

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 INTRODUCTION

The main focus of this study was to determine the change in total fibrinogen and fibrinogen γ' concentrations over a five-year period in a black South African population in transition as well as the extent to which these changes were influenced by genetic factors, environmental factors and/or possible interaction between these factors. In order to determine these gene-environment interactions and understand their effect on the change in total fibrinogen and fibrinogen γ' over time, the cross-sectional and prospective effects of the environmental factors and the genetic factors respectively on the fibrinogen variables were first explored. This chapter, therefore, describes how urbanisation and gender influenced total fibrinogen and a splice variation of the γ chain, fibrinogen γ' , on a cross-sectional level and how it affected the change in these variables over time. This is followed by a discussion of the cross-sectional association of environmental and genetic factors with the fibrinogen variables as well as with the change in concentrations over the five-year study period. The minor allele frequencies of the analysed SNP are also compared with those reported in the literature in order to determine how the distributions in black South Africans compare with what is reported for other ethnicities. Lastly, the possible effect of gene-environment interactions on fibrinogen variables is discussed on both a cross-sectional as well as a prospective level.

5.2 INFLUENCE OF URBANISATION AND GENDER ON TOTAL FIBRINOGEN AND FIBRINOGEN γ'

The following section discusses how urbanisation and gender influenced the fibrinogen variables on a cross-sectional level as well as how they influenced the change in the fibrinogen variables over a five-year period.

5.2.1 Influence of urbanisation on total fibrinogen and fibrinogen γ'

The urban and the rural setting can be considered to represent two distinct clusters of a specific grouping of environmental factors. Comparing the fibrinogen variables of the rural and urban settings, therefore, reflects the effect of a specific cluster/combination of environmental factors on the fibrinogen variables, and, with follow-up analysis, the respective roles of the individual environmental factors can be investigated. Total fibrinogen concentrations in both 2005 and 2010 were significantly higher in the rural than in the urban group. In the THUSA study, however, higher total fibrinogen concentrations were observed in the urban group (James *et al.*, 2000; Pieters & Vorster, 2008). The purpose of the THUSA study was to ascertain the changes in health determinants during urbanisation of Africans in the North West region of South Africa and then formulate preventive strategies, policies and programmes (Vorster *et al.*, 2005). The THUSA study is comparable with the PURE study as the participants of both studies were of the same ethnicity (black South Africans) and from the North West region of South Africa, although the THUSA study was conducted ten years earlier than the PURE study (James *et al.*, 2000). Being an acute-phase protein, fibrinogen increases under conditions of stress and inflammation (Shirom *et al.*, 2010; Steptoe *et al.*, 2003; Toker *et al.*, 2005; Von Känel *et al.*, 2001). This difference in total fibrinogen concentrations between the THUSA and PURE study was partly attributed to psychosocial stress levels that were indicated to be higher in rural than urban participants in the PURE study, and which are known to cause an increase in fibrinogen concentrations (Friedlander *et al.*, 1995; Kakafika *et al.*, 2007; Kamath & Lip, 2003; Steptoe *et al.*, 2003; Von Känel *et al.*, 2001). The reason for the higher total fibrinogen in the rural group could be due to higher levels of inflammation caused by poor access to electricity and running water and higher levels of unemployment and poverty, which was evidenced by a borderline significantly higher CRP in the rural group than in the urban group (Pieters *et al.*, 2011). Total fibrinogen concentrations also increased significantly in both the rural and urban participants from 2005 to 2010. These increases in the rural and urban groups, however, did not differ significantly from each other. In determining what caused the increase in total fibrinogen from 2005 to 2010 in the rural and urban groups, changes in environmental factors over time within the rural and urban settings were considered as possible explanations.

After adjustment for the environmental factors that differed over time and which correlated with total fibrinogen (BMI, SBP, fasting glucose, HbA1c, CRP and blood lipids), the increase in total fibrinogen from 2005 to 2010 in the rural and urban groups remained significant. Thus the increase in total fibrinogen in both the rural and urban settings could be only partially explained by changes in environmental factors (individually or in combination) known to influence fibrinogen. These results agree with those of Pieters *et al.* (2011), who concluded that the variability in total fibrinogen in black South Africans is largely unexplained. The Fibrinogen Studies Collaboration also found that up to 70% of the explained variance in fibrinogen concentrations was attributable to non-modifiable factors whereas only 30% was explained by modifiable environmental factors. The influence of genetic factors, as non-modifiable factors, will be discussed in the following sections.

Although total fibrinogen was higher in the rural than the urban group in both 2005 and 2010, fibrinogen γ' was higher in the rural group in 2010 only, but lower when compared with the urban group in 2005. Similarly, while total fibrinogen increased in both the rural and urban groups from 2005 to 2010, fibrinogen γ' increased significantly only in the rural group. This resulted in a significant reduction in the γ' ratio in the urban group over time. It seems, therefore, that despite the increase in total fibrinogen over time in both areas, fibrinogen γ' concentrations remained unchanged in the urban area, while it increased proportionally with total fibrinogen in the rural area. Although fibrinogen γ' is a constituent of total fibrinogen, variation in fibrinogen γ' is not merely a reflection of changes in total fibrinogen concentrations, but is also a result of independent control mechanisms (Lovely *et al.*, 2011; Alexander *et al.*, 2011). The three environmental factors – CRP, IL-6 and BMI – were the same environmental factors that correlated best with both total fibrinogen and the γ' transcript, suggesting at least partial agreement in control. Pieters *et al.* (2013), however, demonstrated that the association of fibrinogen γ' with several known cardiovascular disease risk factors (BMI, waist circumference, CRP, HbA1c, metabolic syndrome and HDL-cholesterol) remained after adjustment for total fibrinogen and that these associations are probably independent of associations with total fibrinogen.

According to Pieters *et al.* (2013), CRP is the cardiovascular disease risk factor that explained the largest proportion of fibrinogen γ' variance in the PURE population, apart from total fibrinogen. This relationship is also reflected in our correlation data, which indicated that CRP was the environmental factor with the strongest correlation with fibrinogen γ' . Our results also indicate that, as with fibrinogen γ' , CRP increased from 2005 to 2010 in the rural group only, with no change observed in the urban group. This lack of increase in fibrinogen γ' in the urban group is, therefore, explained, at least in part, by its association with CRP, which also did not increase. Evidence from the literature in support of this hypothesis comes from the study of Cheung *et al.* (2008), who suggested that messenger ribonucleic acid (mRNA) processing of the γ' chain may be altered during the acute-phase reaction. It should be mentioned, however, that even after adjustment for CRP (and other environmental factors that differed over time, including total fibrinogen), fibrinogen γ' concentrations were still significantly elevated in the rural group, suggesting that the relationship with CRP and total fibrinogen does not fully explain the fibrinogen γ' results. Future research is needed to identify additional factors that influence fibrinogen γ' concentrations.

From these results it seems clear that environmental factors and combinations thereof, such as specific combinations present in a rural or an urban environment, can significantly influence plasma protein concentrations such as fibrinogen γ' , although the exact mechanistic pathways still need to be elucidated.

5.2.2 Influence of gender on total fibrinogen and fibrinogen γ'

Total fibrinogen concentrations in 2005 and 2010 were significantly higher in women than in men. Other studies have also indicated that women have higher total fibrinogen concentrations than men, irrespective of pregnancy, contraceptive use or urbanisation status (James *et al.*, 2000; Kakafika *et al.*, 2007; Kamath & Lip, 2003; Kannel *et al.*, 1987; Pearson *et al.*, 1997; Pieters & Vorster, 2008).

Total fibrinogen concentrations also increased significantly in both men and women from 2005 to 2010. Higher total fibrinogen in women could be due to the significantly higher BMI, waist circumference, CRP, total cholesterol and LDL-cholesterol and significantly lower HDL-cholesterol in women than men (Swanepoel, 2013). These factors have all been shown to be associated with fibrinogen concentrations (James *et al.*, 2000; Kamath & Lip, 2003). The difference in total fibrinogen concentrations between men and women may also be due to the effect of gender hormones. In the study of Canonico *et al.* (2012), endogenous oestradiol, which is a female sex hormone, was positively associated with total fibrinogen concentrations, whereas testosterone did not show any significant association with total fibrinogen in postmenopausal women not using HRT or anticoagulant therapy. This study indicated, furthermore, that the association between total fibrinogen and oestradiol was even higher in overweight than in lean women (Canonico *et al.*, 2012). Physiological models established that, during periods of hyperoestrogeny like pregnancy and ovarian stimulation, an increase of oestradiol causes a rise in procoagulant factors such as fibrinogen (Canonico *et al.*, 2012). It has been determined that oestradiol is positively associated with CRP in postmenopausal women, indicating that oestradiol can induce a pro-inflammatory state which will very likely also lead to an increase in total fibrinogen (Canonico *et al.*, 2012). The study of Haring *et al.* (2012) reported an inverse association between testosterone in men and total fibrinogen, indicating that testosterone may cause a decrease in fibrinogen concentrations. The precise mechanism between testosterone and fibrinogen is not yet known, but speculations are that exogenous testosterone might change fibrinogen synthesis while metabolised in the liver or it might lessen the inflammatory response in atherosclerotic plaques by possible immune-modulating properties, leading to decreased fibrinogen (Haring *et al.*, 2012).

Fibrinogen γ' in 2005 and 2010 was also significantly higher in women than in men, but it increased significantly from 2005 to 2010 in men only. Other studies also indicated that women had higher fibrinogen γ' than men (Alexander *et al.*, 2011; Lovely *et al.*, 2010; Mannila *et al.*, 2007a). There were other studies, however, that indicated that there was no significant association between gender and fibrinogen γ' (Lovely *et al.*, 2002; Mosesson *et al.*, 2007).

