CHAPTER 4
RESULTS

4.1 INTRODUCTION

The main aim of this study was to determine the change in fibrinogen and fibrinogen γ' concentrations over the five-year period in the black South African population and to determine whether these changes were influenced by genetic factors, environmental factors and/or possible interaction between these factors. In order to achieve this aim, a variety of statistical analyses were performed including basic descriptive analysis as well as exploration of cross-sectional relationships of the fibrinogen variables with these factors, to serve as necessary background for the prospective investigation. This chapter, therefore, firstly presents the analysis of the baseline (2005) and follow-up (2010) data of the PURE population. Differences in the fibrinogen variables associated with urbanisation and gender are also presented as well as associations between the fibrinogen variables and environmental factors. In addition, it includes a comparison of the fibrinogen variables with categorical environmental factors such as tobacco use, HIV status and contraceptive use. The genotype distribution and genetic linkage disequilibrium determination of the SNPs investigated in this study follows next. Differences in the fibrinogen variables related to the different genotypes of the SNPs are then presented. This chapter also presents the results of the influence of gene-environment interactions on the fibrinogen variables cross-sectionally, as well as how environmental or genetic factors influenced the change in the fibrinogen variables over the five years. Lastly this chapter presents gene-environment interactions that possibly influenced the change in the fibrinogen variables over the five year period.

4.2 BASIC DESCRIPTIVE CHARACTERISTICS OF THE PURE POPULATION AT BASELINE AND FOLLOW-UP

Basic demographic and other descriptive characteristics of the PURE population at baseline (2005) and follow-up (2010) are presented in *Table 4.1.* The participants were representative of both rural and urban areas and of both genders. Sixty-two participants (4.84% of the total group) who were HIV negative in 2005 were determined to be HIV positive during the 2010 collection phase.

The median BMI of the PURE population was within the normal range (18.5–24.9 kg/m²) both in 2005 and 2010. However, 367 (18%) and 201 (16%) participants were underweight (BMI less than 18.5 kg/m²), 356 (18%) and 260 (20%) participants were overweight (BMI between 25 and 29.9 kg/m²) and 434 (22%) and 321 (25%) participants were obese (BMI more than 29.9 kg/m²) in 2005 and 2010, respectively (World Health Organization, 1998). The median systolic and diastolic blood pressure of the PURE population in both 2005 and 2010 indicated that the average study participant was pre-hypertensive (systolic blood pressure between 120 and 139 mmHg or diastolic blood pressure between 80 and 89 mmHg). With regard to the blood pressure ranges, 951 (48%) participants in 2005 and 625 (50%) participants in 2010 were hypertensive (systolic blood pressure of more than or equal to 140 mmHg or diastolic blood pressure of more than or equal to 90 mmHg). Furthermore, both fibrinogen and fibrinogen γ' concentrations increased significantly between 2005 and 2010 in the study population.

Most variables presented in *Table 4.1* that could have had possible effects on fibrinogen and fibrinogen γ' (as determined during the literature review) presented with significant changes between 2005 and 2010. Variables reported as affecting fibrinogen and fibrinogen γ' concentrations which increased significantly from 2005 to 2010 included age, BMI, systolic blood pressure, former tobacco use, fasting glucose, HbA1c, CRP, LDL-cholesterol concentrations and contraceptive use. Variables affecting fibrinogen and fibrinogen γ' concentrations that significantly decreased from 2005 to 2010 were current tobacco use, total cholesterol, HDL-cholesterol and heavy alcohol intake. For the sake of simplicity, all variables that are non-genetic variables are grouped under the term: "environmental factors" for the purpose of this study, even though factors such as age and HIV status are considered characteristics of the study population rather than environmental factors. The environmental factors and genetic factors are presented separately in the following sections with the interactions presented as the last section of this chapter.

Table 4.1: Basic descriptive characteristics of environmental factors

Variable	2005	2010	p-value*
	Median (25-75%)	Median (25-75%)	
Total group n	2010	1288	
Urban n (%)	1004 (50%)	589 (46%)	0.0249
Rural n (%)	1006 (50%)	699 (54%)	0.0249
Men n (%)	749 (37%)	435 (34%)	0.0796
Women n (%)	1261 (63%)	853 (66%)	0.0796
Age (yrs)	48.3 (41.9-56.4)	53.7 (47.2-61.4)	<0.0001
HIV positive n (%)	322 (16.1%)	214 (16.7%)	0.6493
BMI (kg/m²)	23.0 (19.3-28.9)	24.0 (19.8-30.2)	<0.0001
WPAI	2.88 (2.56-3.25)	2.96 (2.63-3.17)	0.2994
SBP (mmHg)	130 (116-147)	132 (118-147)	0.0143
DBP (mmHg)	87 (78-97)	87 (79-97)	0.0995
Tobacco use n (%)	Former: 77 (3.85%) Current: 1042 (52.1%) Never: 881 (44.1%)	Former: 128 (10.3%) Current: 592 (47.8%) Never: 519 (41.9%)	<0.0001 0.0160 0.2134
Fasting glucose (mmol/L)	4.80 (4.30-5.30)	4.96 (4.57-5.39)	<0.0001
HbA1c (%)	5.50 (5.30-5.80)	5.90 (5.60-6.20)	<0.0001
CRP (mg/L)	3.29 (0.96-9.34)	3.84 (1.41-8.68)	0.0057
IL-6 (pg/ml)	3.83 (2.42-7.10)	3.68 (2.36-6.16)	0.9561
Total cholesterol (mmol/L)	4.82 (4.01-5.87)	4.81 (4.08-5.66)	<0.0001
HDL-cholesterol (mmol/L)	1.42 (1.06-1.87)	1.32 (1.05-1.68)	<0.0001
LDL-cholesterol (mmol/L)	2.77 (2.07-3.64)	2.81 (2.09-3.62)	0.0464
Triglycerides (mmol/L)	1.08 (0.82-1.55)	1.09 (0.82-1.55)	0.3358
°Alcohol use n (%)	Non-drinkers: 1077 (55.3%) Moderate intake: 518 (26.6%) Heavy intake: 354 (18.2%)	Non-drinkers: 773 (60.5%) Moderate intake: 329 (25.7%) Heavy intake: 176 (13.8%)	0.0032 0.5666 0.0009
Fibrinogen (g/L)	2.90 (2.30-5.00)	3.63 (3.09-4.25)	<0.0001
Fibrinogen γ' (g/L)	0.31 (0.23-0.45)	0.35 (0.27-0.45)	<0.0001
γ' ratio (%)	10.2 (7.15-14.6)	9.80 (7.94-11.9)	0.2113
Contraceptive use n (%)	Use: 451 (37.5%) Do not use: 753 (62.5%)	Use: 367 (45.5%) Do not use: 440 (54.5%)	<0.0001 <0.0001

BMI = body mass index, CRP = C-reactive protein, DBP = diastolic blood pressure, HbA1c = glycated haemoglobin, HIV = human immunodeficiency virus, HDL-cholesterol = high density lipoprotein cholesterol, IL-6 = Interleukin-6, LDL-cholesterol = low density lipoprotein cholesterol, n = population size, SBP = systolic blood pressure, WPAI = weighted physical activity index, γ' = gamma prime

°Alcohol use categories: non-drinkers = 0 g alcohol intake; moderate intake = <15 g alcohol intake in women or <30 g alcohol intake in men; heavy intake = ≥15 g alcohol intake in women or ≥30 g alcohol intake in men

**p-values of continuous variables were calculated from the log-transformed data.*

4.3 TOTAL FIBRINOGEN AND FIBRINOGEN γ' DIFFERENCES RELATED TO URBANISATION AND GENDER

The differences in total fibrinogen, fibrinogen γ' and γ' ratio related to urbanisation and gender are presented in *Table 4.2*. Rural participants had significantly higher total fibrinogen, but lower fibrinogen γ' than the urban group, resulting in a significantly lower γ' ratio in the rural group in 2005. In 2010, however, both total fibrinogen and fibrinogen γ' were higher in the rural than the urban group, resulting in the γ' ratio to remain unchanged. Both total fibrinogen and fibrinogen γ' increased from 2005 to 2010 in the rural group and as a result the γ' ratio remained unchanged. However, in the urban group, total fibrinogen, but not fibrinogen γ' increased over the 5 year period, consequently decreasing the γ' ratio.

In 2005 women had significantly higher total fibrinogen and fibrinogen γ' than men, with a proportionally bigger difference in fibrinogen γ', resulting in a significantly higher γ' ratio in women. In 2010, women again had higher total fibrinogen and fibrinogen γ' than men, but this difference was essentially of the same magnitude, resulting in the γ' ratio to be similar between men and women in 2010. Total fibrinogen and fibrinogen γ' increased in both men and women over the 5 year period, resulting in the γ' ratio to remain unchanged.

Table 4.2: **Between and within-group differences of total fibrinogen, fibrinogen γ' and γ' ratio related to urbanisation and gender**

	Rural		Urban		p-value^	Men		Women		p-value^
	n	Median (25;75%)	n	Median (25;75%)		n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	932	3.00 (2.40;5.40)	850	2.70 (2.20;4.30)	0.0002	673	2.60 (2.10;3.70)	1109	3.10 (2.30;5.50)	<0.0001
Fibrinogen 2010 (g/L)	676	3.81 (3.29;4.37)	573	3.42 (2.89;4.09)	<0.0001	425	3.34 (2.84;4.07)	824	3.76 (3.25;4.37)	<0.0001
Delta fibrinogen (g/L)	626	0.53 (-1.34;1.41)	480	0.32 (-0.81;1.13)	0.5793	387	0.46 (-0.39;1.31)	719	0.39 (-1.50;1.34)	0.0423
p-value*	---	<0.0001	---	0.0001	---	---	<0.0001	---	<0.0001	---
Fibrinogen γ' 2005 (g/L)	927	0.30 (0.21;0.46)	844	0.32 (0.24;0.45)	0.0081	660	0.28 (0.20;0.39)	1111	0.34 (0.25;0.48)	<0.0001
Fibrinogen γ' 2010 (g/L)	675	0.36 (0.27;0.47)	575	0.34 (0.27;0.43)	0.0179	428	0.32 (0.25;0.43)	822	0.36 (0.29;0.46)	0.0001
Delta fibrinogen γ' (g/L)	627	0.06 (-0.09;0.19)	482	0.01 (-0.11;0.10)	0.0002	387	0.05 (-0.07;0.16)	722	0.03 (-0.13;0.14)	0.0068
p-value*	---	<0.0001	---	0.8042	---	---	<0.0001	---	0.1094	---
γ' ratio 2005 (%)	923	10.0 (6.40;14.2)	778	10.6 (7.82;15.0)	<0.0001	631	9.77 (7.09;13.9)	1070	10.3 (7.17;15.1)	0.0486
γ' ratio 2010 (%)	670	9.54 (7.58;12.1)	573	9.98 (8.26;11.7)	0.1737	424	9.74 (7.86;12.0)	819	9.84 (7.97;11.9)	0.5480
Delta γ' ratio (%)	620	-0.03 (-4.81;4.06)	439	-0.57 (-4.69;2.34)	0.0030	367	-0.27 (-4.23;3.48)	692	-0.34 (-5.10;3.32)	0.1572
p-value*	---	0.3262	---	0.0003	---	---	0.8422	---	0.1651	---

**p-values within group between 2005 and 2010 data*
^p-values between groups in each year
p-values were calculated from the log-transformed data, except for the delta variables

In order to be able to interpret the rural-urban differences between the fibrinogen variables, we also investigated rural-urban differences of the environmental factors known to influence fibrinogen. The only environmental factors that differed significantly between the rural and urban participants in 2005 were age, BMI and systolic blood pressure (*Table 4.3*). In 2010 the environmental factors that differed significantly between the rural and urban participants were age, HbA1c, total cholesterol and LDL-cholesterol (*Table 4.3*).

