

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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“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), glycosylated 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 METODEDES

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 proteïene (CRP), interleukin-6 (IL-6), liggaamsmassa-indeks (LMI), geglikosileerde hemoglobien (HbA1c), ouderdom, bloedlipiede, menslike immuniteitsgebrekswirus (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

TABLE OF CONTENTS

Acknowledgements	i
Abstract	ii
Opsomming	iv
Table of Contents	vi
List of Tables	x
List of Figures	xii
List of Addenda	xiii
List of Abbreviations	xiv
Chapter 1 Introduction	1
1.1 Background	1
1.2 Aim and objectives	4
1.3 Research team	5
1.4 Structure of this dissertation	6
Chapter 2 Literature Review	8
2.1 Introduction	8
2.2 Haemostasis	10
2.2.1 Overview of haemostasis	10
2.2.2 Fibrinogen	13
2.2.3 Fibrinogen γ'	19
2.3 Genetic single nucleotide polymorphisms that influence the concentration of fibrinogen and fibrinogen γ'	24
2.4 Gene–environment interactions	34
2.5 Change over time	36
2.6 Conclusion and recommendations	37

Chapter 3 Methodology	38
3.1 Introduction	38
3.2 Ethical approval	39
3.3 Study population	39
3.3.1 Recruitment	39
3.4 Study design	41
3.5 Anthropometrical assessment	41
3.6 Blood pressure measurements	41
3.7 Adult questionnaire	42
3.8 Dietary intake analysis	42
3.9 Assessment of physical activity	42
3.10 Blood sampling	43
3.11 Total cholesterol, HDL-cholesterol and triglycerides	43
3.12 LDL-cholesterol	44
3.13 High sensitivity C-reactive protein	44
3.14 Glucose	45
3.15 Glycated haemoglobin	45
3.16 Interleukin-6	45
3.17 Fibrinogen	45
3.18 Fibrinogen γ'	46
3.19 HIV testing	47
3.20 Genetics	47
3.20.1 DNA isolation	47
3.20.2 DNA amplification	48
3.20.3 DNA sequencing	48
3.20.4 Haplotyping	49
3.20.5 Genotyping of fibrinogen gene single nucleotide polymorphisms	49

3.21 Statistical analysis.....	50
Chapter 4 Results.....	52
4.1 Introduction.....	52
4.2 Basic descriptive characteristics of the pure population at baseline and follow-up	52
4.3 Total Fibrinogen and fibrinogen γ' differences related to urbanisation and gender	55
4.4 Associations between the fibrinogen variables and environmental factors	59
4.5 Genotype distribution of the single nucleotide polymorphisms	63
4.6 Linkage disequilibrium determination	64
4.7 Fibrinogen, fibrinogen γ' and γ' ratio differences related to genotype.....	67
4.8 Cross-sectional gene-environment interactions influencing total fibrinogen and fibrinogen γ' concentrations.....	75
4.9 Influence of genetic or environmental factors on change in total fibrinogen and fibrinogen γ' concentrations over the five year period.....	80
4.10 Genotype-environment interactions influencing change in total fibrinogen and fibrinogen γ' concentrations over the five year period.....	85
Chapter 5 Discussion and Conclusion.....	88
5.1 Introduction.....	88
5.2 Influence of urbanisation and gender on total fibrinogen and fibrinogen γ'	88
5.2.1 Influence of urbanisation on total fibrinogen and fibrinogen γ'	89
5.2.2 Influence of gender on total fibrinogen and fibrinogen γ'	91
5.3 Effect of environmental factors on fibrinogen variables	93
5.3.1 Effect of environmental factors on fibrinogen variables cross-sectionally.....	93
5.3.2 Effect of environmental factors on change in total fibrinogen and fibrinogen γ' over the five-year period	104
5.4 Single nucleotide polymorphisms.....	105
5.4.1 Comparison of minor allele frequencies between different populations.....	105
5.4.2 Comparison of linkage disequilibrium between different studies	107

5.4.3 Cross-sectional effect of genotypes on total fibrinogen and fibrinogen γ'	108
5.4.4 Effect of genotypes on the change over time in the fibrinogen variables.....	116
5.5 Gene-environment interactions	119
5.5.1 Gene-environment interactions that affected the fibrinogen variables on a cross-sectional level	119
5.5.2 Gene-environment interactions that affected change in the fibrinogen variables over time	122
5.6 Strengths and limitations.....	123
5.7 Conclusion.....	124
Bibliography	129
Addenda.....	150