The change in fibrinogen γ' over the five-year period differed significantly between men and women, with a greater increase in men. This difference is probably a reflection of a similar difference observed in total fibrinogen concentration, which also showed a greater increase in men than in women. In support of this hypothesis, there was no significant difference in the γ' ratio over the five-year period in either men or women, suggesting that the rate of increase of fibrinogen γ' and total fibrinogen is similar.

5.3 EFFECT OF ENVIRONMENTAL FACTORS ON FIBRINOGEN VARIABLES

5.3.1 Effect of environmental factors on fibrinogen variables cross-sectionally

This section will focus on the top five environmental factors with which total fibrinogen and fibrinogen γ' had the strongest associations, as many of the other factors showed only weak correlations. It will also include a discussion on the associations with categorical factors such as tobacco use, HIV status and contraceptive use. The environmental factors with the strongest correlations with total fibrinogen in 2005 were CRP, IL-6, BMI, HbA1c and age. In 2010 the environmental factors were CRP, IL-6, BMI, LDL-cholesterol and HbA1c. The environmental factors with the strongest correlations with fibrinogen γ' in 2005 were CRP, BMI, HDL-cholesterol, IL-6 and HbA1c, whereas those with the strongest correlations in 2010 were CRP, LDL-cholesterol, IL-6, BMI and age. Thus the environmental factors having significant correlations with both total fibrinogen and fibrinogen γ' in 2005 and 2010 were CRP, IL-6 and BMI. If the same correlation effects were not found in both total fibrinogen and fibrinogen γ' it would influence the γ' ratio. Fibrinogen γ' , for instance, had a stronger negative association with HDL-cholesterol than did total fibrinogen, resulting in a strong correlation between γ' ratio and HDL-cholesterol in 2005. Total fibrinogen, on the other hand, had a stronger positive association with age than did fibrinogen γ' , resulting in a strong correlation between γ' ratio and age in 2005. In 2010 total fibrinogen also had a stronger positive association with HbA1c than did fibrinogen γ' , resulting in a strong correlation between γ' ratio and HbA1c. The next two sections will discuss the associations of these environmental factors with total fibrinogen and fibrinogen γ' , respectively.

5.3.1.1 Fibrinogen

CRP and IL-6 both had positive correlations with total fibrinogen in 2005 and 2010. The association between fibrinogen and CRP is well known (Black, 2003; Kritchevsky *et al.*, 2005; Redman & Xia, 2000). Fibrinogen and CRP are both positive acute-phase proteins which become elevated during the inflammatory process in response to any type of tissue damage and/or infection (Black, 2003; Horan *et al.*, 2001; Kamath & Lip, 2003; Kritchevsky *et al.*, 2005; Rudež *et al.*, 2009; Toker *et al.*, 2005). The association between IL-6 and fibrinogen is also well known (Black, 2003; Redman & Xia, 2000). IL-6 is a major mediator of the acute-phase response, which is expressed after the production of tumour necrosis factor and interleukin-1 β , the first pro-inflammatory cytokines produced in response to infection and/or tissue damage (Black, 2003; Redman & Xia, 2000). CRP and fibrinogen have IL-6 response elements in the promoter area of their genes and IL-6 is, therefore, considered to be one of the cytokines responsible for the hepatic production of fibrinogen and CRP during the acute-phase response of inflammation (Black, 2003; Horan *et al.*, 2001).

BMI was positively correlated with total fibrinogen in both 2005 and 2010. It has been proved that increasing BMI correlates positively with an increase in fibrinogen (Danesh *et al.*, 2005; Friedlander *et al.*, 1995; Kamath & Lip, 2003). An association exists between increased BMI and inflammatory markers such as IL-6, CRP and fibrinogen (Fransson *et al.*, 2010; Nguyen *et al.*, 2009). Several pro-inflammatory proteins such as fibrinogen are expressed to a greater extent in obese persons than in lean persons (Fransson *et al.*, 2010; Nguyen *et al.*, 2009). IL-6, which is to a large extent responsible for the synthesis of fibrinogen is produced from adipose tissue, which is increased in obese persons (Bo *et al.*, 2004). In the THUSA study there was a significant positive correlation between BMI and total fibrinogen concentrations only in the women (James *et al.*, 2000). The difference in results between the THUSA and PURE study is most likely due to the fact that fewer men were observed to be overweight in the THUSA study than in the PURE study, and as a result the BMI ranges in the men were not wide enough for a good correlation to be detected.

HbA1c also had a positive correlation with total fibrinogen in both 2005 and 2010. Many studies have indicated that diabetic patients have higher fibrinogen concentrations than non-diabetic persons; however, there are also studies that found an absence of effect between diabetes and fibrinogen (Danesh *et al.*, 2005; Dunn & Ariëns, 2004; Lam *et al.*, 2000). In the study of Klein *et al.* (2003), mean total fibrinogen concentrations were lowest in participants in the lower quartile (<7.3 %) of HbA1c and highest in participants in the highest quartile (≥ 9.0 %) of HbA1c. The review of Dunn and Ariëns (2004) has also indicated that total fibrinogen is positively associated with HbA1c. It has been found that improved glycaemic control leads to lower fibrinogen concentrations (Ceriello *et al.*, 1998; D'Elia *et al.*, 2001; Lam *et al.*, 2000). However, not all studies agreed on this result, as some found no effect between glycaemic control and fibrinogen concentrations (Becker *et al.*, 2003; Emanuele *et al.*, 1998; Johansson *et al.*, 2003). The association between diabetes and total fibrinogen was determined to be possibly related to inflammation (Dunn & Ariëns, 2004; Rogowski *et al.*, 2008). Increased concentrations of HbA1c have been found to increase synthesis of cytokines such as IL-6, thus stimulating the production of acute-phase proteins such as fibrinogen (Dunn & Ariëns, 2004; Rogowski *et al.*, 2008). Diabetes mellitus, which is a chronic inflammatory state, can cause endothelial injury through increased oxidative stress and it has been determined that fibrinogen concentrations increase in the presence of endothelial injury (Desai *et al.*, 2012). Fibrinogen can, therefore, respond as an inflammatory and prothrombotic marker in the case of diabetes (Desai *et al.*, 2012). Discussion on the contribution of genetic factors in section 5.5.1 may help to explain the inconsistency mentioned above, as it seems that genetic factors influence the association between total fibrinogen and HbA1c.

Age had a positive correlation with total fibrinogen in 2005. Other studies have also indicated that total fibrinogen concentrations have a strong positive association with age (Danesh *et al.*, 2005; Friedlander *et al.*, 1995; Kakafika *et al.*, 2007; Kamath & Lip, 2003; Pearson *et al.*, 1997). In the study of Kannel *et al.* (1987) there was an increase of about 1.0 g/L in total fibrinogen concentration with every decade of increase in age. The THUSA study also determined that age had a significant influence on total fibrinogen concentrations, but it is important to note that this relationship was not linear as the oldest age group (older than 65 years) had lower fibrinogen concentrations than participants aged between 35 and 64.5 years (James *et al.*, 2000).

The study of Fu and Nair (1998) determined that the synthesis rate of fibrinogen is higher in young individuals than in older individuals, even though the total fibrinogen concentrations increase progressively with age. This indicates that the increase in fibrinogen with increase in age is not due to a higher synthesis of fibrinogen in older individuals, but may be due to the lower disposal rate of total fibrinogen and that total fibrinogen may have a longer half-life in older individuals (Fu & Nair, 1998). It seems that the association between age and total fibrinogen in Caucasians is stronger than the association in Africans. The reason for this difference in association between age and total fibrinogen between different ethnic groups is unknown, hence the importance of this study.

LDL-cholesterol had a positive correlation with total fibrinogen in 2010. This was also the trend observed in other studies, which found an increase in the total fibrinogen concentrations as total cholesterol and LDL-cholesterol increased (Bo *et al.*, 2004; Danesh *et al.*, 2005; James *et al.*, 2000; Pearson *et al.*, 1997). The study of Kannel *et al.* (1987) contradicted this, finding no significant association between total fibrinogen concentrations and total cholesterol. HDL-cholesterol had a weak negative correlation with total fibrinogen concentration in our study. This is in agreement with other studies that indicated that total fibrinogen concentration decreases as HDL-cholesterol increases (Danesh *et al.*, 2005; Pearson *et al.*, 1997; Stec *et al.*, 2000). Triglycerides did not have any significant correlation with total fibrinogen concentration in our study. This is in contrast with other studies which found that triglycerides had a positive association with total fibrinogen concentrations (Danesh *et al.*, 2005; Stec *et al.*, 2000). The mechanism behind the blood lipids and fibrinogen association is possibly inflammatory related. It has been determined that CRP, which is an inflammatory marker, increases as LDL-cholesterol increases (Kannel, 2005). This phenomenon was also observed between fibrinogen and LDL-cholesterol as well as between fibrinogen and total cholesterol (Kannel, 2005; Koenig, 2003).