Table 4.3: **Differences in environmental factors between rural and urban participants in 2005 and 2010**

Variables	Urbanisation differences in 2005 Median (25-75%)		p-value*	Urbanisation differences in 2010 Median (25-75%)		p-value*
	Rural	Urban		Rural	Urban	
Age (yrs)	47.5 (41.3-55.1)	48.9 (42.3-57.7)	0.0005	53.1 (46.7-60.4)	54.6 (47.7-62.8)	0.0013
BMI (kg/m²)	22.4 (19.1-28.0)	23.5 (19.4-29.7)	0.0005	23.6 (19.8-29.4)	24.5 (19.7-30.8)	0.1757
SBP (mmHg)	126 (114-142)	134 (120-151)	<0.0001	131 (118-147)	133 (118-149)	0.7890
Fasting glucose (mmol/L)	4.70 (4.40-5.20)	4.90 (4.30-5.40)	0.0646	4.97 (4.57-5.37)	4.94 (4.58-5.43)	0.6040
HbA1c (%)	5.60 (5.30-5.80)	5.50 (5.20-5.90)	0.5992	5.90 (5.60-6.20)	5.90 (5.60-6.30)	0.0134
CRP (mg/L)	3.33 (0.85-9.02)	3.25 (1.12-9.85)	0.0706	3.97 (1.38-8.68)	3.77 (1.44-8.74)	0.7361
IL-6 (pg/ml)	3.73 (2.47-7.00)	4.06 (2.37-7.17)	0.8703	3.67 (2.45-6.25)	3.68 (2.29-6.06)	0.6948
Total cholesterol (mmol/L)	4.75 (4.02-5.80)	4.89 (4.00-5.97)	0.1867	4.89 (4.15-5.67)	4.77 (3.99-5.65)	0.0282
HDL-cholesterol (mmol/L)	1.41 (1.07-1.87)	1.43 (1.06-1.87)	0.7628	1.27 (1.02-1.59)	1.39 (1.09-1.78)	0.1193
LDL-cholesterol (mmol/L)	2.75 (2.08-3.62)	2.79 (2.04-3.64)	0.8500	2.94 (2.22-3.71)	2.62 (1.95-3.50)	<0.0001

BMI = body mass index, CRP = C-reactive protein, HbA1c = glycated haemoglobin, HDL-cholesterol = high density lipoprotein cholesterol, IL-6 = Interleukin-6, LDL-cholesterol = low density lipoprotein cholesterol, SBP = systolic blood pressure
**p-values were calculated from the log-transformed data.*

In order to interpret the rural-urban differences observed for total fibrinogen and fibrinogen γ' in 2005 and 2010, we did an ANCOVA and adjusted for the environmental factors that differed significantly between the rural and urban setting. Adjustment for the factors individually or combined into one model, did not significantly alter the rural-urban differences observed for total fibrinogen or fibrinogen γ'.

In order to be able to interpret the change in the fibrinogen variables from 2005 to 2010 in the rural and urban participants respectively, we investigated the change from 2005 to 2010 in the environmental factors known to influence fibrinogen in both the rural and urban setting. The environmental factors that differed significantly between 2005 and 2010 in rural participants were BMI, systolic blood pressure, fasting glucose, HbA1c, CRP and HDL-cholesterol (*Table 4.4*). The environmental factors that differed significantly between 2005 and 2010 in urban participants were, BMI, systolic blood pressure, fasting glucose, HbA1c, total cholesterol, HDL-cholesterol and LDL-cholesterol (*Table 4.4*).

Table 4.4: **Differences in environmental factors between 2005 and 2010 in rural and urban participants**

Variables	Rural differences Median (25-75%)		p-value*	Urban differences Median (25-75%)		p-value*
	2005	2010		2005	2010	
Age (yrs)	47.5 (41.3-55.1)	53.1 (46.7-60.4)	<0.0001	48.9 (42.3-57.7)	54.6 (47.7-62.8)	<0.0001
BMI (kg/m²)	22.4 (19.1-28.0)	23.6 (19.8-29.4)	<0.0001	23.5 (19.4-29.7)	24.5 (19.7-30.8)	0.0038
SBP (mmHg)	126 (114-142)	131 (118-147)	<0.0001	134 (120-151)	133 (118-149)	0.0235
Fasting glucose (mmol/L)	4.70 (4.40-5.20)	4.97 (4.57-5.37)	<0.0001	4.90 (4.30-5.40)	4.94 (4.58-5.43)	0.0011
HbA1c (%)	5.60 (5.30-5.80)	5.90 (5.60-6.20)	<0.0001	5.50 (5.20-5.90)	5.90 (5.60-6.30)	<0.0001
CRP (mg/L)	3.33 (0.85-9.02)	3.97 (1.38-8.68)	0.0004	3.25 (1.12-9.85)	3.77 (1.44-8.74)	0.9028
IL-6 (pg/ml)	3.73 (2.47-7.00)	3.67 (2.45-6.25)	0.9131	4.06 (2.37-7.17)	3.68 (2.29-6.06)	0.9735
Total cholesterol (mmol/L)	4.75 (4.02-5.80)	4.89 (4.15-5.67)	0.4963	4.89 (4.00-5.97)	4.77 (3.99-5.65)	<0.0001
HDL-cholesterol (mmol/L)	1.41 (1.07-1.87)	1.27 (1.02-1.59)	<0.0001	1.43 (1.06-1.87)	1.39 (1.09-1.78)	0.0040
LDL-cholesterol (mmol/L)	2.75 (2.08-3.62)	2.94 (2.22-3.71)	0.2349	2.79 (2.04-3.64)	2.62 (1.95-3.50)	0.0001

BMI = body mass index, CRP = C-reactive protein, HbA1c = glycated haemoglobin, HDL-cholesterol = high density lipoprotein cholesterol, IL-6 = Interleukin-6, LDL-cholesterol = low density lipoprotein cholesterol, SBP = systolic blood pressure
**p-values were calculated from the log-transformed data.*

In order to interpret the change in the fibrinogen variables from 2005 to 2010 in both the rural and urban settings we did an ANCOVA, adjusting for the environmental factors that differed significantly between 2005 and 2010 (separately for rural and urban). Adjustment for the factors individually or combined into one model, did not significantly alter the change in the fibrinogen variables from 2005 to 2010 in either the rural or the urban setting.

4.4 ASSOCIATIONS BETWEEN THE FIBRINOGEN VARIABLES AND ENVIRONMENTAL FACTORS

In order to determine the cross-sectional relationship between the fibrinogen variables and environmental factors, Pearson correlations were performed, for the 2005 and 2010 data separately. The fibrinogen variables and environmental factors were log-transformed to improve normality. The results of the correlations for 2005 and 2010 are presented in *Table 4.5* and *Table 4.6*, respectively. Only the top five significant correlations in 2005 and 2010 with total fibrinogen and fibrinogen γ' will be interpreted as the other significant correlations were rather weak (r<0.1) and statistical significance of these correlations is probably a result of the large sample size, but due to the low r-value, likely not of practical significance. The top four environmental factors that had the strongest positive correlations with total fibrinogen in both 2005 and 2010 were CRP, IL-6, BMI and HbA1c. Age and LDL-cholesterol also had statistical significant positive correlations with total fibrinogen in 2005 and 2010, respectively.

Table 4.5: **Correlation between total fibrinogen, fibrinogen γ', γ' ratio and environmental factors in 2005**

Environmental factors (2005)	Total fibrinogen 2005 (g/L)		Fibrinogen γ' 2005 (g/L)		γ' ratio 2005(%)	
	r	P*	r	P*	r	P*
Age (yrs)	0.1451	<0.0001	0.0344	0.1479	-0.1016	<0.0001
BMI (kg/m^2)	0.1567	<0.0001	0.2243	<0.0001	0.0491	0.0432
WPAI	-0.0399	0.1906	-0.1139	0.0002	-0.0602	0.0524
SBP (mmHg)	0.0354	0.1365	-0.0594	0.0128	-0.0811	0.0009
DBP (mmHg)	0.0472	0.0470	-0.0285	0.2330	-0.0628	0.0099
Fasting glucose (mmol/L)	0.0102	0.6741	0.0770	0.0015	0.0482	0.0510
HbA1c (%)	0.1502	<0.0001	0.1807	<0.0001	0.0204	0.4023
CRP (mg/L)	0.4183	<0.0001	0.3727	<0.0001	-0.0568	0.0206
IL-6 (pg/ml)	0.2437	<0.0001	0.1894	<0.0001	-0.0522	0.2231
Total cholesterol (mmol/L)	0.0578	0.0156	-0.0120	0.6171	-0.0731	0.0028
HDL-cholesterol (mmol/L)	-0.0590	0.0136	-0.2123	<0.0001	-0.1332	<0.0001
LDL-cholesterol (mmol/L)	0.0987	<0.0001	0.0861	0.0004	-0.0214	0.3853
Triglyceride (mmol/L)	0.0504	0.0357	0.0889	0.0002	0.0315	0.1995
Alcohol use (g)	-0.0678	0.0583	-0.1605	<0.0001	-0.0805	0.0286

*BMI = body mass index, CRP = C-reactive protein, DBP = diastolic blood pressure, HbA1c = glycated haemoglobin, HDL-cholesterol = high density lipoprotein cholesterol, IL-6 = Interleukin-6, LDL-cholesterol = low density lipoprotein cholesterol, SBP = systolic blood pressure, WPAI = weighted physical activity index, γ' = gamma prime; *p-values were calculated from the log-transformed data.*

Table 4.6: **Correlation between total fibrinogen, fibrinogen γ', γ' ratio and environmental factors in 2010**

Environmental factors (2010)	Total fibrinogen 2010 (g/L)		Fibrinogen γ' 2010 (g/L)		γ' ratio 2010 (%)	
	r	P*	r	P*	r	P*
Age (yrs)	0.1203	<0.0001	0.0885	0.0018	0.0015	0.9589
BMI (kg/m^2)	0.2479	<0.0001	0.1008	0.0004	-0.0644	0.0235
WPAI	-0.0780	0.0062	-0.0267	0.3500	0.0407	0.1547
SBP (mmHg)	-0.0533	0.0642	-0.0552	0.0555	-0.0104	0.7199
DBP (mmHg)	-0.0358	0.2141	-0.0414	0.1511	-0.0019	0.9481
Fasting glucose (mmol/L)	0.0787	0.0054	-0.0156	0.5819	-0.0775	0.0063
HbA1c (%)	0.1629	<0.0001	0.0183	0.5182	-0.1070	0.0002
CRP (mg/L)	0.5070	<0.0001	0.3274	<0.0001	-0.0063	0.8262
IL-6 (pg/ml)	0.2542	<0.0001	0.1255	0.0293	-0.0675	0.2426
Total cholesterol (mmol/L)	0.1123	<0.0001	0.0716	0.0118	0.0162	0.5705
HDL-cholesterol (mmol/L)	-0.1067	0.0002	-0.0647	0.0229	0.0087	0.7615
LDL-cholesterol (mmol/L)	0.2186	<0.0001	0.1502	<0.0001	0.0260	0.3642
Triglyceride (mmol/L)	-0.0244	0.3923	-0.0517	0.0692	-0.0349	0.2207
Alcohol use (g)	-0.1305	0.0037	-0.0803	0.0746	0.0197	0.6633

*BMI = body mass index, CRP = C-reactive protein, DBP = diastolic blood pressure, HbA1c = glycated haemoglobin, HDL-cholesterol = high density lipoprotein cholesterol, IL-6 = Interleukin-6, LDL-cholesterol = low density lipoprotein cholesterol, SBP = systolic blood pressure, WPAI = weighted physical activity index, γ' = gamma prime; *p-values were calculated from the log-transformed data.*

The top three environmental factors that had significant positive correlations with fibrinogen γ' in both 2005 and 2010, were CRP, IL-6 and BMI. In 2005 HbA1c also had a significant positive correlation and HDL-cholesterol a significant negative correlation with fibrinogen γ' while in 2010 age and LDL-cholesterol were the other two factors that had significant positive correlations with fibrinogen γ'.

All the correlations between the environmental factors and γ' ratio in both 2005 and 2010 were rather weak. In 2005 age and HDL-cholesterol had the strongest significant correlations (r>0.1) with γ' ratio and in 2010 HbA1c had the strongest significant correlations (r>0.1) with γ' ratio.

For environmental factors that were categorical by nature, the fibrinogen variables were compared in 2005 and 2010 across the categories using t-tests when comparing two groups and ANOVA with Tukey's honest significant difference *post-hoc* tests when comparing three or more groups (*Table 4.7* and *Table 4.8*). In 2005 the total fibrinogen concentrations were lower in the HIV-positive than in the HIV-negative group, while fibrinogen γ' was higher in the HIV-positive than in the HIV-negative group. Therefore, the γ' ratio differed significantly between the HIV-positive and HIV-negative groups in 2005. In 2010 both the total fibrinogen and fibrinogen γ' concentrations were lower in the HIV-positive than in the HIV-negative group. Therefore, the γ' ratio did not differ significantly between the HIV-positive and the HIV-negative group. In 2005 there were no significant differences in total fibrinogen or fibrinogen γ' concentration between the different groups of tobacco users. However, in 2010 participants who never used tobacco had a significantly higher total fibrinogen concentration and also a slightly, (but statistically significant) higher fibrinogen γ' than participants currently using tobacco. There was no statistical significant difference in 2005 and 2010 in γ' ratio between the different groups of tobacco users. There was no statistical significant difference in the fibrinogen variables between the different groups of contraceptive use.

