CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

Cardiovascular disease is a leading cause of morbidity and mortality in both developing and developed countries (Mathers & Loncar, 2006). In developing countries three times as many deaths due to cardiovascular diseases occur than in developed countries (Gaziano, 2007). Furthermore, cardiovascular disease in developing countries causes twice as many deaths as the human immunodeficiency virus (HIV), malaria and tuberculosis combined (Lopez *et al*., 2006), and affects 1.3 million African individuals per year (Vorster, 2002). In black South Africans, specifically, 23% of deaths are caused by this disease and the number is increasing (Vorster, 2002; Vorster *et al*., 2007). The cardiovascular disease burden in developing countries is caused mostly by the increase in the prevalence of risk factors and low access to preventive interventions (Gaziano, 2007).

Cardiovascular disease is a multifactorial disease that is a result of the interplay of multiple pathogenic mechanisms involving multiple risk factors (Gelehrter *et al*., 1998). The risk factors which are causally involved in cardiovascular disease development will briefly be mentioned. These factors include age, gender, body mass index (BMI), C-reactive protein (CRP), hypertension, physical inactivity, smoking, dyslipidaemia, diabetes mellitus, blood lipids, imprudent dietary intake, hormone replacement therapy (HRT), genetic factors and an abnormal haemostatic process (Ajjan & Grant, 2006; Albert, 2007; Ariëns *et al.*, 2002; Folsom, 2001; Lefevre *et al.,* 2004; Pruissen *et al*., 2008; Vorster, 2002).

This mini-dissertation will focus on haemostatic variables and, specifically, on fibrinogen and a variant of it, fibrinogen gamma prime (γ') as cardiovascular disease risk markers. Haemostasis is responsible for the formation and degradation of blood clots which occur in healthy individuals as a result of injury to the vessel wall. Cardiovascular disease can result if the formation and degradation process of the blood clots is not balanced and formed clots are not appropriately lysed (Ajjan & Grant, 2006; Folsom, 2001; Lefevre *et al.,* 2004).

Fibrinogen is a key haemostatic factor and is important for the formation of a stable fibrin clot or haemostatic plug (Kakafika *et al*., 2007), which ultimately prevents blood loss through the injured vessel wall. According to a review conducted by Pieters and Vorster (2008), the mean fibrinogen concentrations of black South Africans were reported to be above 2.5 g/L, indicating that black South Africans have relatively high fibrinogen concentrations which might predispose them towards the development of cardiovascular disease. Fibrinogen γ' is a variant of fibrinogen which potentially contributes to cardiovascular disease by producing a proatherogenic fibrin clot with smaller pores and thinner fibres that is more resistant to fibrinolysis (Uitte de Willige *et al*., 2009a). However, little is known about this variant in black South Africans.

Many variables associated with cardiovascular disease, including fibrinogen and fibrinogen γ', have their own set of demographic, environmental, lifestyle and genetic determinants, which play a key role in their respective risk of contributing towards the development of cardiovascular disease. This review will outline the role of fibrinogen and fibrinogen γ' in the development of cardiovascular disease and will outline the factors that influence their concentrations. It is known that genetic factors have a major effect on cardiovascular disease development, but because the focus of this review is on fibrinogen and fibrinogen γ', only genetic determinants of these variables (focusing on those measured and reported in the ensuing chapters) will be discussed. It is furthermore known that fibrinogen increases with age. While many of the factors that influence fibrinogen cross-sectionally have been identified, it has not yet been determined which factors influences the increase in levels over time. The last section of the review will therefore be dedicated to an explanation of genetic and environmental factors as well as gene−environment interactions that can potentially influence changes in fibrinogen and fibrinogen γ' concentrations over time.

2.2 HAEMOSTASIS

2.2.1 Overview of haemostasis

Haemostasis, as previously mentioned, is a process that is responsible for the formation and degradation of blood clots by involving a complex system of haemostatic factors (Lefevre *et al*., 2004). The working balance of these haemostatic factors is of great importance in allowing the body to control blood loss and, at the same time, protect the body against tissue ischaemia and necrosis, which can result in myocardial infarction or stroke due to blood clotting in a vessel (Lefevre *et al*., 2004).