Another mechanism that possibly explains the relationship between fibrinogen and blood lipids is that fibrinogen acts as an adsorptive surface for LDL-cholesterol and is also involved in foam cell formation by assisting the relocation of cholesterol from platelets to monocytes or macrophages, thus contributing to plaque formation (Grebe *et al.*, 2010; Koenig, 2003; Navab *et al.*, 2011). HDL-cholesterol, on the other hand, regulates cholesterol efflux from tissues to the liver (Navab *et al.*, 2011). HDL-cholesterol can also remove cholesterol from macrophages, thus being protective against atherosclerosis (Navab *et al.*, 2011). It also has anti-oxidant properties making it anti-inflammatory, causing a decrease in the inflammatory response, which could possibly lead to a decrease in fibrinogen production (Navab *et al.*, 2011). Another link between fibrinogen and cholesterol can be explained by HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase), an enzyme that plays a key role in cholesterol synthesis *via* the mevalonate pathway (Redman & Xia, 2001). The mRNA concentrations of HMG-CoA reductase indicate the level of cholesterol biosynthesis (Redman & Xia, 2001). It has been determined that human liver cells which had overexpression of fibrinogen had higher HMG-CoA reductase mRNA concentrations, thus also synthesising more cholesterol (Redman & Xia, 2001). Apolipoprotein B (ApoB), which is the main structural protein of LDL-cholesterol and very low-density lipoprotein (VLDL) cholesterol, is also synthesised by liver cells that overexpress fibrinogen (Redman & Xia, 2001). Thus overexpression of fibrinogen is associated with increased ApoB secretion, resulting in higher LDL and VLDL-cholesterol, the main carriers of cholesterol and triglycerides (Redman & Xia, 2001). Triglycerides and LDL-cholesterol are also contributing factors to blood viscosity, as is the case with fibrinogen. Thus an increase in blood viscosity may reflect not only high fibrinogen concentrations, but also high triglyceride and LDL-cholesterol concentrations (Rosenson *et al.*, 2002). HDL-cholesterol has been found, moreover, to have an inverse association with blood viscosity (Stamos & Rosenson, 1999). It has also been established that lipid concentrations influence fibrinogen concentrations by lowering the clearance rate of fibrinogen rather than by simply affecting the production rate of fibrinogen (Verschuur *et al.*, 2001).

Total fibrinogen was significantly lower in the HIV-positive group than in the HIV-negative group in both 2005 and 2010. In the THUSA study, the same trend was observed, although this difference was not significant (James *et al.*, 2000). The study of Jahoor *et al.* (1999) contradicted this, as even the symptom-free HIV-positive group had significantly higher fibrinogen and CRP concentrations than the HIV-negative group, indicating an increase in acute-phase proteins during asymptomatic HIV. This means that infection with HIV, even in the absence of secondary infections, activates the acute-phase protein response, although to a smaller extent than when accompanied by secondary infections (Jahoor *et al.*, 1999). The study of Madden *et al.* (2008) also ascertained that HIV-positive men had higher total fibrinogen concentrations than men who were HIV negative, whereas the difference in fibrinogen concentrations between HIV-positive and HIV-negative women was not significant. It is interesting to note that not all anti-retroviral (ARV) treatments have the same effect on fibrinogen. In the study of Madden *et al.* (2008), different ARV treatments were given to the HIV-positive participants *i.e.* protease inhibitors (PIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs) and a combination of PIs and NNRTIs. The PIs were associated with increased fibrinogen concentrations (Madden *et al.*, 2008). Contrary to the effect of PIs on fibrinogen concentrations, the NNRTIs were associated with decreased fibrinogen concentrations (Madden *et al.*, 2008). When a combination of PIs and NNRTIs was given to the HIV-positive participants, no significant differences were found between the fibrinogen concentrations of the HIV-positive subjects and controls (Madden *et al.*, 2008). In the study of Martin *et al.* (2010), HIV-positive participants already on ARV treatment such as PIs and NNRTIs were also given nucleoside reverse transcriptase inhibitors (NRTIs). When the NRTIs were added to the current ARV treatment, a decrease in fibrinogen concentrations was observed (Martin *et al.*, 2010). Thus it seems that the ARV treatment affects total fibrinogen concentrations, as the administration of PIs was associated with increased fibrinogen concentrations (Madden *et al.*, 2008), whereas the administration of both NRTIs and NNRTIs was associated with decreased fibrinogen concentrations (Madden *et al.*, 2008; Martin *et al.*, 2010).

In the study of Baker *et al.* (2010) there was no statistically significant difference in fibrinogen concentrations between the HIV-positive and HIV-negative groups. This difference in results was ascribed to the difference in ARV therapy, since, in the study of Baker *et al.* (2010), the subjects received no ARV treatment, while in the studies mentioned above, ARV treatment was given. It was suggested that increased fibrinogen concentrations in HIV-positive subjects should be ascribed rather to the ARV treatment than to the HIV infection itself (Baker *et al.*, 2010). This could be the reason for the lower total fibrinogen concentrations in the HIV-positive group than in the HIV-negative group of the PURE population, as the HIV-positive individuals in the PURE study, being newly diagnosed, were not yet using ARVs, at least in 2005 (Fourie *et al.*, 2011). The newly diagnosed HIV-positive participants in 2005 were referred to nearby clinics where ARV treatment was given only if participants qualified according to the eligibility criteria for starting ARV treatment (Department of Health, 2010). The ARV treatment administered was a combination of two NRTIs, Stavudine and Lamivudine, as well as one NNRTI, Efavirenz or Nevirapine. In 2010, persons already diagnosed with HIV in 2005 would have been given ARV treatment, while those newly diagnosed in 2010 would not have been given this treatment, complicating the interpretation of the results. Therefore, the fact that some individuals were using NRTIs and NNRTIs in 2010 may partly explain the lower total fibrinogen concentrations observed in 2010. Another factor that needs to be considered is that the studies mentioned above were conducted in non-African populations; it is known that there are different subtypes of HIV in different countries (Gaschen *et al.*, 2002; Peeters, 2001). In South and East African countries, which include countries such as South Africa, the predominant HIV subtype is HIV-1 subtype C, whereas in North America, Europe and Australia the predominant HIV subtype is HIV-1 subtype B (Gaschen *et al.*, 2002; Peeters, 2001). There is a difference of approximately 30% in the genomes of HIV-1 subtype B and C (Freire, 2006). The study of Jahoor *et al.* (1999) was conducted among Europeans, the study of Martin *et al.* (2010) among Australians and the studies of Madden *et al.* (2008) and Baker *et al.* (2010) were both conducted among Americans; the HIV subtype investigated differed from that in our study and this could, therefore, also contribute to the difference in results.

Increased CD4 cell count is another factor that is associated with decreased total fibrinogen concentrations (Madden *et al.*, 2008). In the PURE population, however, there was no significant correlation between CD4 cell count and total fibrinogen. The lower total fibrinogen concentrations in the HIV-positive group are therefore probably not due to the CD4 cell counts, but rather due to the ARV treatment or difference in HIV subtype. A discussion on the contribution of genetic factors in section 5.5.1 may also help to explain this inconsistency, as it seems that genetic factors also influence the association between total fibrinogen and HIV status.

In 2010 the participants who had never used tobacco had a significantly higher total fibrinogen concentration than participants who were currently using tobacco. There was, however, no significant difference observed in 2005 between the different groups of tobacco users. This is in agreement with a study of Friedlander *et al.* (1995), which also found no significant difference in total fibrinogen concentrations between smokers and non-smokers. Other studies, however, contradicted these results, finding that the subjects who smoked had higher total fibrinogen concentrations than the non-smokers (Bladbjerg *et al.*, 2006; Danesh *et al.*, 2005; James *et al.*, 2000; Kakafika *et al.*, 2007; Kamath & Lip, 2003; Kannel *et al.*, 1987; Pearson *et al.*, 1997). The higher fibrinogen concentrations in smokers were possibly caused by an increased inflammatory response due to lung damage and respiratory infections (Kakafika *et al.*, 2007; Kamath & Lip, 2003). The difference in results could be ascribed to the fact that the other studies included only participants who smoked tobacco, whereas the PURE study also included participants who use snuff and chewed tobacco. In these instances the lungs are not predominantly affected by the tobacco use and thus there is no increased inflammatory response caused by lung damage.

Total fibrinogen concentrations did not differ significantly between women who used contraceptives and those who did not. This is in contrast with other studies that indicated that total fibrinogen concentrations increased when contraceptives were used (Kamath & Lip, 2003; Kluft *et al.*, 2002; Machado *et al.*, 2004; Wiegratz *et al.*, 2004). Oral contraceptives have been found to induce increases in acute-phase proteins such as CRP and fibrinogen (Kluft *et al.*, 2002).

It has been suggested that oestrogens from oral contraceptives may have a pro-inflammatory effect, activating the acute-phase response (Kluft *et al.*, 2002). However, it has also been reported that oestrogens could have an anti-inflammatory effect on the vascular wall (Kluft *et al.*, 2002). It is difficult to predict whether the net effect will be pro- or anti-inflammatory, as this can be influenced by different chronic diseases and may also differ among individuals (Kluft *et al.*, 2002). It is also important to note that not all contraceptives always cause an increase in total fibrinogen concentrations. In the study of Wiegratz *et al.* (2004), the use of the contraceptives ethinylestradiol (EE) in combination with dienogest (DNG) or EE in combination with DNG and estradiol valerate (EV) consistently resulted in increased total fibrinogen concentrations (Wiegratz *et al.*, 2004). The contraceptive EE in combination with levonorgestrel (LNG) also resulted in an increase, although not consistently (Wiegratz *et al.*, 2004). Some combinations of contraceptives therefore caused a greater increase in fibrinogen concentrations than others. In the PURE study, data were unfortunately not collected on the types of contraceptives used. A possible explanation for the lack of any significant correlation of contraceptive use with total fibrinogen in our study may, therefore, be due to the type of contraceptive used, although this cannot be proved.