Table 4.7: **The effect of categorical environmental factors on total fibrinogen, fibrinogen γ' and γ' ratio in 2005**

Environmental factors (2005)	Total fibrinogen 2005 (g/L)		Fibrinogen γ' 2005 (g/L)		γ' ratio 2005 (%)	
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)
HIV status						
Positive	299	2.60 (2.10;3.30)	294	0.33 (0.25;0.46)	289	12.7 (9.18;16.5)
Negative	1477	3.00 (2.30;5.30)	1471	0.31 (0.23;0.45)	1406	9.70 (6.77;14.0)
p-value*		<0.0001		0.0088		<0.0001
Tobacco use						
Former	66	2.70 (2.40;4.80)	65	0.29 (0.22;0.39)	62	10.9 (7.57;13.4)
Current	934	2.80 (2.20;4.80)	928	0.31 (0.23;0.45)	892	10.2 (7.29;14.8)
Never	774	3.00 (2.30;5.40)	771	0.32 (0.23;0.46)	740	10.1 (7.05;14.5)
p-value*		0.3597		0.4948		0.6648
Contraceptive use						
Use	407	3.00 (2.30;5.70)	405	0.34 (0.25;0.49)	394	10.4 (7.13;15.4)
Do not use	647	3.10 (2.40;5.50)	651	0.34 (0.25;0.47)	622	10.4 (7.27;14.8)
p-value*		0.8290		0.8851		0.9356

HIV = human immunodeficiency virus, γ' = gamma prime
**p-values were calculated from the log-transformed data of the fibrinogen variables*

Table 4.8: **The effect of categorical environmental factors on total fibrinogen, fibrinogen γ' and γ' ratio in 2010**

Environmental factors (2010)	Total fibrinogen 2010 (g/L)		Fibrinogen γ' 2010 (g/L)		γ' ratio 2010 (%)	
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)
HIV status						
Positive	212	3.26 (2.84;3.76)	212	0.32 (0.25;0.41)	212	9.86 (7.83;12.3)
Negative	1032	3.71 (3.18;4.32)	1032	0.35 (0.28;0.46)	1025	9.79 (7.97;11.9)
p-value*		<0.0001		0.0001		0.7614
Tobacco use						
Former	127	3.53 (2.96;4.29)	126	0.36 (0.27;0.49)	126	10.5 (7.97;12.4)
Current	575	3.55 (3.01;4.14)•	578	0.34 (0.26;0.43)•	574	9.64 (7.91;11.9)
Never	502	3.72 (3.21;4.32)•	501	0.36 (0.29;0.46)•	498	9.87 (7.98;11.9)
p-value*		0.0114		0.0197		0.1490
Contraceptive use						
Use	360	3.76 (3.25;4.40)	360	0.36 (0.28;0.47)	358	9.65 (8.00;11.9)
Do not use	423	3.76 (3.26;4.31)	420	0.36 (0.29;0.45)	419	9.94 (7.91;12.0)
p-value*		0.7308		0.5161		0.5152

HIV = human immunodeficiency virus, γ' = gamma prime
**p-values were calculated from the log-transformed data of the fibrinogen variables*

4.5 GENOTYPE DISTRIBUTION OF THE SINGLE NUCLEOTIDE POLYMORPHISMS

The genotype distributions of the SNPs analysed in this study are presented in *Table 4.9*. All the SNPs were calculated to adhere to the assumptions of Hardy-Weinberg equilibrium. The purpose of this study was not to identify novel SNPs, but to examine the effects of currently reported SNPs on the fibrinogen variables.

Table 4.9: **Genotype distributions of the investigated SNPs**

SNP	Genotype	Genotype count	Genotype (%)	95% CI of SNP frequency (%)	MAF	HW (p-value)
FGA 2224 G>A (rs2070011)	GG	1105	68.6	66.4-70.9	0.17	0.82
	GA	462	28.7	26.5-30.9		
	AA	43	2.70	1.88-3.46		
FGA 6534 A>G (rs6050)	AA	801	49.9	47.5-52.4	0.30	0.21
	AG	644	40.2	37.7-42.5		
	GG	159	9.90	8.45-11.4		
FGB Arg448Lys (rs4220)	GG	1367	84.6	82.9-86.4	0.08	0.87
	GA	236	14.6	12.9-16.3		
	AA	12	0.80	0.32-1.16		
FGB -148 C>T (rs1800787)	CC	1397	88.3	86.7-89.9	0.06	0.41
	CT	176	11.1	9.57-12.7		
	TT	9	0.60	0.20-0.94		
FGB 40 A>G (rs2227385)	AA	1773	94.7	93.6-95.7	0.03	0.49
	AG	100	5.3	4.32-6.36		
	GG	0	0	0		
FGB 749 A>G (rs2227388)	AA	1316	70.2	68.1-72.2	0.16	0.94
	AG	513	27.3	25.3-29.4		
	GG	47	2.50	1.80-3.21		
FGB 1038 G>A (rs1800791)	GG	1580	83.6	81.9-85.3	0.09	0.74
	GA	299	15.8	14.2-17.5		
	AA	11	0.600	0.24-0.93		
FGB 1643 C>T (rs1800788)	CC	1706	90.26	88.9-91.6	0.05	0.73
	CT	181	9.58	8.25-10.9		
	TT	3	0.16	0.00-0.43		
FGG 10034 C>T (rs2066865)	CC	876	54.6	52.1-57.0	0.27	0.09
	CT	596	37.1	34.8-39.5		
	TT	133	8.30	6.94-9.64		
FGG 9340 T>C (rs1049636)	TT	1154	71.4	69.2-73.6	0.16	0.35
	TC	414	25.6	23.5-27.8		
	CC	48	3.00	2.14-3.80		

A = adenine; Arg = arginine; C = cytosine; CI = confidence interval; FGA = fibrinogen α gene; FGB = fibrinogen β gene; FGG = fibrinogen γ gene; G = guanine; HW = Hardy-Weinberg equilibrium; Lys = lysine; MAF = minor allele frequency; SNP = single nucleotide polymorphism; T = thymine

4.6 LINKAGE DISEQUILIBRIUM DETERMINATION

Linkage disequilibrium (LD) is non-random association of alleles at two or more loci (Ardlie *et al.,* 2002). Loci are also in LD if an allele at one locus is found on the same chromosome with a specific allele at another locus more often than would be expected if the loci were segregating independently in a population (Ardlie *et al.,* 2002). LD was determined between the investigated SNPs to establish whether a single independently inherited SNP, or SNPs inherited together, were responsible for the observed phenotypes. In *Figure 4.1*, the Haploview LD structure of the ten SNPs used in this study is presented with the D' (95% confidence bounds) and r^2 values. The colour gradient indicates the level of LD, where the black boxes indicate strong evidence for LD and the white boxes indicate little to no LD. These colours are chosen based on the confidence intervals of the D' values. If the upper confidence bound on D' is less than 0.9 and/or the lower bound are less than 0.7, the SNPs are estimated to have strong evidence of recombination, therefore, indicating strong LD (Gabriel *et al.,* 2002).

D' is calculated by dividing the D (disequilibrium) by its maximum possible value, determined by the allele frequencies at the two loci (Ardlie *et al.,* 2002). A D' value of 1, which is known as complete LD, indicates that two SNPs have not been separated by recombination (Ardlie *et al.,* 2002). Values of D' less than 1 indicate that the LD have been disturbed (Ardlie *et al.,* 2002). There is difficulty, however, in interpreting D' values less than 1 (Ardlie *et al.,* 2002). The sample size strongly influences the D' values, as small sample sizes can cause high D' values even if there is linkage equilibrium between the SNPs (Ardlie *et al.,* 2002). Intermediate values of D' should not be used to determine the strength of LD; only statistically significant D' values near 1 should be used (Ardlie *et al.,* 2002). For the determination of LD for our study the entire population was used, thus the sample size was sufficient to prevent the D' being influenced by a small sample size.

The r^2 value determines the correlation of the alleles at the two loci (Ardlie *et al.,* 2002). The r^2 is calculated by dividing the D^2 by the product of the four allele frequencies at the two loci (Ardlie *et al.,* 2002). The r^2 value ranges from 0 to 1; if the r^2 is 0 the SNPs are in complete linkage equilibrium whereas if the r^2 is 1, the SNPs are in complete LD (Ardlie *et al.,* 2002).

If the r^2 is 1 it means that information at one SNP will provide complete information about the other SNP (Ardlie *et al.,* 2002). An r^2 value of 0.33 or higher can be accepted as strong LD (Ardlie *et al.,* 2002). Intermediate r^2 values are more easily interpretable than intermediate values of D' (Ardlie *et al.,* 2002). The value of r^2 is related to the amount of information provided by one locus about the other (Ardlie *et al.,* 2002). The influence of sample size on the r^2 value is also far smaller than it is on the D' (Ardlie *et al.,* 2002).

With regard to the SNPs established as being in LD, it is important to remember that these formulas that establish LD determine only the likelihood of the SNPs being linked (Klug *et al.,* 2009). Both the D' and r^2 were included in the Haploview plot; however, the plot was based on the confidence bounds of the D' as the study population analysed was of sufficient size.

From the Haploview plot it was found that there was strong evidence that the following SNPs were in LD:

- FGA 2224 G>A with FGB 749 A>G
- FGB Arg448Lys with FGG10034 C>T with FGA 6534 A>G with FGG 9340 T>C
- FGG 10034 C>T with FGG 9340 T>C with FGB 40 A>G
- FGA 6534 A>G with FGB 40 A>G
- FGB 40 A>G with FGB 749 A>G
- FGB 749 A>G with FGB 1038 G>A with FGB 1643 C>T

Due to the relatively low r^2 values, however, which is most likely due to the differences in MAF between these SNPs, it can be assumed that these SNPs may not have been in complete linkage disequilibrium and may still have differing effects on total fibrinogen and fibrinogen γ' concentration. Therefore the results of the individual SNPs will be presented instead of only making use of representative tagging SNPs as determined from the LD plot.

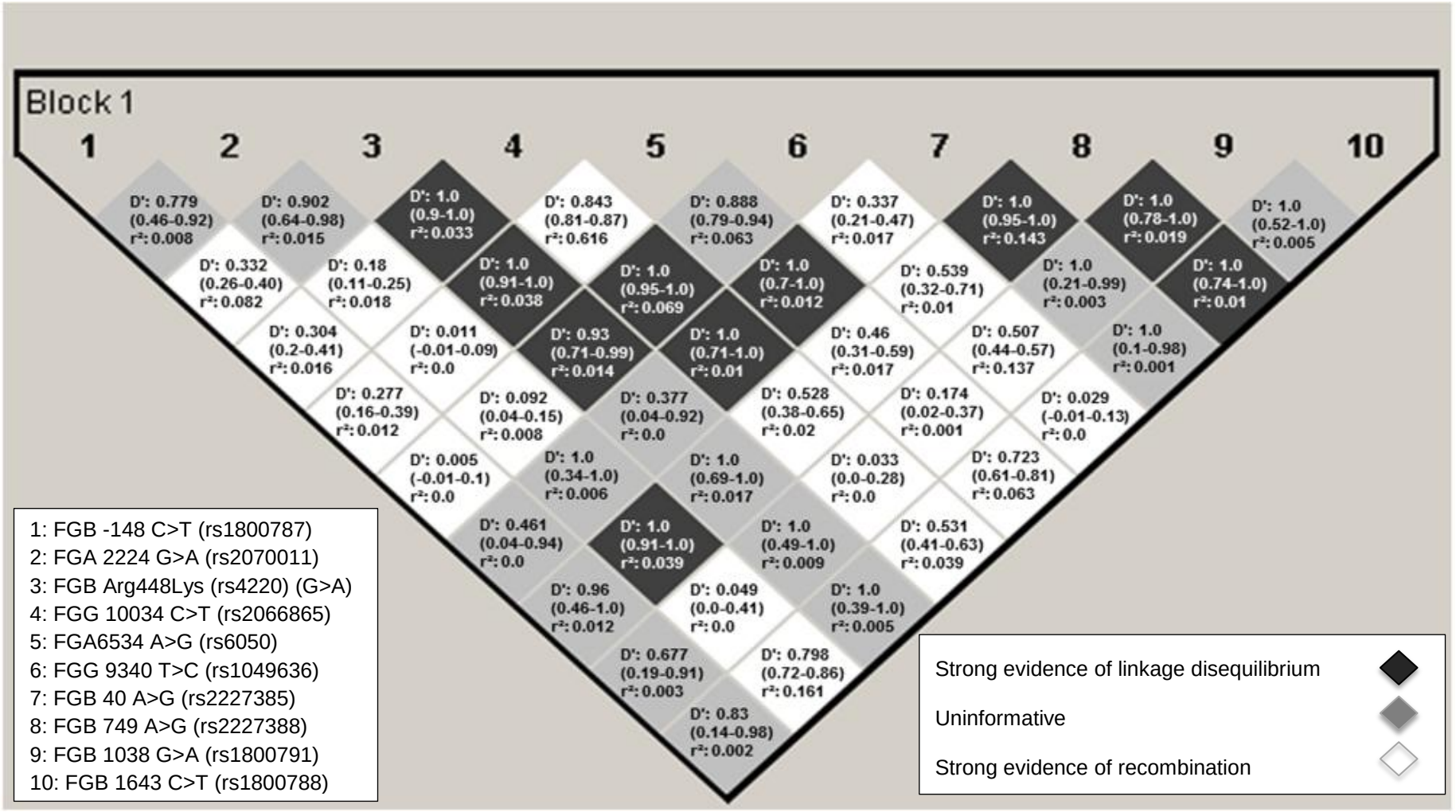

Figure 4.1: **Pair-wise linkage disequilibrium structure presenting the D' (95% confidence bounds) and the r^2**

4.7 FIBRINOGEN, FIBRINOGEN γ' AND γ' RATIO DIFFERENCES RELATED TO GENOTYPE

The effect of the different genotypes on total fibrinogen, fibrinogen γ' and γ' ratio is presented in *Table 4.10*. In this section, the levels of the three fibrinogen variables are compared across the genotypes of each SNP, for each of the two time points (2005 and 2010). It also presents the differences in fibrinogen variables between 2005 and 2010 within each genotype. Lastly, this section also demonstrates whether the change in the fibrinogen variables over the five-year period differed between the genotypes (between-group comparisons).