The endothelium plays an important role in the haemostatic process as the endothelial cells protect blood vessels by providing a mechanical lining and controlling vascular tone through the release of vasodilators such as nitric oxide and prostacyclin (Ajjan & Grant, 2006). Upon activation, endothelial cells also play a role in the coagulation process by secreting prothrombotic agents like von Willebrand factor, factor V, plasminogen activator inhibitor (PAI) and tissue factor (Ajjan & Grant, 2006). However, the endothelial cells also produce anticoagulant factors, including nitric oxide, prostacyclin, tissue plasminogen activator (t-PA), protein C, protein S and thrombomodulin (Ajjan & Grant, 2006; Lefevre *et al*., 2004). There is a balanced secretion of these factors in a normal, healthy environment and this maintains the integrity of the surface, which ensures protection of the vessel wall and provides a healthy blood flow (Ajjan & Grant, 2006). The occurrence of endothelial damage disturbs this balance and this leads to events that play a role in the progression of the atherosclerotic process (Ajjan & Grant, 2006).

Apart from the endothelium, haemostasis involves three other biological systems, namely platelet aggregation (primary haemostasis), coagulation cascade (secondary haemostasis) and fibrinolysis, as depicted in *Figure 2.1* (Lefevre *et al*., 2004). Platelet aggregation takes place when blood vessels are damaged, by platelets adhering to the damaged endothelium (Lefevre *et al*., 2004).

Platelets are primarily responsible for starting events which lead to blood clotting (Lefevre *et al*., 2004). The platelets then become activated by binding to the subendothelial structural proteins at the site of the wound and this results in the release of biologically activated compounds such as factor V, factor VIII and factor XI, which are important for the propagation phase of coagulation (Ajjan & Grant, 2006; Lefevre *et al*., 2004; Löwenberg *et al*., 2010). Activation causes platelets to change in shape and results in aggregation *via* cross-linking by intact fibrinogen (Lefevre *et al*., 2004; Löwenberg *et al*., 2010). Platelets also play a role in the development of the atherosclerotic lesion. When platelets are activated, platelet-derived growth factor is released, which stimulates the migration and proliferation of underlying smooth muscle cells into the intimae of the injured artery segments (Lefevre *et al*., 2004).

Coagulation involves three phases: initiation, amplification and propagation (Monroe & Hoffman, 2006). Coagulation requires that haemostatic factors in the blood become activated, and starts when factor VII binds to tissue factor (TF) which is released from the subendothelium during the coagulation process (Ajjan & Grant, 2006; Lefevre *et al*., 2004). This brings about the activation of other procoagulant enzymes, such as factors IX, X and XI, and results in prothrombin being cleaved to form thrombin (Ajjan & Grant, 2006; Lefevre *et al*., 2004; Scott *et al*., 2004). Thrombin then binds to fibrinogen, from which the haemostatic plug or fibrin clot develops (Lefevre *et al*., 2004; Scott *et al*., 2004).

After the formation of the haemostatic plug, fibrinolysis takes place, causing the haemostatic plug to break up so that it can be removed from the vasculature once the endothelium has healed (Lefevre *et al*., 2004). Plasminogen, the enzyme responsible for lysis of the haemostatic plug, is activated by t-PA and urokinase plasminogen activator (u-PA) to form active plasmin (Lefevre *et al*., 2004; Mondino & Blasi, 2004). Plasmin causes the fibrin network to break down by cleaving fibrin fibres at C-terminal lysines and releasing soluble fragments, referred to as fibrin degradation products (Lefevre *et al*., 2004; Lord, 2011). As the fibrin clot degrades, more C-terminal lysine residues become exposed and serve as additional binding sites for the plasmin, which enhances the rate of fibrinolysis (Lord, 2011).

Given the important role of fibrinogen in coagulation, as described previously, an increase in this variable might predispose to cardiovascular disease risk (Ajjan & Grant, 2006). To contribute to an understanding of the variations in the concentration of fibrinogen and its variant, fibrinogen γ', a detailed outline of their respective biochemistries, as well as their roles in cardiovascular disease development, will follow, after which the genetic determinants of fibrinogen and fibrinogen γ' will be presented in *Table 2.1*.