5.3.1.2 Fibrinogen γ'

Of all the environmental factors, CRP had the strongest significant positive correlation with fibrinogen γ' in 2005 and 2010, thus suggesting a link between fibrinogen γ' and inflammation. Other studies corroborate our finding (Lovely *et al.*, 2013), especially with CRP concentrations higher than 3 mg/L (Alexander *et al.*, 2011). In the study of Alexander *et al.* (2011), it was suggested that fibrinogen γ' may be more elevated with chronic than with acute inflammation. The ratio of fibrinogen γ' was 18.6% in their study, which is higher than the accepted normal range of 8-15% indicating that fibrinogen γ' increased with chronic disease (periodontal disease and history of cardiovascular disease) (Alexander *et al.*, 2011). Cheung *et al.* (2009), however, determined that there was a significant association between CRP and γ' ratio in subjects in the acute phase of ischaemic stroke. As mentioned in Section 5.2.1, this association was ascribed to altered mRNA processing of fibrinogen γ' during the acute phase (Cheung *et al.*, 2009).

Another inflammatory marker significantly associated with fibrinogen γ' in both 2005 and 2010 is IL-6. IL-6 also had a positive correlation with fibrinogen γ' in other studies (Lovely *et al.*, 2013; Rein-Smith *et al.*, 2013). The study of Rein-Smith *et al.* (2013) determined that an increase in IL-6 up-regulated the synthesis of fibrinogen γ' . This indicates that fibrinogen γ' also acts as an acute-phase reactant during inflammation (Rein-Smith *et al.*, 2013).

BMI correlated positively with fibrinogen γ' in both 2005 and 2010. This is consistent with the results of some studies (Lovely *et al.*, 2010; Lovely *et al.*, 2013), but contradicts others which found that BMI had no significant interaction with fibrinogen γ' (Alexander *et al.*, 2011; Mannila *et al.*, 2007a). In the study of Lovely *et al.* (2013), fibrinogen γ' decreased significantly after three months of exercise in obese children, while the fibrinogen γ' concentrations in the obese children who did not exercise seemed to increase after three months. The reduction of fibrinogen γ' in these children after physical activity was ascribed to a decreased obesity-related inflammatory state, the same mechanism as determined for total fibrinogen (Lovely *et al.*, 2013). There is uncertainty, however, as to whether the same response of reduction in fibrinogen γ' after physical activity will also be observed in adults (Lovely *et al.*, 2013).

Another environmental factor showing a significant positive correlation with fibrinogen γ' was age (in 2010). This is in agreement with some studies (Drouet *et al.*, 1999; Lovely *et al.*, 2010), but in contrast with other studies where fibrinogen γ' did not show any significant association with age (Alexander *et al.*, 2011; Lovely *et al.*, 2002; Mosesson *et al.*, 2007). The mechanism behind the association between fibrinogen γ' and age is not clear and whether it is an independent association or a reflection of the association of total fibrinogen with age still needs to be established.

HbA1c also had a significant positive correlation with fibrinogen γ' in 2005. Although less information is available on the relationship between glucose metabolism and fibrinogen γ' , Lovely *et al.* (2010) found that fasting glucose had a significant association with fibrinogen γ' .

Other studies indicated that insulin concentrations had a strong positive correlation with fibrinogen γ' (Lovely *et al.*, 2013; Mannila *et al.*, 2007a) and it has been suggested that insulin possibly plays a role in the regulation of fibrinogen γ' (Lovely *et al.*, 2013).

HDL-cholesterol had a negative correlation with fibrinogen γ' in 2005, which is consistent with the results of other studies which indicated that HDL-cholesterol had an inverse association with fibrinogen γ' (Lovely *et al.*, 2010; Mannila *et al.*, 2007a). LDL-cholesterol had a positive correlation with fibrinogen γ' in 2010. In the study of Lovely *et al.* (2010), total cholesterol did not have a significant association with fibrinogen γ' . Again, due to the lack of information available for fibrinogen γ' , it is not clear whether these associations reflect an independent association with fibrinogen γ' or whether they are a reflection of the association with total fibrinogen. Additional research, focusing specifically on this aspect, is required to identify the underlying relationships.

In 2005 the fibrinogen γ' concentrations were significantly higher in HIV-infected than HIV-uninfected individuals, resulting in the higher γ' ratio in those who were HIV infected in 2005. In 2010, however, the fibrinogen γ' concentrations were significantly lower in the HIV-infected than the HIV-uninfected individuals. This phenomenon may possibly be due to the same factors reported for total fibrinogen, but there may also be other reasons which remain to be elucidated.

In 2005 there were no significant differences in fibrinogen γ' concentration between the different groups of tobacco users, and in 2010 the statistically significant difference between participants who had never used tobacco and participants currently using tobacco was also small. In other studies it was reported that fibrinogen γ' was not significantly affected by tobacco use (Alexander *et al.*, 2011; Mannila *et al.*, 2007a). This is in contrast, however, with the study of Lovely *et al.* (2010), which determined that fibrinogen γ' does have a significant association with smoking. This illustrates yet again the lack of (and the need for) scientific evidence regarding the association of fibrinogen γ' with other biological factors.

5.3.2 Effect of environmental factors on change in total fibrinogen and fibrinogen γ' over the five-year period

Following the discussion on the cross-sectional relationships of total fibrinogen and fibrinogen γ' with environmental factors, we now provide details on the environmental factors that may influence the change in total fibrinogen and fibrinogen γ' over time. To the best of our knowledge, this has not previously been investigated. The only environmental factor that influenced change in total fibrinogen concentrations over the five-year period was CRP. It was determined that fibrinogen decreased over the five-year period in individuals who had higher CRP concentrations in 2005. A possible reason for the decrease in total fibrinogen over time in participants with the higher CRP concentrations could be that, in participants who had higher CRP concentrations in 2005, the total fibrinogen concentrations were also acutely increased due to a possible acute-phase reaction, indicating that the 2005 total fibrinogen concentrations were probably not the baseline concentrations. If these particular participants did not have a similar acute-phase response in 2010 during the blood collection period, then their total fibrinogen concentrations may have been at basal concentration, reflecting a decrease from 2005 to 2010. In support of this theory the change in CRP concentrations over time also showed a negative association with CRP concentrations in 2005, indicating that, in individuals with high CRP concentrations in 2005, in agreement with the decrease in fibrinogen, CRP concentrations also decreased over the five-year period. CRP and fibrinogen have a significant short-term association since both are acute-phase proteins which can, therefore, increase within minutes upon activation of the acute-phase response. This short-term association might, therefore, explain the apparent association found in the prospective data. Additionally, because fibrinogen is an acute-phase protein, the concentrations of which can be altered over a short term, such as a few days, establishing longer-term associations becomes extremely complex as its concentrations can be altered significantly over the short term.

None of the investigated environmental factors significantly predicted/contributed to the change in fibrinogen γ' over the five years, using our models.

5.4 SINGLE NUCLEOTIDE POLYMORPHISMS

5.4.1 Comparison of minor allele frequencies between different populations

Table 5.1 presents the MAF of our study population compared with other populations according to the 1000genomes database.

Table 5.1: Difference in minor allele frequency between various populations

SNP	MAF of our black South African population	MAF of African Americans	MAF of whites (North America, Northern and Western Europe)	MAF of Asians	Reference*
FGA 2224 G>A (rs2070011)	17%	26%	35%	54%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155511397-155512397;v=rs2070011;vdb=variation;vf=1643217
FGA 6534 A>G (rs6050)	30%	39%	18%	49%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155507090-155508090;v=rs6050;vdb=variation;vf=2013
FGB Arg448Lys (rs4220)	8%	4%	21%	18%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155491259-155492259;v=rs4220;vdb=variation;vf=9358
FGB -148 C>T (rs1800787)	6%	13%	22%	18%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155483515-155484515;v=rs1800787;vdb=variation;vf=1366673
FGB 40 A>G (rs2227385)	3%	2%	0%	0%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155481811-155482811;v=rs2227385;vdb=variation;vf=1803141
FGB 749 A>G (rs2227388)	16%	2%	0%	0%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155482520-155483520;v=rs2227388;vdb=variation;vf=1803143
FGB 1038 G>A (rs1800791)	9%	14%	12%	4%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155482809-155483809;v=rs1800791;vdb=variation;vf=1366677

Table 5.1 (continued)

SNP	MAF of our black South African population	MAF of African Americans	MAF of whites (North America, Northern and Western Europe)	MAF of Asians	Reference*
FGB 1643 C>T (rs1800788)	5%	11%	19%	62%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155483414-155484414;v=rs1800788;vdb=variation;vf=1366674
FGG 10034 C>T (rs2066865)	27%	37%	16%	51%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155524776-155525776;v=rs2066865;vdb=variation;vf=1640060
FGG 9340 T>C (rs1049636)	16%	20%	32%	18%	http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=4:155525470-155526470;v=rs1049636;vdb=variation;vf=835269

*References obtained from website: <http://www.1000genomes.org/>

A = adenine; Arg = arginine; C = cytosine; FGA = fibrinogen α gene; FGB = fibrinogen β gene; FGG = fibrinogen γ gene; G = guanine; Lys = lysine; MAF = minor allele frequency; SNP = single nucleotide polymorphism; T = thymine

From Table 5.1 it seems that there is a larger agreement between the MAFs of black South Africans and African Americans than with the other ethnicities. Two SNPs present in our black South African population are also prevalent in the African American population, but not in the other populations, which are FGB 40 A>G and FGB 749 A>G. Research has indicated that the African population has the greatest genetic diversity of all ethnic groups in the world (Campbell & Tishkoff, 2008). This might be the reason for the difference in MAFs of the SNPs between the black South African population and the non-African populations. It is also suggested that all other populations arose from this population as many Africans migrated out of Africa to other countries (Campbell & Tishkoff, 2008).