Firstly, to investigate the cross-sectional effect of the genotypes on the fibrinogen variables, the median fibrinogen variables between the genotypes were compared for each time point (2005 and 2010). The 2005 total fibrinogen concentration differed significantly only between the genotypes of the FGG 9340 T>C SNP. The 2010 total fibrinogen concentration differed significantly between the different genotypes of the FGA 2224 G>A, FGB Arg448Lys, FGG 10034 C>T, FGG 9340 T>C and FGB 1643 C>T SNPs.

The 2005 fibrinogen γ' concentration differed significantly between the genotypes of the FGA 2224 G>A SNP. The 2010 fibrinogen γ' concentration differed significantly between the FGA 6534 A>G, FGB -148 C>T, FGG 10034 C>T, FGB 1038 G>A and FGG 9340 T>C genotypes. There was no difference in the 2005 γ' ratio between any of the analysed genotypes. The 2010 γ' ratio differed significantly between FGB Arg448Lys, FGA 6534 A>G, FGB -148 C>T, FGB 749 A>G, FGG 10034 C>T, FGG 9340 T>C and FGB 1643 C>T genotypes.

To determine whether the genotypes influenced the change over the five-year time period for the fibrinogen variables, the change from 2005 to 2010 was determined for each genotype (within-group comparison). It was also determined whether the change over the five-year period differed between the genotypes (between-group comparisons). Total fibrinogen increased significantly from 2005 to 2010 in almost all the genotypes except in participants harbouring the mutant homozygous genotypes of the FGA 2224 G>A, FGA 6534 A>G, FGB 749 A>G and FGG 9340 T>C, as well as in those carrying the heterozygous genotypes of FGB Arg448Lys and FGB -148 C>T polymorphisms. As seen

in *Figure 4.1*, it was determined that FGA 2224 G>A and FGB 749 A>G were in LD with each other, which could explain why these SNPs have the same effect on total fibrinogen. While the above-mentioned differences over time within each specified genotype were observed, the change between the genotypes of each respective genetic polymorphism over the five-year period was only borderline significant for FGB -148 C>T (p=0.0561) and significant for FGB 1643 C>T (p=0.0246), indicating that these genetic polymorphisms may influence change in total fibrinogen concentrations over time.

Fibrinogen γ' increased significantly from 2005 to 2010 in almost all the genotypes except in participants harbouring the mutant homozygous genotypes of FGA 2224 G>A, FGA 6534 A>G, FGB 749 A>G and FGG 9340 T>C, as well as those harbouring the heterozygous genotypes of FGA 2224 G>A, FGA 6534 A>G, FGB -148 C>T, FGB 40 A>G, FGG 10034 C>T and FGB 1643 C>T. It was determined that FGA 2224 G>A and FGB 749 A>G, FGG 10034 C>T and FGB 40 A>G, as well as FGA 6534 A>G and FGB 40 A>G, are in LD (see *Figure 4.1*) and it is possible that this factor may be responsible for these SNPs having the same effect on fibrinogen γ'. Fibrinogen γ' decreased significantly from 2005 to 2010 in participants harbouring the mutant homozygous genotype at the FGG 10034 C>T locus. It is important to note that while fibrinogen γ' decreased over the five years in participants harbouring the homozygous mutant genotype, it increased over the five years in participants harbouring the homozygous wild-type genotype at the FGG 10034 C>T locus. While the above-mentioned differences within each specified genotype over time were observed, the change over the five-year period between the genotypes of each respective genetic polymorphism was significant only at the FGB -148 C>T (p=0.0235), and FGG 10034 C>T (p=0.0001) loci, and borderline significant at the FGB Arg448Lys (p=0.0746) and FGA 6534 A>G (p=0.0655) loci, indicating that these genetic polymorphisms may influence change in fibrinogen γ' over time. A possible reason for the similar effects of the SNPs FGB Arg448Lys and FGG 10034 C>T, as well as the FGB Arg448Lys and FGA 6534 A>G SNPs, on the change in fibrinogen γ' over time can likely be attributed to the fact that they were in LD (see *Figure 4.1*).

Fibrinogen γ' ratio decreased significantly from 2005 to 2010 in participants harbouring the wild-type homozygous genotypes of FGB Arg448Lys and FGG 9340 T>C; in participants harbouring the heterozygous genotypes of FGA 2224 G>A, FGA 6534 A>G, FGG 10034 C>T and FGB 1643 C>T; and in participants harbouring the mutant homozygous genotypes of FGA 6534 A>G and FGG 10034 C>T. It was determined that FGB Arg448Lys and FGG 9340 T>C are in LD, which could explain why these SNPs have the same effect on γ' ratio (see *Figure 4.1*). Fibrinogen γ' ratio increased significantly from 2005 to 2010 in participants harbouring the wild-type homozygous genotype of FGG 10034 C>T. It is important to note that the γ' ratio decreased over the five years in participants harbouring the mutant homozygous and heterozygous genotypes of FGG 10034 C>T, but increased over the five years in those harbouring the wild-type homozygous genotype of FGG 10034 C>T. While the above-mentioned differences over time within each specified genotype were observed, the change over the five-year period between the genotypes of each respective genetic polymorphism was significant only for FGA 2224 G>A (p=0.0220), FGB Arg448Lys (p=0.0126) and FGG 10034 C>T (p=0.0020), indicating that these genetic polymorphisms may influence change in γ' ratio over time. The SNPs FGB Arg448Lys and FGG 10034 C>T were determined to be in LD, which could explain why these SNPs have the same effect on change in γ' ratio over time (see *Figure 4.1*).

Table 4.10: Between-group differences and effect of genotypes on change of total fibrinogen, fibrinogen γ' and γ' ratio over time

FGA 2224 G>A (rs2070011)	WT homozygous		Heterozygous		MT homozygous		ANOVA p-value^	FGB Arg448Lys (rs4220)	WT homozygous		MT homozygous & Heterozygous		p-value ^
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)			n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	1031	2.80 (2.30;5.00)	422	2.90 (2.20;5.00)	37	3.70 (2.80;6.20)	0.1063	**Fibrinogen 2005 (g/L)**	1256	2.90 (2.20;5.00)	239	2.90 (2.20;5.00)	0.8328
Fibrinogen 2010 (g/L)	679	3.56 (3.02;4.20)•	292	3.66 (3.13;4.37)	32	4.05 (3.49;4.65)•	0.0077	**Fibrinogen 2010 (g/L)**	853	3.64 (3.10;4.24)	153	3.48 (2.97;4.07)	0.0338
Delta fibrinogen (g/L)	632	0.36 (-1.21;1.23)	267	0.56 (-0.91;1.56)	28	0.12 (-1.63;1.80)	0.3160	**Delta fibrinogen (g/L)**	780	0.45 (-1.15;1.36)	150	0.31 (-1.15;1.12)	0.4990
p-value*		<0.0001		<0.0001		0.4237		**p-value***		<0.0001		0.1234	
Fibrinogen γ' 2005 (g/L)	1021	0.30 (0.23;0.44)•	428	0.32 (0.23;0.46)♦	40	0.39 (0.33;0.55)•♦	0.0084	**Fibrinogen γ' 2005 (g/L)**	1263	0.31 (0.23;0.45)	231	0.31 (0.22;0.45)	0.7445
Fibrinogen γ' 2010 (g/L)	683	0.35 (0.27;0.45)	288	0.34 (0.27;0.44)	32	0.37 (0.29;0.46)	0.6025	**Fibrinogen γ' 2010 (g/L)**	855	0.35 (0.27;0.44)	152	0.37 (0.29;0.45)	0.1108
Delta fibrinogen γ' (g/L)	634	0.03 (-0.11;0.16)	267	0.03 (-0.12;0.12)	31	-0.06 (-0.10;0.14)	0.7604	**Delta fibrinogen γ' (g/L)**	790	0.03 (-0.12;0.14)	145	0.05 (-0.07;0.16)	0.0746
p-value*		0.0008		0.2121		0.6909		**p-value***		0.0151		0.0001	
γ' ratio 2005 (%)	987	10.1 (7.15;14.7)	405	10.3 (7.42;15.0)	36	11.1 (6.55;17.6)	0.5008	**γ' ratio 2005 (%)**	1207	10.3 (7.21;14.8)	226	9.95 (7.04;14.7)	0.7165
γ' ratio 2010 (%)	679	10.0 (8.00;12.1)	287	9.40 (7.84;11.4)	32	9.16 (7.27;11.8)	0.2878	**γ' ratio 2010 (%)**	851	9.54 (7.83;11.8)	151	10.7 (8.62;12.3)	0.0035
Delta γ' ratio (%)	609	-0.03 (-4.26;3.57)•	253	-0.70 (-5.93;2.46)•	28	-1.71 (-8.43;2.84)	0.0220	**Delta γ' ratio (%)**	750	-0.49 (-5.14;3.13)	143	0.57 (-3.49;4.0)	0.0126
p-value*		0.7281		0.0029		0.3235		**p-value***		0.0279		0.1311	

***Table 4.10* (continued)**

FGA 6534 A>G (rs6050)	WT homozygous		Heterozygous		MT homozygous		ANOVA p-value^	FGB -148 C>T (rs1800787)	WT homozygous		MT homozygous & Heterozygous		p-value ^
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)			n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	742	2.90 (2.20;5.30)	594	2.80 (2.20;4.50)	148	3.00 (2.40;5.00)	0.6599	**Fibrinogen 2005 (g/L)**	1294	2.90 (2.20;4.80)	169	3.00 (2.20;5.90)	0.1334
Fibrinogen 2010 (g/L)	513	3.56 (3.04;4.18)	396	3.65 (3.16;4.23)	89	3.85 (3.16;4.53)	0.1496	**Fibrinogen 2010 (g/L)**	877	3.61 (3.06;4.22)	109	3.69 (3.25;4.20)	0.5558
Delta fibrinogen (g/L)	474	0.35 (-1.46;1.33)	364	0.47 (-0.81;1.33)	83	0.43 (-0.71;1.23)	0.2643	**Delta fibrinogen (g/L)**	810	0.43 (-0.99;1.33)	100	0.39 (-2.13;1.44)	0.0561
p-value*		0.0004		<0.0001		0.0529		**p-value***		<0.0001		0.3953	
Fibrinogen γ' 2005 (g/L)	742	0.31 (0.22;0.46)	594	0.31 (0.24;0.45)	147	0.32 (0.23;0.44)	0.7651	**Fibrinogen γ' 2005 (g/L)**	1297	0.31 (0.23;0.44)	167	0.34 (0.22;0.47)	0.4075
Fibrinogen γ' 2010 (g/L)	515	0.36 (0.29;0.47)•	394	0.34 (0.27;0.44)♦	89	0.29 (0.22;0.38)•♦	<0.0001	**Fibrinogen γ' 2010 (g/L)**	878	0.35 (0.28;0.45)	108	0.32 (0.26;0.42)	0.0391
Delta fibrinogen γ' (g/L)	480	0.05 (-0.09;0.15)	364	0.02 (-0.12;0.14)	82	-0.03 (-0.13;0.08)	0.0655	**Delta fibrinogen γ' (g/L)**	821	0.04 (-0.10;0.15)	96	-0.01 (-0.15;0.10)	0.0235
p-value*		<0.0001		0.2537		0.2129		**p-value***		0.0001		0.6238	
γ' ratio 2005 (%)	712	10.3 (7.04;15.0)	566	10.3 (7.46;14.5)	144	9.41 (7.32;15.1)	0.8004	**γ' ratio 2005 (%)**	1244	10.3 (7.26;14.7)	160	9.53 (6.27;14.8)	0.2627
γ' ratio 2010 (%)	511	10.5 (8.58;12.3)•♠	393	9.43 (7.76;11.7)♦♠	89	7.81 (6.19;8.96)•♦	<0.0001	**γ' ratio 2010 (%)**	873	9.85 (8.04;12.1)	108	9.01 (7.15;10.9)	0.0015
Delta γ' ratio (%)	457	0.04 (-4.56;4.03)	345	-0.36 (-5.11;2.62)	82	-1.29 (-6.81:1.15)	0.2019	**Delta γ' ratio (%)**	781	-0.31 (-4.64;3.37)	94	-0.63 (-5.39;2.62)	0.4809
p-value*		0.4360		0.0430		0.0027		**p-value***		0.2205		0.4482	