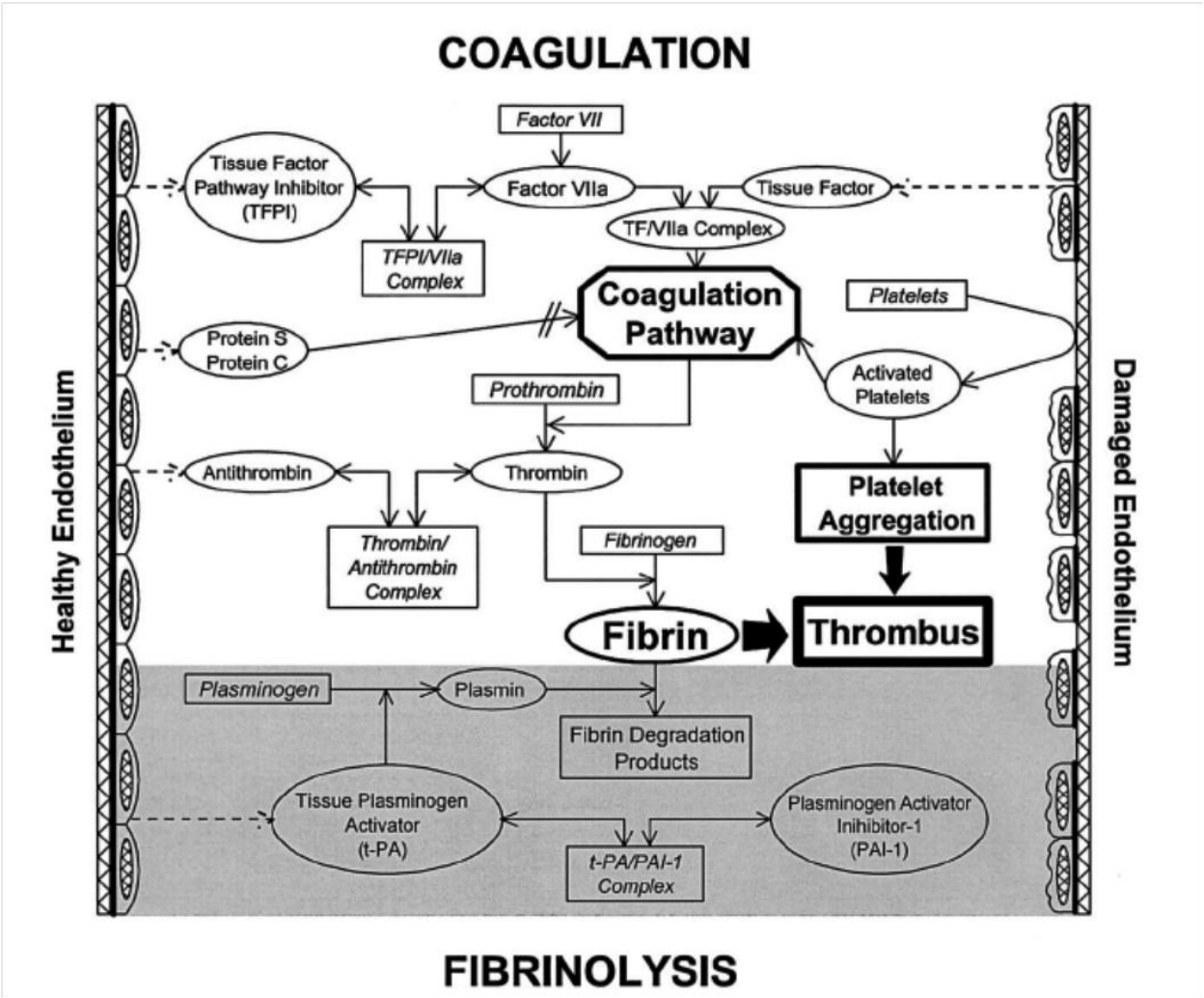

Figure 2.1: **The haemostatic pathway (taken from Lefevre *et al*., 2004)**

2.2.2 Fibrinogen

2.2.2.1 Biochemistry of fibrinogen

Fibrinogen is a soluble glycoprotein circulating in the blood (Cooper *et al*., 2003; Jensen *et al*., 2007; Kaijzel *et al*., 2006; Kamath & Lip, 2003). Fibrinogen molecules are 45 nm structures that consist of a central E region which is connected by coiled-coil segments to two outer D regions, as seen in *Figure 2.2* (Ajjan & Grant, 2006; Cooper *et al*., 2003; Uitte de Willige *et al*., 2009a). It consists of three non-identical polypeptide chains, which are the A alpha (Aα), B beta (Bβ) and gamma (γ) chains linked to one another by disulphide bonds (Ajjan & Grant, 2006; Cooper *et al*., 2003; Kamath & Lip, 2003).