5.4.2 Comparison of linkage disequilibrium between different studies

The fibrinogen genes (FGA, FGB and FGG) are all clustered on chromosome 4q23-q32 (Kamath & Lip, 2003) and found on a 50 kb region of DNA (Connor *et al.*, 1992). Thus strong LD is expected between these genes (Connor *et al.*, 1992; Uitte de Willige *et al.*, 2009a). However, lower levels of LD were reported in African than in non-African populations (Campbell & Tishkoff, 2008). It has been determined that the strong LD that exists in non-African populations extends over wider genomic distances than in African populations (Campbell & Tishkoff, 2008). The reason for this is that more recombination occurred in African populations as the ancestral Africans maintained larger population sizes (Campbell & Tishkoff, 2008). There is also evidence, however, that diversity exists in levels and patterns of LD among subpopulations in Africa, and therefore all African subpopulations have their own distinct pattern of LD (Campbell & Tishkoff, 2008).

According to our study, FGB 1038 G>A was in LD with FGA 2224 G>A, but no LD existed between FGB 1038 G>A and FGG 9340 T>C as indicated by the D' , which contradicts the findings of the study by Mannila *et al.* (2006). According to the literature there is strong LD between the FGA 6534 A>G and FGG 10034 C>T SNPs; consequently, the functional effects of the SNPs cannot be distinguished (Ariëns, 2013; Uitte de Willige *et al.*, 2009b). This is in contrast with results of our study, as the Haploview LD plot indicated that these SNPs are probably not in LD. According to the study of Jacquemin *et al.* (2008), strong LD existed between the SNPs FGA 6534 A>G and FGA 2224 G>A, between the SNPs FGB 1038 G>A, FGB 1643 C>T and FGB Arg448Lys as well as between the SNPs FGG 10034 C>T and FGG 9340 T>C. In our study there was no LD between the SNPs FGA 6534 A>G and FGA 2224 G>A, or between the SNPs FGB 1038 G>A, FGB 1643 C>T and FGB Arg448Lys. However, our study determined that LD existed between the SNPs FGG 10034 C>T and FGG 9340 T>C.

The differences between the studies regarding LD were expected, however, as the studies were conducted in different populations. As previously mentioned, the levels of LD may differ between populations, especially between African and non-African populations (Campbell & Tishkoff, 2008).

The studies of Jacquemin *et al.* (2008) and Mannila *et al.* (2006) were conducted among Europeans, while the study of Uitte de Willige *et al.* (2009b) was conducted among African Americans and Caucasians. Therefore, it was anticipated that the levels of LD would differ from those in our study, which was performed in black South Africans. It should also be kept in mind that due to the relatively low r^2 values, which is most likely due to the differences in MAFs between the SNPs indicated to be in LD, it can be assumed that these SNPs may not have been in complete LD and may still have differing effects on total fibrinogen and fibrinogen γ' concentration.

5.4.3 Cross-sectional effect of genotypes on total fibrinogen and fibrinogen γ'

The effect of the measured SNPs on total fibrinogen and fibrinogen γ' concentrations in this study is presented in *Tables 5.2* and *5.3*, together with a comparison with the available literature.

In general, the results of this study are consistent with those reported in the literature. In cases where a SNP showed an association with total fibrinogen and/or fibrinogen γ' in either 2005 or 2010 but not the same association in both years, the literature also indicates variability in the association of the SNP with the fibrinogen variables, where some studies found that the mutant allele caused an increase, some found a decrease and others found that it had no effect. For selected SNPs the results of this study differed from those of other studies. FGB -148 C>T in our study, for instance, had no effect on total fibrinogen, while the majority of the studies in the literature (Cook *et al.*, 2001; Titov *et al.*, 2012; Wong *et al.*, 2008; Wypasek *et al.*, 2012) indicated increased fibrinogen concentration in the presence of the mutant allele. FGB 1643 C>T and FGG 10034 C>T, on the other hand, showed increased fibrinogen concentration in carriers of the mutant allele in this study, while the literature indicates decreased concentrations or no effect (Carty *et al.*, 2008; Jacquemin *et al.*, 2008; Ken-Dror *et al.*, 2012; Titov *et al.*, 2012; Uitte de Willige *et al.*, 2005). FGB 1643 C>T in this study also had no effect on fibrinogen γ' whereas the literature indicates decreased concentrations in mutant allele carriers (Lovely *et al.*, 2011). For many of the SNPs, this study provides the first evidence for possible effects on fibrinogen γ' , as no evidence in the literature is available for these associations.

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

Alteration name	Rs number	Mutation	Effect of SNPs on total fibrinogen in current study in 2005 and 2010		Effect of mutant allele of SNPs on total fibrinogen concentration according to the literature		Studies that reported no effect on total fibrinogen concentration
			2005	2010	Increase	Decrease	
FGA 2224 G>A	rs2070011	UTR-5*	No significant difference between genotypes	Significantly higher in homozygous MT than homozygous WT genotype	Mannila <i>et al.</i> (2006) Mannila <i>et al.</i> (2007a)	Carty <i>et al.</i> (2008) Jacquemin <i>et al.</i> (2008) Ken-Dror <i>et al.</i> (2012)	Mannila <i>et al.</i> (2007b)
FGA 6534 A>G Or FGA Thr312Ala	rs6050	Missense mutation – transversion	No significant difference between genotypes	No significant difference between genotypes	Lim <i>et al.</i> (2003) Scott <i>et al.</i> (2004) Uitte de Willige <i>et al.</i> (2005)	Carty <i>et al.</i> (2008)	Jacquemin <i>et al.</i> (2008) Titov <i>et al.</i> (2012)
FGB Arg448Lys (A>G)	rs4220	Missense mutation – transversion	No significant difference between alleles	Significantly lower in MT allele than WT allele	Jacquemin <i>et al.</i> (2008) Ken-Dror <i>et al.</i> (2012) Leung Ong <i>et al.</i> (2010)	Dehghan <i>et al.</i> (2009)	Lim <i>et al.</i> (2003) Scott <i>et al.</i> (2004)
FGB -148 C>T	rs1800787	nearGene-5 Untranslated region*	No significant difference between alleles	No significant difference between alleles	Cook <i>et al.</i> (2001) Titov <i>et al.</i> (2012) Wong <i>et al.</i> (2008) Wypasek <i>et al.</i> (2012)	None	Schmidt <i>et al.</i> (1998)
FGB 40 A>G	rs2227385	nearGene-5 Untranslated region*	No significant difference between alleles	No significant difference between alleles	Not determined	Not determined	Not determined
FGB 749 A>G	rs2227388	nearGene-5 Untranslated region*	No significant difference between genotypes	No significant difference between genotypes	Not determined	Not determined	Not determined

Table 5.2 (continued)

Alteration name	Rs number	Mutation	Effect of SNPs on total fibrinogen in current study in 2005 and 2010		Effect of mutant allele of SNPs on total fibrinogen concentration according to the literature		Studies that reported no effect on total fibrinogen concentration
			2005	2010	Increase	Decrease	
FGB 1038 G>A	rs1800791	nearGene-5 Untranslated region*	No significant difference between alleles	No significant difference between alleles	None	None	Carty <i>et al.</i> (2008) Jacquemin <i>et al.</i> (2008) Mannila <i>et al.</i> (2006) Uitte de Willige <i>et al.</i> (2005)
FGB 1643 C>T	rs1800788	nearGene-5 Untranslated region*	No significant difference between alleles	Significantly higher in MT allele than WT allele	None	Ken-Dror <i>et al.</i> (2012)	Jacquemin <i>et al.</i> (2008) Titov <i>et al.</i> (2012) Uitte de Willige <i>et al.</i> (2005)
FGG 10034 C>T	rs2066865	nearGene-5 Untranslated region*	No significant difference between genotypes	Significantly higher in homozygous MT than homozygous WT genotype	None	Carty <i>et al.</i> (2008)	Jacquemin <i>et al.</i> (2008)
FGG 9340 T>C	rs1049636	UTR-3*	Significantly higher in homozygous MT than homozygous WT and heterozygous genotype	Significantly higher in homozygous MT than heterozygous, but not significantly different from homozygous WT genotype	Lovely <i>et al.</i> (2011)	None	Jacquemin <i>et al.</i> (2008) Mannila <i>et al.</i> (2006) Mannila <i>et al.</i> (2007b) Uitte de Willige <i>et al.</i> (2005)

* = dbSNP, 2012; A = adenine; Ala = alanine; Arg = arginine; C = cytosine; FGA = fibrinogen α ; FGB = fibrinogen β ; FGG = fibrinogen γ ; G = guanine; Lys = lysine; MT = mutant; nearGene-5 = Includes the upstream promoter region and untranslated 5' mRNA; rs = reference SNP; T = thymine; Thr = threonine; UTR-3 = 3' untranslated region; UTR-5 = 5' untranslated region; WT = wild-type

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

Alteration name	Rs number	Mutation	Effect of SNPs on fibrinogen γ' in current study in 2005 and 2010		Effect of mutant allele of SNPs on fibrinogen γ' concentration according to literature		Studies that reported no effect on fibrinogen γ' concentration
			2005	2010	Increase	Decrease	
FGA 2224 G>A	rs2070011	UTR-5*	Significantly higher in homozygous MT than homozygous WT genotype	No significant difference between genotypes	Mannila <i>et al.</i> (2007a)	Lovely <i>et al.</i> (2011)	None
FGA 6534 A>G Or FGA Thr312Ala	rs6050	Missense mutation – transversion	No significant difference between genotypes	Significantly lower in homozygous MT than homozygous WT genotype	None	Lovely <i>et al.</i> (2011)	None
FGB Arg448Lys (A>G)	rs4220	Missense mutation – transversion	No significant difference between alleles	No significant difference between alleles	Not determined	Not determined	Not determined
FGB -148 C>T	rs1800787	nearGene-5 Untranslated region*	No significant difference between alleles	Significantly lower in MT allele than WT allele	Not determined	Not determined	Not determined
FGB 40 A>G	rs2227385	nearGene-5 Untranslated region*	No significant difference between alleles	No significant difference between alleles	Not determined	Not determined	Not determined
FGB 749 A>G	rs2227388	nearGene-5 Untranslated region*	No significant difference between genotypes	No significant difference between genotypes	Not determined	Not determined	Not determined