Table 4.10 (continued)

FGB 749 A>G (rs2227388)	WT homozygous		Heterozygous		MT homozygous		ANOVA p-value^	FGB 40 A>G (rs2227385)	WT homozygous		Heterozygous		p-value ^
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)			n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	1221	2.90 (2.20;5.30)	479	2.90 (2.30;4.80)	44	2.75 (2.30;5.65)	0.5794	**Fibrinogen 2005 (g/L)**	1651	2.90 (2.20;5.00)	90	2.90 (2.40;5.00)	0.8923
Fibrinogen 2010 (g/L)	817	3.65 (3.12;4.27)	318	3.54 (2.96;4.23)	33	3.56 (3.12;4.43)	0.1097	**Fibrinogen 2010 (g/L)**	1102	3.62 (3.07;4.27)	62	3.62 (3.09;4.12)	0.8177
Delta fibrinogen (g/L)	756	0.42 (-1.29;1.32)	296	0.36 (-1.12;1.36)	32	0.83 (-1.86;1.35)	0.9109	**Delta fibrinogen (g/L)**	1025	0.42 (-1.13;1.32)	55	0.74 (-0.69;1.43)	0.1952
p-value*		<0.0001		0.0003		0.2555		**p-value***		<0.0001		0.0037	
Fibrinogen γ' 2005 (g/L)	1220	0.31 (0.23;0.46)	470	0.31 (0.22;0.44)	44	0.27 (0.19;0.42)	0.3186	**Fibrinogen γ' 2005 (g/L)**	1638	0.31 (0.23;0.45)	92	0.32 (0.22;0.48)	0.8660
Fibrinogen γ' 2010 (g/L)	817	0.35 (0.27;0.45)	318	0.35 (0.28;0.46)	33	0.34 (0.27;0.41)	0.7586	**Fibrinogen γ' 2010 (g/L)**	1101	0.35 (0.27;0.45)	63	0.38 (0.29;0.46)	0.6212
Delta fibrinogen γ' (g/L)	766	0.03 (-0.11;0.14)	290	0.04 (-0.12;0.16)	31	0.11 (-0.03;0.19)	0.2913	**Delta fibrinogen γ' (g/L)**	1025	0.03 (-0.11;0.15)	57	0.04 (-0.09;0.16)	0.8844
p-value*		0.0058		0.0051		0.3625		**p-value***		0.0002		0.3312	
γ' ratio 2005 (%)	1167	10.1 (7.16;14.6)	455	10.4 (7.22;14.9)	43	9.22 (6.30;12.5)	0.3008	**γ' ratio 2005 (%)**	1575	10.2 (7.21;14.6)	87	10.3 (6.38;15.4)	0.9137
γ' ratio 2010 (%)	812	9.54 (7.86;11.7)•	317	10.1 (8.25;12.4)•	33	10.1 (7.96;12.1)	0.0330	**γ' ratio 2010 (%)**	1096	9.66 (7.92;11.9)	62	10.3 (8.31;11.9)	0.1463
Delta γ' ratio (%)	728	-0.31 (-4.55;3.07)	279	-0.31 (-5.18:3.92)	31	0.92 (-2.99;2.96)	0.6436	**Delta γ' ratio (%)**	981	-0.32 (-4.74;3.20)	53	-1.00 (-6.41;4.36)	0.8599
p-value*		0.0831		0.9250		0.7111		**p-value***		0.1392		0.6459	

Table 4.10 (continued)

FGG 10034 C>T (rs2066865)	WT homozygous		Heterozygous		MT homozygous		ANOVA p-value^	FGB 1038 G>A (rs1800791)	WT homozygous		MT homozygous & Heterozygous		p-value ^
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)			n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	816	2.90 (2.30;5.00)	547	2.80 (2.20;5.30)	122	2.90 (2.20;4.50)	0.9226	**Fibrinogen 2005 (g/L)**	1460	2.90 (2.20;5.00)	297	3.00 (2.30;5.30)	0.3502
Fibrinogen 2010 (g/L)	560	3.56 (3.03;4.20)•	365	3.66 (3.17;4.21)	76	3.91 (3.19;4.69)•	0.0248	**Fibrinogen 2010 (g/L)**	986	3.60 (3.07;4.25)	192	3.70 (3.14;4.26)	0.4014
Delta fibrinogen (g/L)	519	0.36 (-1.32;1.31)	336	0.42 (-1.27;1.32)	69	0.54 (-0.44;1.41)	0.5173	**Delta fibrinogen (g/L)**	911	0.44 (-1.24;1.34)	182	0.35 (-1.08;1.20)	0.9282
p-value*		0.0001		0.0001		0.0122		**p-value***		<0.0001		0.0063	
Fibrinogen γ' 2005 (g/L)	813	0.31 (0.22;0.45)	551	0.32 (0.24;0.46)	120	0.32 (0.23;0.43)	0.1804	**Fibrinogen γ' 2005 (g/L)**	1462	0.31 (0.23;0.45)	285	0.32 (0.23;0.47)	0.1181
Fibrinogen γ' 2010 (g/L)	560	0.38 (0.30;0.47)•♦	365	0.33 (0.26;0.42)♦♠	76	0.27 (0.20;0.34)•♠	<0.0001	**Fibrinogen γ' 2010 (g/L)**	987	0.35 (0.27;0.44)	191	0.36 (0.28;0.50)	0.0441
Delta fibrinogen γ' (g/L)	523	0.07 (-0.08;0.18)•♦	339	-0.01 (-0.14;0.11)•	67	-0.05 (-0.14;0.06)♦	0.0001	**Delta fibrinogen γ' (g/L)**	918	0.03 (-0.11;0.15)	178	0.03 (-0.11;0.16)	0.8646
p-value*		<0.0001		0.5356		0.0252		**p-value***		0.0005		0.0486	
γ' ratio 2005 (%)	783	10.3 (7.04;14.8)	522	10.3 (7.39;14.3)	118	9.34 (7.58;15.2)	0.6247	**γ' ratio 2005 (%)**	1402	10.2 (7.21;14.5)	276	10.1 (7.06;15.1)	0.7749
γ' ratio 2010 (%)	556	10.7 (8.93;12.5)♦•	364	8.92 (7.55;10.8)♦♠	76	7.14 (6.03;8.47)•♠	<0.0001	**γ' ratio 2010 (%)**	982	9.65 (7.95;11.8)	190	10.2 (8.09;12.1)	0.2706
Delta γ' ratio (%)	498	0.50 (-4.14;4.43)•♦	322	-0.75(-5.39;2.30)•	67	-1.93 (-8.18;0.74)♦	0.0020	**Delta γ' ratio (%)**	876	-0.20 (-4.74;3.26)	171	-0.53 (-4.77;3.86)	0.6539
p-value*		0.0433		0.0033		<0.0001		**p-value***		0.2669		0.5786	

Table 4.10 (continued)

FGG 9340 T>C (rs1049636)	WT homozygous		Heterozygous		MT homozygous		ANOVA p-value^	FGB 1643 C>T (rs1800788)	WT homozygous		MT homozygous & Heterozygous		p-value^
	n	Median (25;75%)	n	Median (25;75%)	n	Median (25;75%)			n	Median (25;75%)	n	Median (25;75%)	
Fibrinogen 2005 (g/L)	1069	2.80 (2.20;4.50)•	383	3.00 (2.20;5.30)♦	44	5.00 (2.80;6.25)•♦	0.0009	**Fibrinogen 2005 (g/L)**	1590	2.90 (2.30;5.00)	167	3.00 (2.10;4.80)	0.5133
Fibrinogen 2010 (g/L)	725	3.63 (3.11;4.23)	255	3.57 (2.97;4.16)•	26	4.03 (3.45;4.85)•	0.0284	**Fibrinogen 2010 (g/L)**	1059	3.61 (3.07;4.23)	119	3.77 (3.18;4.50)	0.0174
Delta fibrinogen (g/L)	671	0.44 (-0.94;1.36)	237	0.37 (-1.42;1.20)	22	-0.07 (-2.61;1.69)	0.2035	**Delta fibrinogen (g/L)**	986	0.37 (-1.26;1.29)	107	0.67 (-0.69;1.62)	0.0246
p-value*		<0.0001		0.0447		0.7979		**p-value***		<0.0001		<0.0001	
Fibrinogen γ' 2005 (g/L)	1058	0.31 (0.23;0.45)	392	0.31 (0.23;0.45)	45	0.32 (0.22;0.57)	0.6067	**Fibrinogen γ' 2005 (g/L)**	1577	0.31 (0.23;0.46)	170	0.32 (0.23;0.41)	0.2143
Fibrinogen γ' 2010 (g/L)	725	0.34 (0.27;0.43)•	256	0.35 (0.28;0.47)♦	26	0.52 (0.38;0.59)•♦	<0.0001	**Fibrinogen γ' 2010 (g/L)**	1058	0.35 (0.27;0.45)	120	0.34 (0.26;0.45)	0.5554
Delta fibrinogen γ' (g/L)	667	0.03 (-0.11;0.14)	245	0.03 (-0.09;0.14)	23	0.09 (-0.11;0.37)	0.1334	**Delta fibrinogen γ' (g/L)**	984	0.03 (-0.11;0.15)	112	0.03 (-0.07;0.12)	0.6280
p-value*		0.0431		0.0052		0.1011		**p-value***		0.0002		0.1960	
γ' ratio 2005 (%)	1020	10.3 (7.33;14.8)	371	10.3 (7.05;15.1)	43	8.30 (5.67;13.6)	0.0815	**γ' ratio 2005 (%)**	1520	10.2 (7.17;14.7)	158	10.1 (6.67;13.4)	0.7335
γ' ratio 2010 (%)	723	9.51 (7.80;11.7)•♦	253	10.1 (8.26;12.2)♦	26	11.6 (10.3;13.3)•	0.0002	**γ' ratio 2010 (%)**	1054	9.91 (8.00;12.0)	118	9.01 (7.69;11.2)	0.0332
Delta γ' ratio (%)	641	-0.42 (-5.15;3.04)	231	0.11 (-4.37;3.61)	21	1.99 (-1.00;4.83)	0.1927	**Delta γ' ratio (%)**	946	-0.13 (-4.75;3.53)	101	-1.13 (-4.64;1.86)	0.1217
p-value*		0.0253		0.7936		0.2387		**p-value***		0.5689		0.0078	

*When fewer than 30 subjects harboured the mutant genotype, they were grouped together with the heterozygotes.; *p-values within group between 2005 and 2010 data; ^p-values between groups in each year; p-values were calculated from the log-transformed data, except for the delta variables; •♦♣Medians with the same symbol differed significantly; A = adenine; C = cytosine; FGA = fibrinogen α gene; FGB = fibrinogen β gene; FGG = fibrinogen γ gene; γ = gamma prime; G = guanine; MT = mutant; T = thymine; WT = wild-type*

4.8 CROSS-SECTIONAL GENE-ENVIRONMENT INTERACTIONS INFLUENCING TOTAL FIBRINOGEN AND FIBRINOGEN γ' CONCENTRATIONS

In order to determine whether gene–environment interactions influenced total fibrinogen and fibrinogen γ' concentrations cross-sectionally, gene–environment interaction terms were entered into a mixed-model method, including both the 2005 and 2010 data to increase statistical power. For details regarding the statistical methodology, please refer back to Section 3.21, page 50. There were four significant cross-sectional gene–environment interactions that affected total fibrinogen, *i.e*. FGA 2224 G>A with age, FGB 1038 G>A with HbA1c, FGB Arg448Lys with HIV status and FGB 1643 C>T with area of residence. *Table 4.11* presents the significant gene–environment interactions of continuous environmental factors influencing total fibrinogen concentrations cross-sectionally. No gene–environment interactions existed for fibrinogen γ'.