LIST OF TABLES

Table 2.1: Genetic single nucleotide polymorphisms of fibrinogen and fibrinogen γ'	26
Table 2.2: Four tagging SNPs identified by Haplotype analysis	29
Table 2.3: Variables of study populations in studies mentioned in Table 2.1 and 2.2.....	30
Table 3.1: Sequencing primers for the β -fibrinogen gene	48
Table 4.1: Basic descriptive characteristics of environmental factors.....	54
Table 4.2: Between and within-group differences of total fibrinogen, fibrinogen γ' and γ' ratio related to urbanisation and gender.....	56
Table 4.3: Differences in environmental factors between rural and urban participants in 2005 and 2010.....	57
Table 4.4: Differences in environmental factors between 2005 and 2010 in rural and urban participants	58
Table 4.5: Correlation between total fibrinogen, fibrinogen γ' , γ' ratio and environmental factors in 2005.....	59
Table 4.6: Correlation between total fibrinogen, fibrinogen γ' , γ' ratio and environmental factors in 2010.....	60
Table 4.7: The effect of categorical environmental factors on total fibrinogen, fibrinogen γ' and γ' ratio in 2005	61
Table 4.8: The effect of categorical environmental factors on total fibrinogen, fibrinogen γ' and γ' ratio in 2010	62
Table 4.9: Genotype distributions of the investigated SNPs	63
Table 4.10: Between-group differences and effect of genotypes on change of total fibrinogen, fibrinogen γ' and γ' ratio over time	70
Table 4.11: Cross-sectional gene–environment interactions for total fibrinogen of continuous environmental factors	75
Table 4.12: Environmental factors influencing change in total fibrinogen over time	80
Table 4.13: Genetic polymorphisms influencing change in total fibrinogen and fibrinogen γ' over time.....	83
Table 4.14: Gene–environment interactions that affected total fibrinogen concentration over time	85
Table 5.1: Difference in minor allele frequency between various populations	105

Table 5.2: Comparison between literature and PURE study on effect of SNPs on total fibrinogen concentrations.....109

Table 5.3: Comparison between literature and current study on effect of SNPs on fibrinogen γ' ..111

Table 5.4: SNPs that significantly influenced change in the fibrinogen variables over time, using ANOVA117

LIST OF FIGURES

Figure 2.1: The haemostatic pathway (taken from Lefevre et al., 2004).....	12
Figure 2.2: Fibrinogen molecule (taken from McDowall, 2006)	13
Figure 2.3: Schematic diagram of fibrinogen, indicating the structural domains and the association sites that participate in fibrin polymerisation and cross-linking (adapted from Mosesson et al., 2001)	16
Figure 2.4: Correlation between fibrinogen concentration, clot structure and disease (adapted from Lord, 2011).....	17
Figure 2.5: Polyadenylation of the γ A and γ' chain (adapted from Uitte de Willige et al., 2009a)	19
Figure 2.6: Gene–environment interactions (taken from Voetsch & Loscalzo, 2004)	35
Figure 4.1: Pair-wise linkage disequilibrium structure presenting the D' (95% confidence bounds) and the r^2	66
Figure 4.2: The interaction effect of the FGA 2224 genotypes with age on total fibrinogen.....	76
Figure 4.3: The interaction effect of FGB 1038 genotype with HbA1c on total fibrinogen.....	77
Figure 4.4: The interaction effect of FGB Arg448Lys genotypes with HIV status on total fibrinogen	78
Figure 4.5: The interaction effect of FGB 1643 genotype with urbanisation on total fibrinogen	79
Figure 4.6: Effect of C-reactive protein on change in total fibrinogen over time	81
Figure 4.7: Association between C-reactive protein and change in total fibrinogen over time	81
Figure 4.8: Association between change in CRP over time and CRP in 2005.....	82
Figure 4.9: Effect of FGG 10034 on change in γ' ratio over time	84
Figure 4.10: The interaction effect of FGG 9340 with HbA1c on total fibrinogen over time	86
Figure 4.11: Association between change in HbA1c over time and HbA1c in 2005	87

LIST OF ADDENDA

Addendum A: Ethical approval 2005.....	151
Addendum B: Ethical approval 2010.....	151
Addendum C: Information to communities 2005	152
Addendum D: Information to communities 2010	153
Addendum E: Informed consent form 2005-phase 1.....	154
Addendum F: Informed consent form 2005-phase 2.....	154
Addendum G: Informed consent form 2010.....	156
Addendum H: Family census questionnaire.....	157
Addendum I: Household questionnaire.....	162
Addendum J: Adult questionnaire.....	165
Addendum K: Quantitative food frequency questionnaire	185
Addendum L: Physical activity questionnaire.....	193
Addendum M: Fibrinogen γ' analysis	194

LIST OF ABBREVIATIONS

A	Adenine
α	Alpha
Å	Angstrom
A α	A alpha
α C	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
B β	B beta
BMI	Body mass index
BSA	Bovine serum albumin
°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 ₂ O	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 ₂ SO 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