Table 5.3 (continued)

Alteration name	Rs number	Mutation	Effect of SNPs on fibrinogen γ' in current study in 2005 and 2010		Effect of mutant allele of SNPs on fibrinogen γ' concentration according to literature		Studies that reported no effect on fibrinogen γ' concentration
			2005	2010	Increase	Decrease	
FGB 1038 G>A	rs1800791	nearGene-5 Untranslated region*	No significant difference between alleles	Significantly higher in MT allele than WT allele	Not determined	Not determined	Not determined
FGB 1643 C>T	rs1800788	nearGene-5 Untranslated region*	No significant difference between alleles	No significant difference between alleles	None	Lovely <i>et al.</i> (2011)	None
FGG 10034 C>T	rs2066865	nearGene-5 Untranslated region*	No significant difference between genotypes	Significantly lower in homozygous MT than homozygous WT and heterozygous genotype	None	Carty <i>et al.</i> (2008) Grünbacher <i>et al.</i> (2007) Lovely <i>et al.</i> (2011) Uitte de Willige <i>et al.</i> (2005) Uitte de Willige <i>et al.</i> (2007) Uitte de Willige <i>et al.</i> (2009b)	None
FGG 9340 T>C	rs1049636	UTR-3*	No significant difference between genotypes	Significantly higher in homozygous MT than homozygous WT and heterozygous genotype	Lovely <i>et al.</i> (2011) Mannila <i>et al.</i> (2007a) Uitte de Willige <i>et al.</i> (2009b)	None	None

* = dbSNP, 2012; A = adenine; Ala = alanine; Arg = arginine; C = cytosine; FGA = fibrinogen α ; FGB = fibrinogen β ; FGG = fibrinogen γ ; G = guanine; Lys = lysine; MT = mutant; nearGene-5 = Includes the upstream promoter region and untranslated 5' mRNA; rs = reference SNP; T = thymine; Thr = threonine; UTR-3 = 3' untranslated region; UTR-5 = 5' untranslated region; WT = wild-type

The differences observed in the effect of the SNPs on the total fibrinogen and fibrinogen γ' concentrations between the literature and our study might be due to the difference in environment. It has been reported that the effect of the fibrinogen SNPs differs between racial and ethnic groups (Lee *et al.*, 1999). Most of the studies mentioned above were conducted in Caucasian individuals with a European ethnicity. A few studies included populations of South Asian, West African, Afro-Caribbean and Chinese ethnicity, as well as American individuals of European descent and African descent. See section 2.3 and *Table 2.3* in chapter 2 for a detailed discussion regarding the differences between the populations used in the above-mentioned studies. This is the first study that was conducted on genetic influences on total fibrinogen and fibrinogen γ' concentrations in a black South African population. Thus the value of studying diverse ethnic groups does not lie only in genetic diversity, but also in differences in their environment which can influence the phenotypic expression of the same genotype. Therefore it is also important to explore possible gene-environment interactions which can influence the total fibrinogen and fibrinogen γ' concentrations. This will be discussed in section 5.5.

A genetic variation within an untranslated (non-coding) region of the gene could change gene expression but not the amino acid or protein as this region is not translated (Iacoviello *et al.*, 2001; Roche & Mensik, 2003). This includes polymorphisms in the promoter area of a gene. Almost all the SNPs included in this study are located in the untranslated region of the fibrinogen gene. These SNPs can potentially influence gene expression of the fibrinogen gene, which can influence the functional effect of SNPs in the coding region of the gene, leading to increased or decreased total fibrinogen and fibrinogen γ' concentrations. Genomics data are, however, required to investigate the effect of SNPs on expression. When a genetic variation occurs in the coding region of the gene (missense mutation), it may cause a change in the amino acid sequence and therefore possibly alter protein composition (Roche & Mensik, 2003). FGA 6534 A>G (Thr312Ala) and FGB Arg448Lys were the only two missense mutations in this study

Research conducted on fibrinogen genes is focused mostly on the FGB gene, and the promoter area in particular, as the β -chain seems to be rate-limiting for fibrinogen synthesis (Humphries *et al.*, 1997; Iacoviello *et al.*, 2001; Koenig, 2003). Thus it is plausible that SNPs that affect the synthesis of the β -chain have an impact on total fibrinogen concentrations (Humphries *et al.*, 1997; Iacoviello *et al.*, 2001). In our study, four of the five SNPs in the promoter area of the FGB gene, however, had no effect on total fibrinogen concentration cross-sectionally. Increased fibrinogen concentrations were observed only in the mutant allele carriers of FGB 1643 C>T. Ken-Dror *et al.* (2012), however, found decreased fibrinogen concentrations in the presence of the mutant allele. Three of the five SNPs in the FGB promoter area also had no effect on fibrinogen γ' concentrations. Increased fibrinogen γ' concentrations were found in the FGB 1038 G>A mutant allele carriers, however, with decreased concentrations in the FGB -148 C>T mutant allele group. A possible reason for the apparent lack of effect of most of the FGB SNPs in the promoter area may be that the SNPs were not present within a known transcription factor binding site (as determined by means of the UCSC genome browser, using the TFBS conserved and ENCODE regulation tracks). The FGB -148 C>T was the only SNP that was present in a transcription factor binding site, but the transcription factor (Forkhead-Related transcription factor) known to bind to this site has not been associated with fibrinogen concentrations (as determined by the NCBI database). Both missense mutations have undergone alternative splicing (as determined by the UCSC genome browser), which is a regulatory mechanism which allows the production of a variety of different proteins from one gene (Kashyap and Tripathi, 2008). Alternative splicing can cause protein-concentration alterations by introducing an alternate protein (Kashyap and Tripathi, 2008). Thus the reason that the FGB Arg448Lys and FGA Thr312Ala missense mutations result in lower total fibrinogen and fibrinogen γ' concentrations respectively may be the alternative splicing, causing a fibrinogen concentration alteration.

In our study, the SNPs that caused higher total fibrinogen concentrations in carriers of the mutant allele were FGA 2224 G>A, FGB 1643 C>T, FGG 10034 C>T and FGG 9340 T>C. The MAF of FGB 1643 C>T was, however, only 5%, which is much lower than the other three SNPs. Therefore, at a population level, the SNPs that could possibly explain the higher total fibrinogen concentrations observed in black South Africans are FGA 2224 G>A, FGG 10034 C>T and FGG 9340 T>C, because of their higher frequency within our population. There was only one SNP that caused lower total fibrinogen concentrations in carriers of the mutant allele in our population: FGB Arg448Lys; however, the MAF of this SNP was only 8%, indicating that frequency of the mutant allele was probably not high enough to influence fibrinogen concentration at a population level. We can, therefore, speculate that the SNPs FGA 2224 G>A, FGG 10034 C>T and FGG 9340 T>C could contribute to future cardiovascular disease risk in our population as elevated total fibrinogen concentrations have been identified as an independent risk factor for cardiovascular disease (Ajjan & Grant, 2006; Kakafika *et al.*, 2007). The SNPs that caused higher fibrinogen γ' concentrations in carriers of the mutant allele were FGA 2224 G>A, FGB 1038 G>A and FGG 9340 T>C. The MAF of FGB 1038 G>A was only 9%, which is again lower than the other two SNPs; therefore, the SNPs that had the greatest likelihood of influencing fibrinogen γ' concentrations were FGA 2224 G>A and FGG 9340 T>C. SNPs that caused lower fibrinogen γ' concentrations in carriers of the mutant allele in our population were FGA 6534 A>G, FGB -148 C>T and FGG 10034 C>T. FGB -148 probably did not significantly influence fibrinogen γ' on a population level, also because of its comparatively low MAF of 6%. As explained in section 2.2.3.2 in chapter 2, it has been determined that increased fibrinogen γ' concentrations are associated with arterial disease while decreased fibrinogen γ' concentrations associated with venous disease. Thus we can speculate that the SNPs FGA 2224 G>A and FGG 9340 T>C may contribute to increased risk of arterial disease and the SNPs FGA 6534 A>G and FGG 10034 C>T may contribute to an increased risk of venous disease in our population.

According to the GWA study of Dehghan *et al.* (2009) other genes have also been identified that possibly influence the total fibrinogen concentrations: the IRF1 gene on chromosome 5, the NLRP3 gene on chromosome 1, the interleukin-1 receptor antagonist (IL-1RN) gene on chromosome 2 and the PCCB gene on chromosome 3. According to the GWA study of Lovely *et al.* (2011) SNPs on the pleiotropic regulator 1 (PLRG1) gene have been identified as SNPs that possibly influence the fibrinogen γ' concentrations. Thus, in future studies, it will be important also to investigate the effects of these genes on the total fibrinogen and fibrinogen γ' concentrations of black South African populations.

5.4.4 Effect of genotypes on the change over time in the fibrinogen variables

In the previous section, the cross-sectional association of the measured SNPs with the fibrinogen variables was discussed. This section focuses on identifying and discussing SNPs that influenced the change in the fibrinogen variables over the five-year period. *Table 5.4* presents the SNPs for which the change in the fibrinogen variables over the five-year period differed significantly between the respective genotypes (using ANOVA).