Table 4.11: **Cross-sectional gene–environment interactions for total fibrinogen of continuous environmental factors**

Significant interaction term	Model dimension	Reference	Estimate β (95% CI)		p-value
			Comparison	Value (g/L)	
FGA 2224–Age	All SNPs, all environmental factors and significant interactions	Age effect in WT	Extra age effect in MT homozygotes	0.04 (-0.002;0.07)	0.062
			Extra age effect in heterozygotes	-0.02 (-0.03;-0.001)	0.038
FGB 1038–HbA1c		HbA1c effect in WT	Extra HbA1c effect in MT homozygotes and heterozygotes	0.31 (0.03;0.58)	0.029
FGA 2224–Age	Significant SNPs, significant environmental factors and significant interactions	Age effect in WT	Extra age effect in MT homozygotes	0.04 (-0.003;0.07)	0.052
			Extra age effect in heterozygotes	-0.02 (-0.03;-0.002)	0.026
FGB 1038–HbA1c		HbA1c effect in WT	Extra HbA1c effect in MT homozygotes and heterozygotes	0.32 (0.05;0.58)	0.021

CI = confidence interval; FGA = Fibrinogen α gene; FGB = Fibrinogen β gene; HbA1c = glycated haemoglobin; MT = mutant; SNPs = single nucleotide polymorphisms; WT = wild-type genotype

Total fibrinogen concentrations generally increase with increasing age, although the increase differed across the three genotypes of the FGA 2224 G>A polymorphism. The effect of age on total fibrinogen was 0.02 g/L less per one unit of change in age in the heterozygous allele carriers of FGA 2224 G>A (p=0.026) than in the homozygous wild-type allele carriers (*Figure 4.2*). The effect of age on total fibrinogen is, however, 0.04 g/L more per one unit of change in age in the homozygous mutant allele carriers (p=0.052) than in the homozygous wild-type allele carriers of FGA 2224 G>A (*Figure 4.2*). This effect is also evident when comparing the total fibrinogen concentrations between the three genotypes of FGA 2224 G>A in 2005. The total fibrinogen concentrations of the homozygous wild type genotype were lower than the total fibrinogen concentrations of the homozygous mutant genotype in 2005, thus also indicating a slower increase in total fibrinogen concentrations in the homozygous wild type genotype in the past (*Table 4.10*).

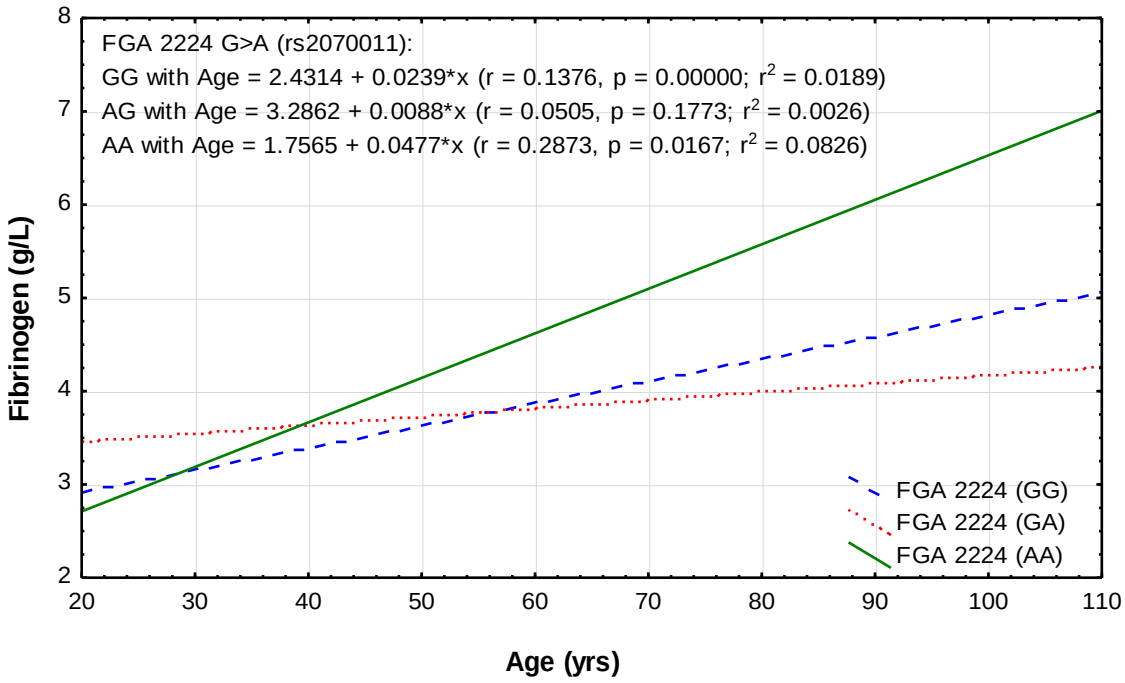

Figure 4.2: **The interaction effect of the FGA 2224 genotypes with age on total fibrinogen**

FGA 2224 (GG): Wild type homozygous
FGA 2224 (GA): Heterozygous
FGA 2224 (AA): Mutant homozygous

The next cross-sectional gene–environment interaction found to influence total fibrinogen concentrations was FGB 1038 G>A with HbA1c. Total fibrinogen concentrations generally increase with an increase in HbA1c although this increase differed statistically between the different alleles of the FGB 1038 G>A genotype. Total fibrinogen concentrations increased by 0.32 g/L more per one unit of change in HbA1c in carriers of the mutant allele, than in those harbouring the wild-type allele (p=0.021). This can be observed in *Figure 4.3*.

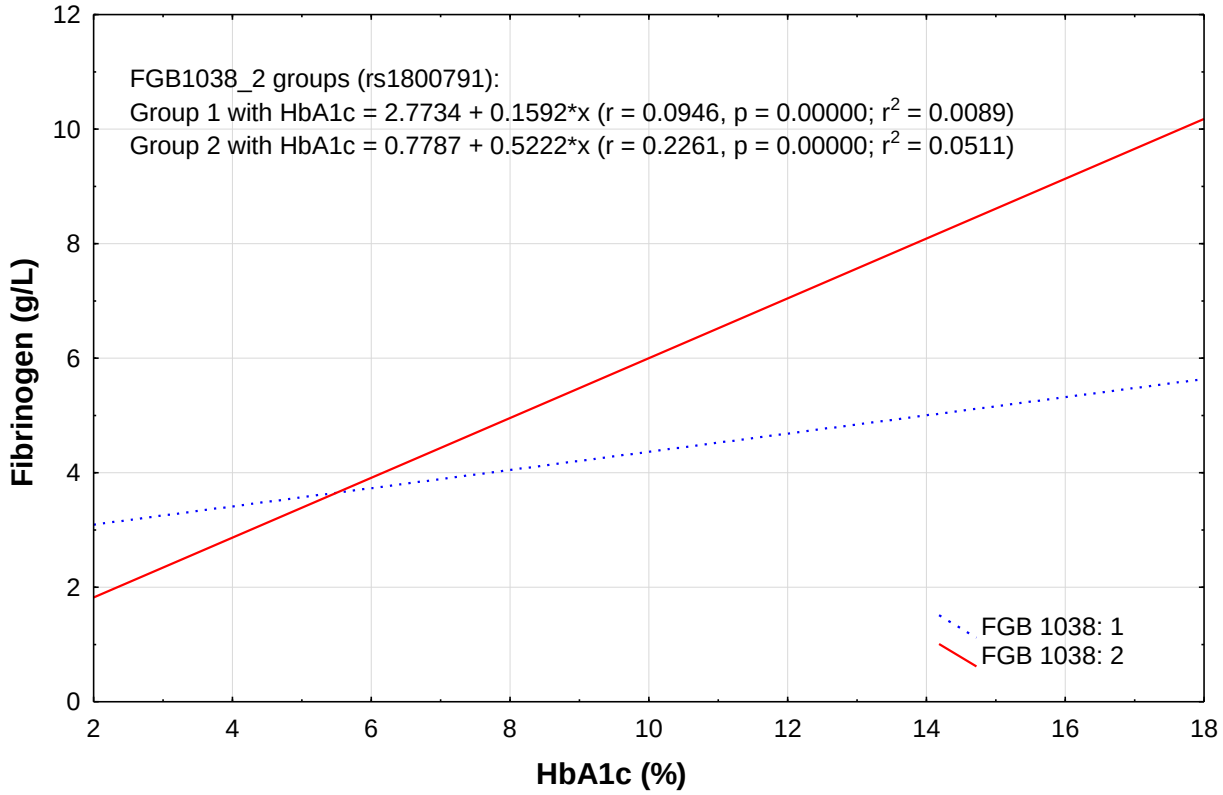

Figure 4.3: **The interaction effect of FGB 1038 genotype with HbA1c on total fibrinogen**

FGB 1038 group 1: Wild type homozygous
FGB 1038 group 2: Mutant homozygous and heterozygous

The significant gene-environment interactions of categorical environmental factors influencing total fibrinogen concentrations cross-sectionally are presented in *Figure 4.4* and *Figure 4.5*. Total fibrinogen concentrations were influenced by an FGB Arg448Lys-HIV status interaction. Total fibrinogen concentrations were significantly lower in HIV-infected compared with HIV-uninfected individuals in the homozygous wild-type allele carriers, while no difference was observed in the carriers of the mutant allele (*Figure 4.4*). Total fibrinogen concentrations of the mutant allele carriers, irrespective of HIV status, were similar to total fibrinogen concentrations of HIV-negative homozygous wild-type allele carriers, while they were higher than concentrations observed in HIV-positive homozygous wild-type allele carriers (*Figure 4.4*).

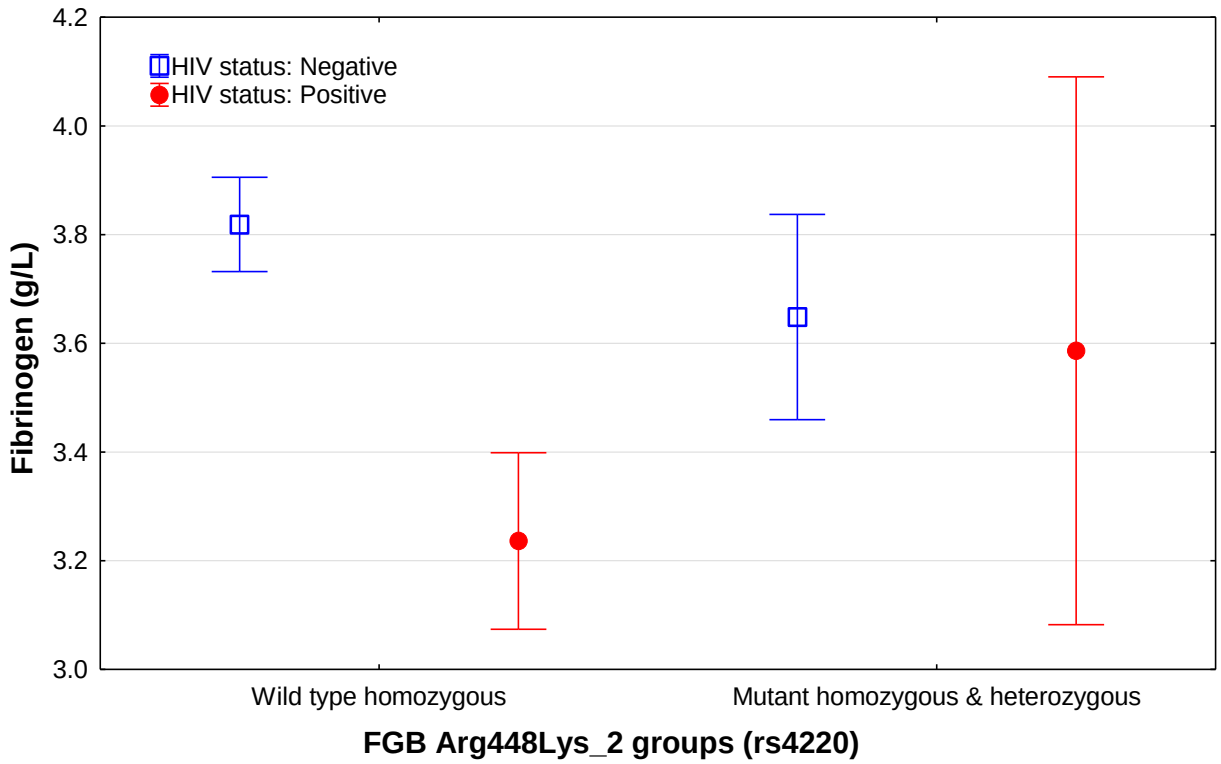

Figure 4.4: **The interaction effect of FGB Arg448Lys genotypes with HIV status on total fibrinogen**

Lastly there was a statistically significant interaction between FGB 1643 C>T and urbanisation in relation to total fibrinogen. In the homozygous wild-type carriers, total fibrinogen concentrations were significantly lower in the urban compared with the rural participants, while no difference was observed for the carriers of the mutant allele (*Figure 4.5*).

