inhibitor
PLRG1	Pleiotropic regulator 1
PURE	Prospective Urban and Rural Epidemiology
QFFQ	Quantitative food frequency questionnaires
r	Correlation coefficient
r^2	Correlation coefficient squared
R	Reverse
rs	Reference SNP
rtPCR	Real-time polymerase chain reaction
SBP	Systolic blood pressure
SMAC	Sequential Multiple Analyser Computer
SNP	Single nucleotide polymorphism
T	Thymine
TC	Total cholesterol
TEA	Triethanolamine
TF	Tissue factor
TFBS	Transcription factor binding site
TG	Triglycerides
tHcy	Total homocysteine
Thr	Threonine
THUSA	Transition and Health during Urbanisation in South Africa
t-PA	Tissue plasminogen activator
UNAIDS/WHO	Joint United Nations Program on HIV/AIDS and World health Organization
u-PA	Urokinase plasminogen activator

UTR	Untranslated region
VLDL	Very low-density lipoprotein
vs	Versus
WH-II	Whitehall-II study
WHO	World Health Organization
WPAI	Weighted physical activity index
WT	Wild-type
x g	Multiplied by gravitational force
yrs	Years