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

SNP	Total fibrinogen		Fibrinogen γ'		γ' ratio	
	WT-allele	MT-allele	WT-allele	MT-allele	WT-allele	MT-allele
FGA 2224 G>A	-	-	-	-	↓	↓
FGA 6534 A>G	-	-	↑	Hez ↑ Hoz ↓	-	-
FGB Arg448Lys	-	-	↑	↑	↓	↑
FGB -148 C>T	↑	↑	↑	↓	-	-
FGB 1643 C>T	↑	↑	-	-	-	-
*FGG 10034 C>T	-	-	↑	↓	↑	↓

*FGG 10034 C>T also significantly influenced fibrinogen γ' in the mixed-models approach

Direction of arrow indicates increase (↑) or decrease (↓) over the 5-year period.

Size of arrow indicates size of increase or decrease: ↓/↑ - comparatively smaller increase/decrease; ↓/↑ - comparatively larger increase/decrease

A = adenine, Arg = arginine, C = cytosine, FGA = Fibrinogen α gene, FGB = Fibrinogen β gene, FGG = Fibrinogen γ gene, Hez = heterozygote, Hoz = homozygote, G = guanine, Lys = lysine, MT = mutant, SNP = single nucleotide polymorphism, T = thymine, WT = wild-type

As can be seen from Table 5.4, FGB -148 C>T and FGB 1643 C>T significantly influenced the change in total fibrinogen concentration over time. FGA 6534 A>G, FGB Arg448Lys, FGB -148 C>T and FGG 10034 C>T significantly influenced the change in fibrinogen γ' over time. The respective changes in total fibrinogen and fibrinogen γ' also differed between genotypes of the FGA 2224 G>A, FGB Arg448Lys and FGG 10034 C>T SNPs, resulting in differences in the γ' ratio over time.

The above-mentioned results were obtained by performing t-tests and ANOVAs without adjusting for any environmental factors. As stated in chapter 2 in section 2.5, it was hypothesised that the effect of genetic factors decreases with increasing age, as the effect of biological factors, such as inflammatory factors, increases as a result of age-related diseases (Bladbjerg *et al.*, 2006; De Maat *et al.*, 2004). For this reason we not only compared the fibrinogen variables across the genotypes, we also constructed a mixed-models approach which included the possible effects of environmental factors, in order to determine the contribution of the SNPs to the fibrinogen variables in the presence of environmental factors known to influence them. The mixed-models approach indicated that none of the SNPs significantly affected change in total fibrinogen concentration over time once the environmental factors were taken into account. For fibrinogen γ' , the only SNP for which a significant association remained was the FGG 10034 C>T. We determined that the change in γ' ratio over the five years decreased when harbouring the heterozygous genotype and decreased even more when harbouring the homozygous mutant genotype, when compared with the homozygous wild-type genotype (which showed an increase). These results are also reflected in the γ' ratio which increased in the homozygous wild-type carriers, but actually decreased in the heterozygous and homozygous mutant carriers. The reason for the decrease in fibrinogen γ' concentrations in the mutant allele of the FGG 10034 C>T polymorphism is possibly that it is located in a cleavage stimulatory factor sequence close to the polyadenylation site of the fibrinogen γ A exon 10 (Grünbacher *et al.*, 2007; Uitte de Willige *et al.*, 2007). Therefore, the mutant allele of the FGG 10034 C>T polymorphism is possibly responsible for the production of more fibrinogen γ A and less fibrinogen γ' (Grünbacher *et al.*, 2007; Uitte de Willige *et al.*, 2007). In our black South African population the MAF of FGG 10034 C>T was 27%, indicating that 27% of our population had possibly decreased fibrinogen γ' concentrations and increased fibrinogen γ A concentrations owing to this polymorphism.

5.5 GENE-ENVIRONMENT INTERACTIONS

In the previous sections, we identified the environmental and genetic factors that influenced the fibrinogen variables in our study population and discussed possible mechanisms for these observations. The next two sections will focus on gene-environment interactions that could possibly influence the fibrinogen variables cross-sectionally (Section 5.5.1) and over time (Section 5.5.2).

5.5.1 Gene-environment interactions that affected the fibrinogen variables on a cross-sectional level

There were four significant gene-environment interactions that affected total fibrinogen on a cross-sectional level. The first significant gene-environment interaction that influenced total fibrinogen concentrations was FGA 2224 G>A with Age. From this interaction it was evident that the total fibrinogen concentrations increased more with an increase in age in participants harbouring the homozygous mutant genotype, than in participants harbouring the homozygous wild-type genotype. A similar gene-age interaction has also been reported in the study of Humphries *et al.* (1997) for FGB -455 G>A. Humphries *et al.* (1997) indicated that with increasing age and in subjects harbouring the mutant allele of FGB -455 G>A there was a rise in total fibrinogen concentrations, compared with those harbouring the wild-type allele. The MAF of FGA 2224 G>A in our black South African population was 17%, indicating that 17% of our population will possibly have a more rapid increase than the rest of the population in total fibrinogen concentrations as they grew older. Thus there is a possibility that participants harbouring the mutant allele of FGA 2224 G>A might have an increased risk of developing cardiovascular disease as they age, as elevated total fibrinogen concentrations have been identified as an independent risk factor for cardiovascular disease (Ajjan & Grant, 2006; Kakafika *et al.*, 2007).

The second significant gene-environment interaction that influenced total fibrinogen concentrations was FGB 1038 G>A with HbA1c. Total fibrinogen concentrations increased more per one unit change in HbA1c in carriers of the mutant allele than in those harbouring the wild-type allele. Thus the increase in total fibrinogen concentrations associated with increased HbA1c was greater in participants harbouring the mutant allele than participants harbouring the wild-type allele. This interaction may partly explain why total fibrinogen is increased in some diabetics while in others not. Apart from the severity of the disease, it may also be that individuals harbouring the mutant allele may have increased fibrinogen, as there is an increase in HbA1c concentrations in these individuals in comparison with those with the wild-type. This phenomenon has also been reported for another SNP in the study of Lam *et al.* (1999), where it was determined that the FGB -455 G>A polymorphism had an influence on fibrinogen concentrations in type 2 diabetic patients. It seemed that the A-allele associated with higher fibrinogen concentrations than the G-allele in type 2 diabetic patients, although this difference was not significant (Lam *et al.*, 1999). Dunn and Ariëns (2004) have also reported that the fibrinogen concentrations of diabetic patients are determined by an interaction between the genotype of the individual and glycometabolic control. The MAF of FGB 1038 G>A in our black South African population was 9%, indicating that 9% of our population will possibly have a more rapid increase in total fibrinogen concentrations if their HbA1c concentrations increase. We can speculate, therefore, that in our population, those diabetic participants who are harbouring the mutant allele of FGB 1038 G>A could have higher total fibrinogen concentrations than those harbouring the wild-type allele, thus increasing their risk of development of cardiovascular disease.

The third significant gene-environment interaction that influenced total fibrinogen concentrations was FGB Arg448Lys-HIV status. Total fibrinogen concentrations were significantly decreased 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.

To the best of our knowledge, the effect of an interaction between a SNP and HIV status on total fibrinogen concentrations has not previously been investigated. In section 5.3.1 the reason for the lower total fibrinogen concentrations in the HIV-positive group was partly ascribed to the ARV treatment or the subtype of HIV, rather than to the HIV infection itself. The MAF of FGB Arg448Lys in our black South African population was 8%, indicating that the majority (92%) of our population is harbouring the wild-type allele of this SNP. Thus we can speculate that an additional reason for the lower total fibrinogen concentrations in the HIV-infected population might be the high prevalence of the wild-type allele of the FGB Arg448Lys polymorphism.

The last significant gene-environment interaction that influenced total fibrinogen concentrations was FGB 1643 C>T with urbanisation. In the homozygous wild-type carriers, total fibrinogen concentrations were significantly lower in the urban than in the rural participants while no difference was observed for the carriers of the mutant allele. To the best of our knowledge, the effect of an interaction between a SNP and urbanisation on total fibrinogen concentrations has not previously been investigated. In section 5.2.1 it was determined that environmental factors known to influence total fibrinogen could only partially explain the lower total fibrinogen concentration in the urban than the rural participants. It was also mentioned that up to 70% of the explained variance in total fibrinogen concentrations was attributable to non-modifiable factors, such as genetic factors. The MAF of FGB 1643 C>T in our black South African population was 5%, indicating that the majority (95%) of our population is harbouring the wild-type allele of this SNP. The significant difference in the total fibrinogen concentrations between the rural and urban participants was observed only in the wild-type allele of this SNP. We can speculate, therefore, that the specific clustering of environmental factors present in the urban and rural settings could significantly influence gene-expression of the wild-type allele of FGB 1643 C>T, while it does not do so for the mutant allele.

There were no significant gene-environment interactions affecting fibrinogen γ' on a cross-sectional level, indicating that no such interactions existed that significantly contributed to or explained the cross-sectional associations determined for fibrinogen γ' . This suggests that the cross-sectional associations observed for fibrinogen γ' probably reflect independent associations with each environmental or genetic factor.

5.5.2 Gene-environment interactions that affected change in the fibrinogen variables over time

The FGG 9340 T>C with HbA1c and time interaction was the only significant gene-environment interaction that affected total fibrinogen concentration over the five-year period. In general there was a negative association between change in total fibrinogen over time and HbA1c in 2005 (*Figure 4.10*, Chapter 4). It seems that 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 mutant than in the wild-type genotype, reaching statistical significance in the homozygous mutant group. It should be kept in mind that the change in HbA1c concentrations over time also showed a negative association with HbA1c concentrations in 2005, indicating that, in individuals with high HbA1c concentrations in 2005, HbA1c concentrations decreased over the five-year period. It should also be mentioned that participants diagnosed with diabetes in 2005 were newly diagnosed, as known diabetes status was an exclusion criterion. Individuals diagnosed with diabetes at the start of the study were referred to local clinics for diabetic treatment. This may therefore explain the lower HbA1c concentrations in 2010, as they would have been receiving treatment since 2005. It is, unfortunately not known how well these individuals complied with their treatment, so this remains speculation only.