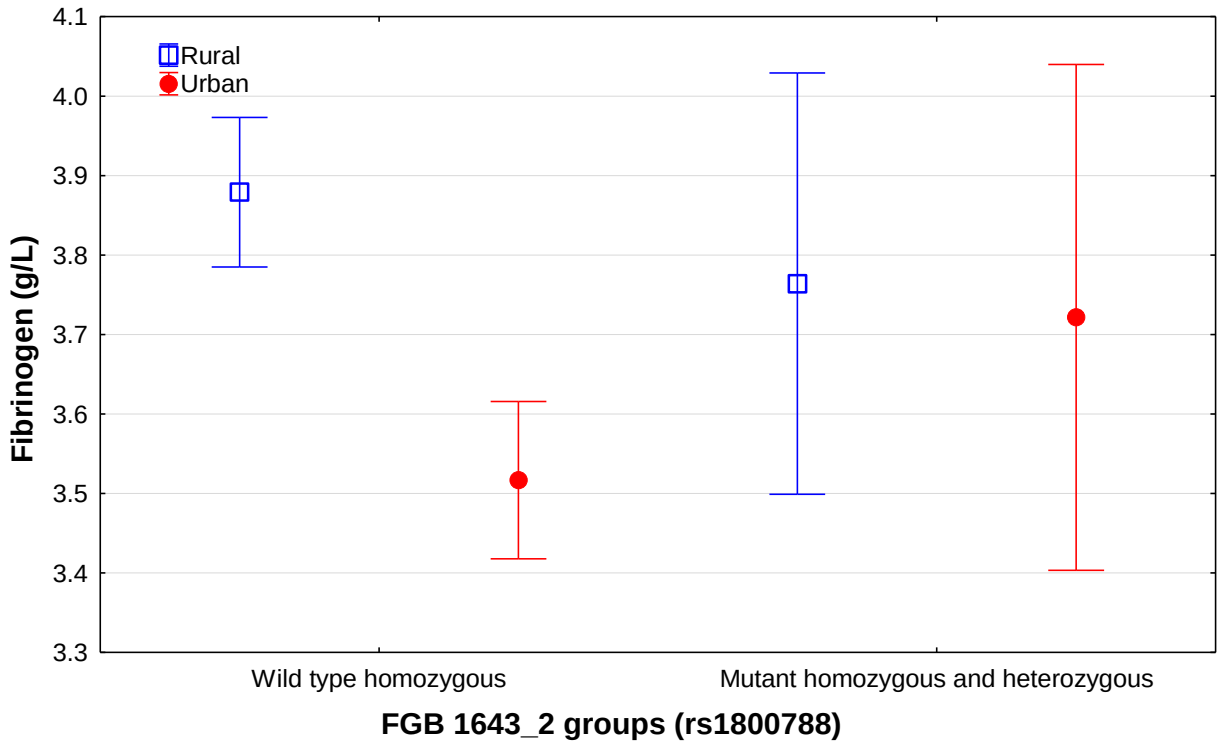

Figure 4.5: **The interaction effect of FGB 1643 genotype with urbanisation on total fibrinogen**

4.9 INFLUENCE OF GENETIC OR ENVIRONMENTAL FACTORS ON CHANGE IN TOTAL FIBRINOGEN AND FIBRINOGEN γ' CONCENTRATIONS OVER THE FIVE YEAR PERIOD

In order to determine whether the analysed genetic polymorphisms or environmental factors influenced the change in total fibrinogen and fibrinogen γ' concentrations over the five-year period, interaction terms between the genotype or environmental factors and time were entered into a mixed-models method. None of the analysed environmental factors significantly influenced the change in fibrinogen γ' concentrations over the five-year period. The only environmental factor influencing change in total fibrinogen over the five-year period was CRP concentration (*Table 4.12*).

Table 4.12: **Environmental factors influencing change in total fibrinogen over time**

Significant interaction term	Model dimension	Reference	Estimate β (95% CI)		p-value
			Comparison	Value (g/L)	
CRP–time	All SNPs, all environmental factors and significant interaction term	Effect of CRP in 2005	Extra effect of CRP in 2010	-0.05 (-0.06;-0.04)	<0.0001
CRP–time	Significant SNPs, significant environmental factors and significant interaction term	Effect of CRP in 2005	Extra effect of CRP in 2010	-0.05 (-0.06;-0.04)	<0.0001

CI = confidence interval; CRP = C-reactive protein; SNPs = single nucleotide polymorphisms

This interaction indicates that if participants have a difference of one unit in CRP, the participant with the higher CRP will have a 0.05 g/L decrease in total fibrinogen over time compared with the individual with the lower CRP. This is illustrated in *Figure 4.6*, in which the study population was divided into quartiles based on CRP concentrations. This phenomenon can clearly be seen in participants falling within the highest CRP quartile (CRP>9.34 mg/L), indicating a decrease in delta fibrinogen (difference between 2005 and 2010 concentrations) as CRP concentrations increase. It is also evident in Figure 4.7 where change in fibrinogen is plotted against CRP concentrations (as continuous variable) in 2005 depicting a negative correlation, which indicates a decrease in fibrinogen concentrations over the five-year period as CRP concentrations increase in 2005.

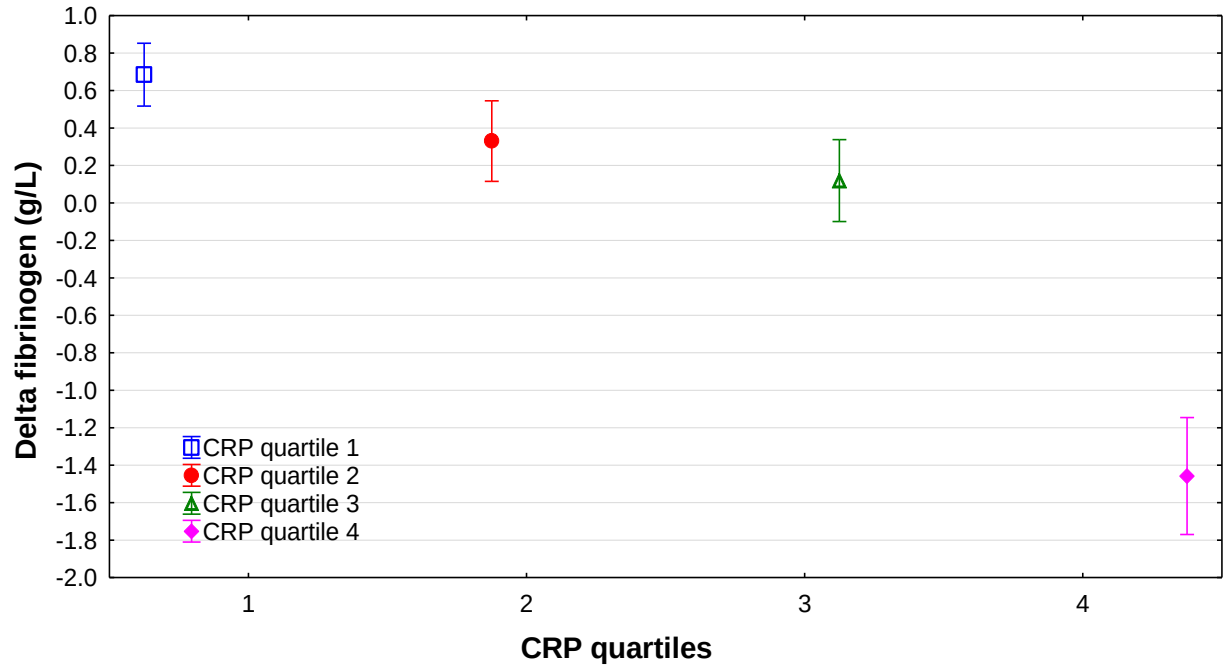

Figure 4.6: **Effect of C-reactive protein on change in total fibrinogen over time**

CRP quartiles calculated from 2005 data
CRP quartile 1: <0.964
CRP quartile 2: >0.964 and <=3.286
CRP quartile 3: >3.286 and <=9.34
CRP quartile 4: >9.34

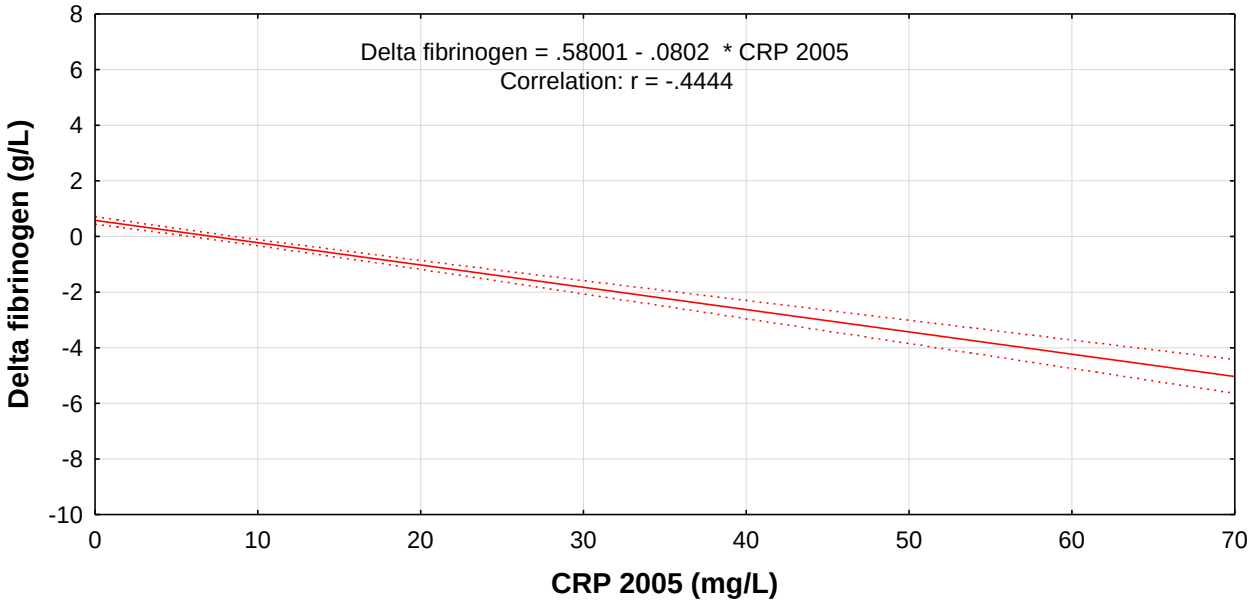

Figure 4.7: **Association between C-reactive protein and change in total fibrinogen over time**

In order to try and explain the reason for the decrease in fibrinogen concentrations over the five-year period as CRP concentrations increase in 2005, change in CRP over the five years was also plotted against CRP concentrations in 2005 (*Figure 4.8*). From this figure it is evident that the higher the CRP concentration in 2005, the greater is the decrease in the CRP concentration over the five year period. This is in agreement with the negative association observed between CRP in 2005 and change in total fibrinogen.

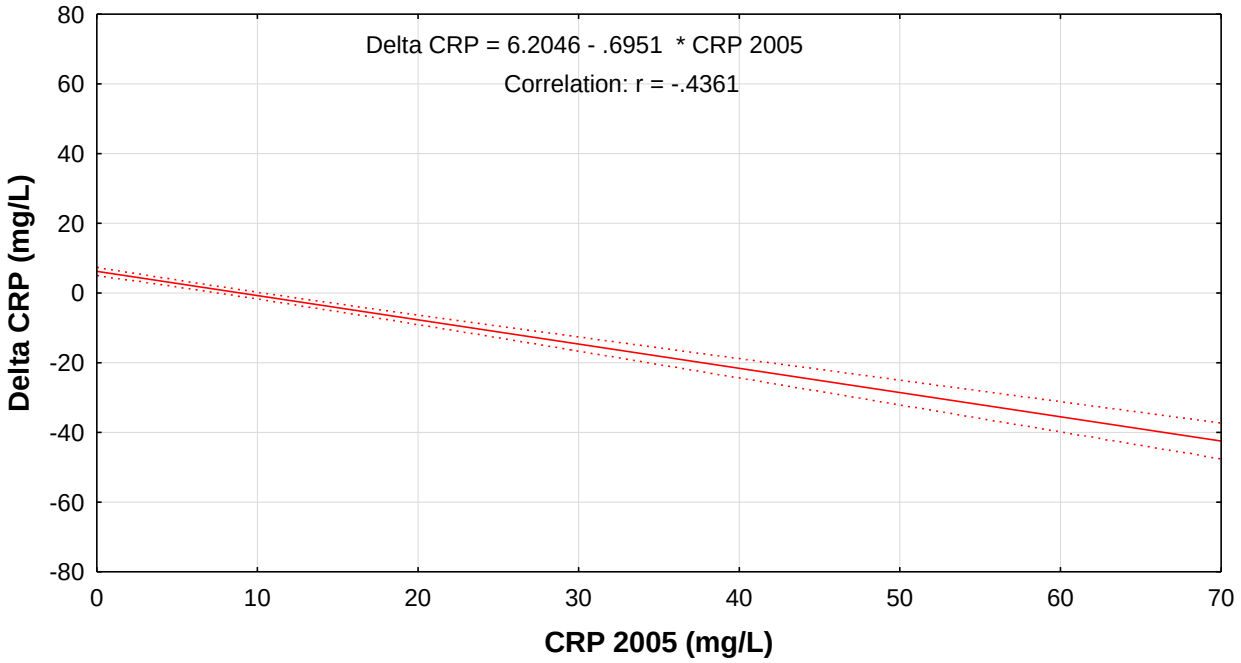

Figure 4.8: **Association between change in CRP over time and CRP in 2005**

While differences existed in the genotypes of several polymorphisms regarding change in total fibrinogen and fibrinogen γ' concentrations over the five-year period when analysed with ANOVA, as discussed in Section 4.7 (*Table 4.10*), none of the genetic polymorphisms seemed to influence the change in total fibrinogen concentrations over the five-year period when entered into the mixed models, which included the environmental factors and the other genotypes analysed. Only one genetic polymorphism influenced change in fibrinogen γ' concentrations over time and that was the FGG 10034 C>T genetic polymorphism (*Table 4.13*).

Table 4.13: **Genetic polymorphisms influencing change in total fibrinogen and fibrinogen γ' over time**

Significant interaction term	Model dimension	Reference	Estimate β (95% CI)		p-value
			Comparison	Value (g/L)	
Total fibrinogen					
None					
Fibrinogen γ'					
FGG 10034–time	Significant SNP, significant environmental factors and significant interaction term	Effect of WT in 2005	Extra effect of MT homozygotes in 2010	-3.72 (-6.85;-0.59)	0.020
			Extra effect of heterozygotes in 2010	-2.09 (-4.31;0.12)	0.063

CI = confidence interval; FGG = Fibrinogen γ gene; MT = mutant; SNPs = single nucleotide polymorphisms; WT = wild-type genotype

This model determined that the change in γ' ratio over time when harbouring the heterozygous genotype was 2.09 g/L less (borderline significance, p=0.063) than when harbouring the homozygous wild-type genotype of FGG 10034 C>T polymorphism. This difference became significant (p=0.020) in individuals carrying the homozygous mutant genotype of FGG 10034 C>T, where the change in γ' ratio was 3.72 g/L less than in the homozygous wild-type genotype carriers. These differences are illustrated in *Figure 4.9*. The results are also in agreement with the results presented in Section 4.7, page 73 in which it can be clearly seen that total fibrinogen increased from 2005 to 2010 in all three genotypes, while fibrinogen γ' increased in the homozygous wild-type carriers, but actually decreased in the heterozygous and homozygous mutant carriers.

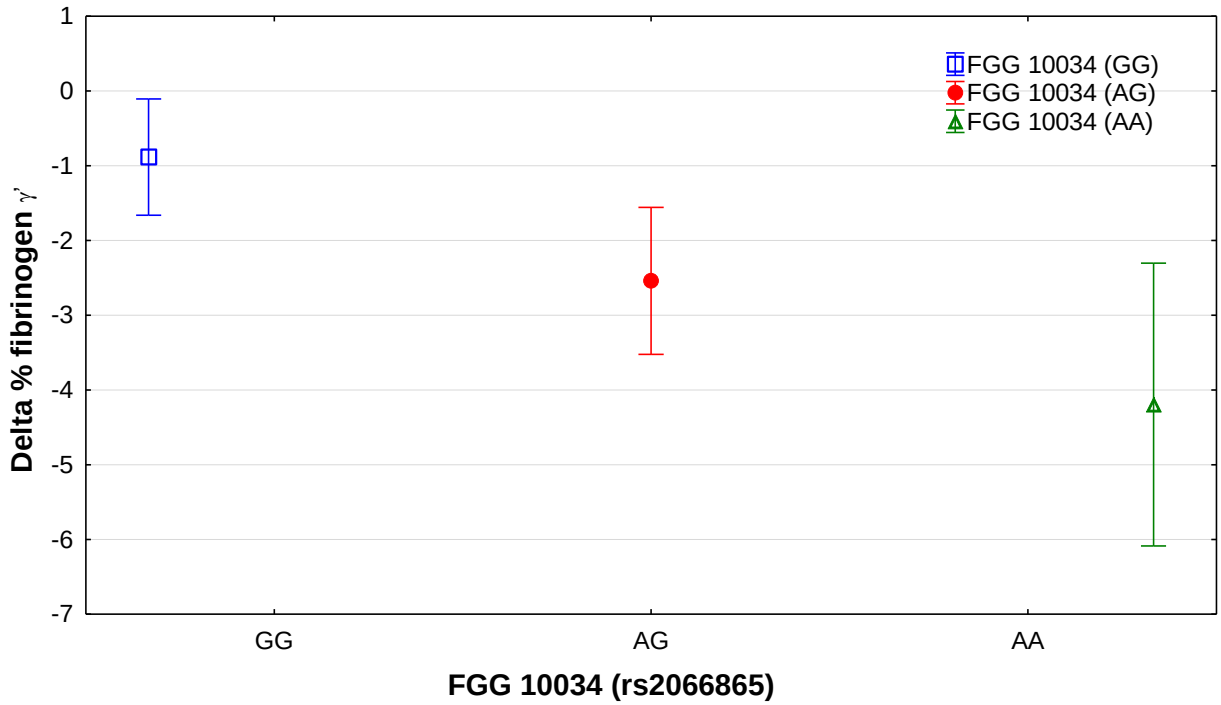

Figure 4.9: **Effect of FGG 10034 on change in γ' ratio over time**

FGG 10034 (GG): Wild-type homozygous
FGG 10034 (AG): Heterozygous
FGG 10034 (AA): Mutant homozygous

4.10 GENOTYPE-ENVIRONMENT INTERACTIONS INFLUENCING CHANGE IN TOTAL FIBRINOGEN AND FIBRINOGEN γ' CONCENTRATIONS OVER THE FIVE YEAR PERIOD

Mixed models were again used to create gene–environment–time interactions to determine whether there were any gene–environment interactions that influenced the change in total fibrinogen or fibrinogen γ' concentrations over the five-year period. There was only one significant gene–environment interaction that affected total fibrinogen concentration over the five-year period, *i.e*. the FGG 9340 with HbA1c and time (*Table 4.14*).

Table 4.14: **Gene–environment interactions that affected total fibrinogen concentration over time**

Significant interaction term	Model dimension	Reference	Estimate β (95% CI)		p-value
			Comparison	Value (g/L)	
FGG 9340–HbA1c–time	All SNPs, all environmental factors and significant interaction terms	Time HbA1c interaction in the WT	Extra Time HbA1c interaction in the MT homozygotes	0.83 (0.21;1.44)	0.008
			Extra Time HbA1c interaction in the heterozygotes	0.003 (-0.27;0.27)	0.984

CI = confidence interval; FGG = Fibrinogen γ gene; HbA1c = glycated haemoglobin; MT = mutant; WT = wild-type genotype

In general there is a negative association between change in total fibrinogen over time and HbA1c in 2005. It seems as if the higher the HbA1c in 2005 the greater the decrease in total fibrinogen over time. However, this decrease differs between the genotypes of FGG 9340 T>C as there is a stronger negative association between change in total fibrinogen and HbA1c in the homozygous mutant than in the homozygous wild-type genotype. Although not significantly so (p=0.984), the reduction in total fibrinogen was 0.003g/L more with one unit change in HbA1c in the heterozygous genotype carriers compared to the wild-type carriers. In the homozygous mutant genotype carriers the reduction in total fibrinogen was 0.83 g/L more with one unit of change in HbA1c compared to the wild-type carriers. This difference was statistically significant (p=0.008) and is illustrated in *Figure 4.10*.

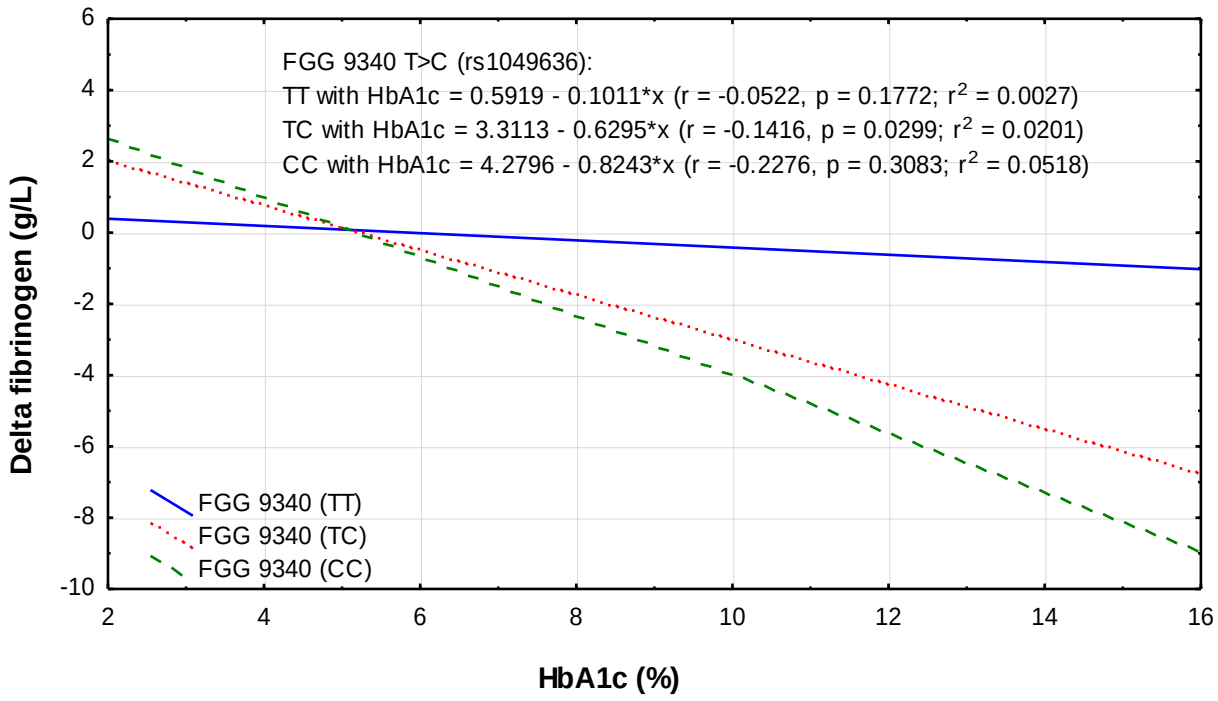

Figure 4.10: **The interaction effect of FGG 9340 with HbA1c on total fibrinogen over time**

FGG 9340 (TT): Wild type homozygous
FGG 9340 (TC): Heterozygous
FGG 9340 (CC): Mutant homozygous

In order to try and explain this observation, change in HbA1c over the five years were also plotted against HbA1c concentrations in 2005 (*Figure 4.11*).

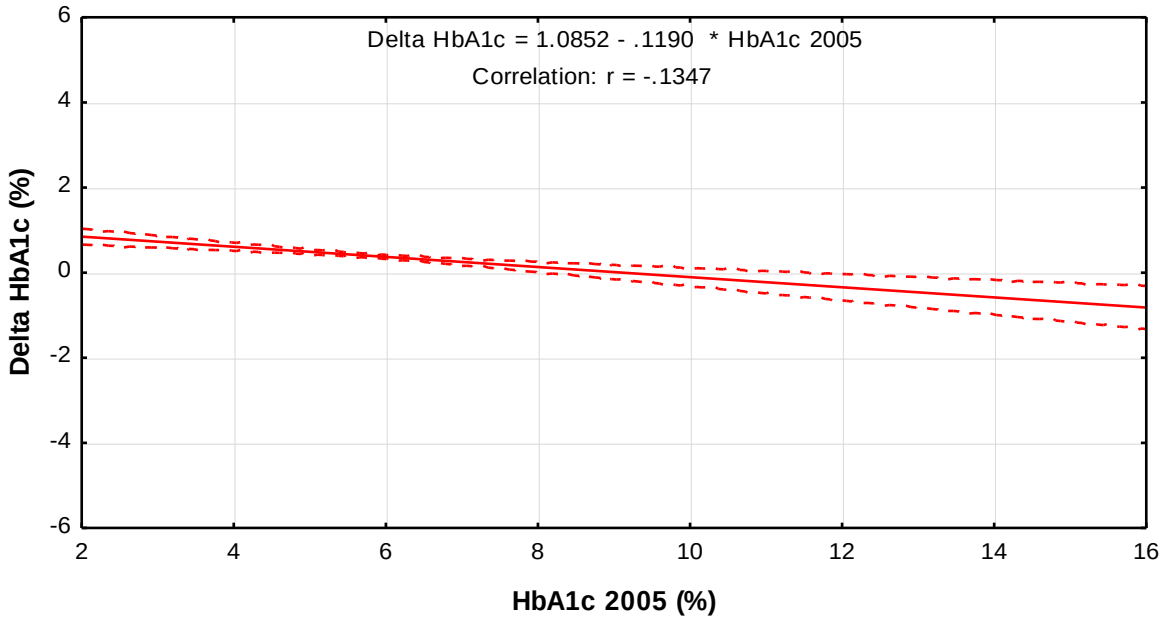

Figure 4.11: **Association between change in HbA1c over time and HbA1c in 2005**

From *Figure 4.11* it is evident that the higher the HbA1c concentration in 2005, the greater is the decrease in the HbA1c concentration over the five-year period. This is in agreement with the negative association observed between HbA1c in 2005 and change in total fibrinogen.

There were no significant gene–environment interactions influencing change in fibrinogen γ' concentrations over the five-year period.

The results reported in this chapter are interpreted and discussed in Chapter 5, where they are placed in the context of the literature available in this field.