A similar gene-HbA1c interaction over a time period of three months which influenced total fibrinogen was previously explored by Ceriello *et al.* (1998) with a different SNP. In the study of Ceriello *et al.* (1998) baseline concentrations of total fibrinogen were higher in the homozygous mutant genotype, than in the other genotypes of the FGB -148 C>T SNP.

There was also a strong positive correlation between the baseline and end values of fibrinogen and HbA1c in the genotypes of this polymorphism (Ceriello *et al.*, 1998). Total fibrinogen decreased over time with glycaemic control (reduction in HbA1c after insulin therapy) in the heterozygous and homozygous mutant genotypes of the FGB -148 C>T polymorphism, but not in the homozygous wild type carriers (Ceriello *et al.*, 1998). The decrease in fibrinogen was moderate in the heterozygous genotype, but more noticeable in the homozygous mutant genotype (Ceriello *et al.*, 1998). This indicates a gene-environment interaction between the homozygous mutant genotype and HbA1c that is, at least in part, responsible for the regulation of total fibrinogen concentrations in diabetic patients (Ceriello *et al.*, 1998).

5.6 STRENGTHS AND LIMITATIONS

A strength of this study is that it is the first to explore the effects of the SNPs investigated in this study in a black South African population. The purpose was not to identify novel SNPs but rather to compare the prevalence of the SNPs and the effect of the SNPs on fibrinogen variables with what has been reported for Caucasians. Because of the higher variability in the genetic make-up of Africans, it was necessary to ascertain whether similar associations existed. The association between many of these SNPs and fibrinogen γ' was established for the first time in this study and we found that two of these SNPs (FGB -148 C>T and FGB 1038 G>A) had significant effects on fibrinogen γ' concentration. Another significant contribution made by this study to the field of knowledge, is that this is the first study that has aimed to identify factors (both environmental and genetic) that influence change in these fibrinogen variables over time. A limitation of the study is that the background information necessary to interpret some of our findings, such as ARV and anti-diabetic treatment, were not documented in detail, as this was not the main focus of the PURE study. Another limitation of this study is that some of the homozygous mutant genotypes had very small cohorts (less than 30 participants) and, therefore, had to be clustered with the heterozygous genotype during statistical analysis.

5.7 CONCLUSION

The main aim of this study was to determine the change in fibrinogen and fibrinogen γ' concentrations over a five-year period in a black South African population, subdivided according to genotypes previously reported to associate with fibrinogen and fibrinogen γ' concentrations to ascertain whether the observed changes were influenced by genotype and whether they were modulated by environmental factors. Specific objectives were:

- To determine the baseline (2005) and follow-up (2010) concentrations of total fibrinogen and fibrinogen γ' in a black South African population residing in the North West Province.
- To determine the genotype distributions of the FGA 2224 G>A [rs2070011], FGA 6534 A>G [rs6050], FGB 1038 G>A [rs1800791], FGB Arg448Lys [rs4220], FGB -148 C>T [rs1800787], FGB 1643 C>T [rs1800788], FGB 40 A>G [rs2227385], FGB 749 A>G [rs2227388], FGG 10034 C>T [rs2066865] and FGG 9340 T>C [rs1049636] SNPs in the black South African cohort.
- To determine the change in fibrinogen and fibrinogen γ' concentrations over the five-year period in participants harbouring the different genotypes, as mentioned above, of a black South African population in the North West Province.
- To assess the major lifestyle determinants of fibrinogen and fibrinogen γ' concentrations in black South Africans.
- To determine whether the hypothesised changes in the fibrinogen and fibrinogen γ' in subjects harbouring the different genotypes were modulated by identified environmental factors.

From our results it is clear that although most of the SNPs identified from the literature are also present in our African population, the frequencies of the respective genotypes differ significantly. The mutant allele of two SNPs found in the promoter area of the FGB gene was found not to be present in populations of European descent, but was present in populations of African ethnicity. This again confirms the assertion that Africans have a larger genetic variability than individuals of European descent.

There is also general agreement between what we found to be the effects of these SNPs on fibrinogen and fibrinogen γ' concentration and what is documented in the literature. The SNPs with the strongest cross-sectional influence on total fibrinogen concentrations in carriers of the mutant allele were FGA 2224 G>A, FGG 10034 C>T and FGG 9340 T>C. The SNPs having the strongest influence on fibrinogen γ' concentrations were FGA 2224 G>A and FGG 9340 T>C (increased concentrations), and FGA 6534 A>G and FGG 10034 C>T (decreased concentrations). For some of the SNPs, however, our results differed from what was reported in the literature, probably due to the ethnic differences between our population and populations in the literature and/or differences in environmental factors between the African and European settings. The presence of significant gene-environment interactions indicated that environmental factors significantly influence genetic effects on fibrinogen concentration in Africans.

Our results also indicated that both total fibrinogen and fibrinogen γ' increased significantly from 2005 to 2010 in both the rural and urban study populations as well as in both men and women, although the exact mechanistic pathways still need to be elucidated. We also demonstrated that environmental factors significantly influenced both total fibrinogen and fibrinogen γ' . The major environmental factors that affected total fibrinogen and fibrinogen γ' concentrations in the PURE population were CRP, IL-6, BMI, HbA1c, age, blood lipids, HIV status and tobacco use. CRP, IL-6, BMI, HbA1c, age and LDL-cholesterol had a positive association with total fibrinogen and fibrinogen γ' , while HDL-cholesterol had a negative association with the fibrinogen variables.

Environmental factors can influence total fibrinogen and fibrinogen γ' individually or in combination, as can be seen from the rural-urban differences, in which the rural and urban settings can be considered to represent a specific clustering of environmental factors. It was also clear from the results that the influence of the environmental factors was mediated by genetic polymorphisms.

The gene-environment interaction between FGB 1643 C>T and urbanisation for example indicates that the specific clustering of environmental factors present in the urban and rural settings could significantly influence gene expression of the wild-type allele of FGB 1643 C>T while it does not do so for the mutant allele.

With regard to the genotypes of the different SNPs, total fibrinogen increased consistently from 2005 to 2010 in all 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 although in some instances decreases were observed, the most notable of these being in the mutant allele carriers of FGG 10034 C>T.

In studying the possible influence of single SNPs or individual environmental factors on change in fibrinogen and fibrinogen γ' over time, it became clear that many of them associated with total fibrinogen and fibrinogen γ' concentration. *In vivo* these factors do not occur in isolation, however, but in the presence of a whole array of other biological factors. We therefore employed the mixed-models technique which enabled us to investigate the associations of each genotype and the environmental factor while taking into account the effect of the other genotypes and environmental factors present. Using this approach, we found four gene-environment interactions that significantly influenced total fibrinogen on a cross-sectional level. These were: FGA 2224 G>A with age, FGB Arg448Lys with HIV status, FGB 1643 C>T with urbanisation and FGB 1038 G>A with HbA1c. This interaction may partly explain why total fibrinogen is increased in some diabetics and not in others.

Using a two-way interaction term we were able to identify individual genetic or environmental factors that influenced the change in total fibrinogen and fibrinogen γ' over time, while taking into account the influence of other genetic and environmental factors. There was no polymorphism that significantly influenced change in total fibrinogen, indicating that the change in the total fibrinogen concentrations in the investigated SNPs over the five years was possibly to a greater extent due to environmental factors rather than genetic factors. The only environmental factor that significantly influenced change in total fibrinogen over time was CRP.

Fibrinogen and CRP are both acute-phase proteins, the concentrations of which can be altered over a short-term, such as a few days; establishing longer-term associations therefore becomes extremely complex as the concentrations can be altered significantly over the short term. There were no environmental factors that significantly influenced change in fibrinogen γ' , while the FGG 10034 C>T SNP was the only SNP that remained as a significant predictor of change in fibrinogen γ' . The influence of this polymorphism on fibrinogen γ' concentrations can be ascribed to its location on the FGG gene, which is close to the polyadenylation site of the fibrinogen γ A.

Lastly, we employed a three-way interaction, in which we determined whether there was any gene-environment interaction that influenced change in the fibrinogen variables over time. The only gene-environment interaction found to significantly predict change in total fibrinogen concentrations was the FGG 9340 T>C with HbA1c, again pointing to a gene-environment interaction affecting the (complex) relationship of fibrinogen with diabetes. No interaction was found for change in fibrinogen γ' .

In conclusion, our results indicate that gene-environment interactions can significantly influence the fibrinogen variables cross-sectionally and also influence the change in concentrations over time. Although this research contributed significantly to our current knowledge base of factors influencing fibrinogen and fibrinogen γ' in Africans, many questions on the role of determinants of fibrinogen and fibrinogen γ' concentrations in health and disease remain unanswered and should be studied.

One recommendation is that future studies should be conducted among South African individuals who are HIV infected, as this will help to clarify the uncertainty regarding the effect of the different subtypes of HIV on the fibrinogen variables. More studies should also be done regarding the effect of different types of ARV treatments on the fibrinogen variables in black South African populations. More longitudinal studies of even longer duration are also needed to get a better understanding of the effect of environmental and genetic factors on the fibrinogen variables over time.

Novel polymorphisms that can influence fibrinogen and/or fibrinogen γ' should be identified in Africans, as the higher total fibrinogen concentrations in Africans in comparison with individuals of European descent remains largely unexplained. Additional studies should also be done investigating the determinants of fibrinogen γ' and the related mechanisms. Lastly studies investigating genes, outside the fibrinogen gene, known to influence total fibrinogen concentration should also be performed in Africans.