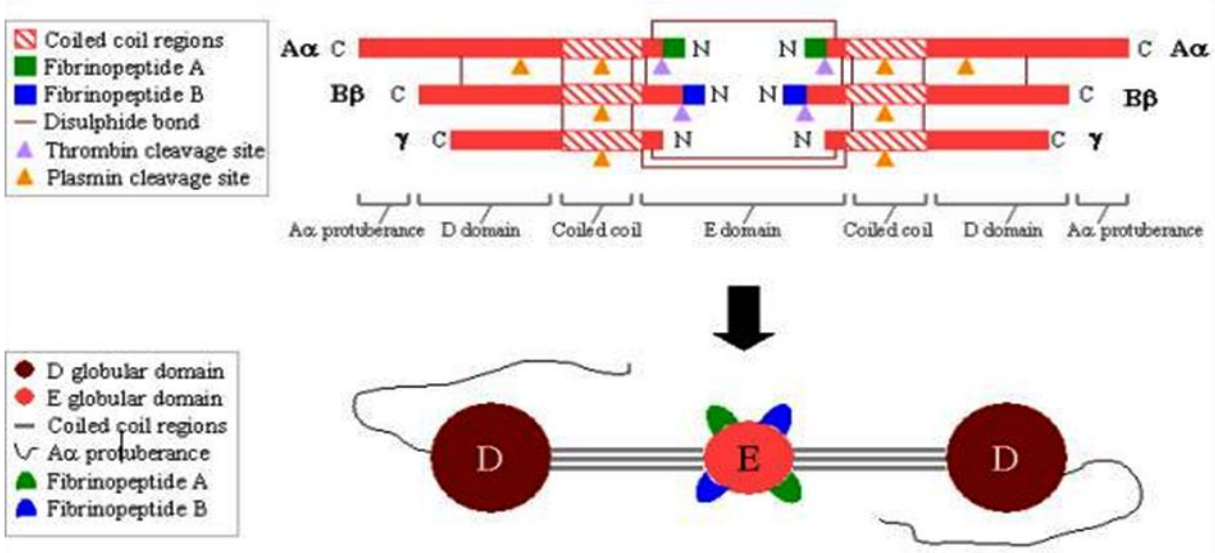

Figure 2.2: **Fibrinogen molecule (taken from McDowall, 2006)**

Fibrinogen is a heterogeneous protein, as common variants exist *in vivo.* These variants include the high molecular weight (340 kDa) form, with the presence of both carboxyl termini of the Aα-chain, the partially degraded low molecular weight (305 kDa) form, where one of the carboxyl termini of the Aα-chain is lost, and the very low molecular weight (270 kDa) form, where both the carboxyl termini of the Aα chain are lost (Jensen *et al*., 2007; Kaijzel *et al*., 2006). The Bβ chain of fibrinogen also has different variants, referred to as the normal $B\beta^A$ chain (54.182 kDA) and the $B\beta^X$ chain (53.629 kDA), which is an aberrant form of the Bβ chain (Brennan *et al*., 2009).

The $B\beta^X$ chain differs from the $B\beta^A$ chain owing to deletion of five amino acids from the centre of the coiled-coil regions on the $B\beta^X$ chain (Brennan *et al*., 2009). Additionally, the γ chain of fibrinogen also has two different variants, termed the γA chain and the γ' chain. These two chains differ regarding the number of amino acids present in the chains. Thus the γA chain consists of 411 amino acids and the γ' chain consists of 427 amino acids (Lovely *et al*., 2007; Uitte de Willige *et al*., 2009a). The γ chain of fibrinogen will be discussed in more detail later in this review.

The fibrinogen molecule has three structural regions, namely the E domain, which contains fibrinopeptides A and B as well as the amino (N) termini of all six chains, the two distal regions (D) and the alpha C (αC) domains (Ajjan & Grant, 2006). The carboxyl termini of the β and γ chains are located in the D regions, while the carboxyl termini of the α chain extend from the D regions to the αC domains, as indicated in *Figure 2.2* (Ajjan & Grant, 2006; Cooper *et al*., 2003).

The three polypeptide chains of the fibrinogen molecule are encoded by three different genes: the fibrinogen alpha (FGA), fibrinogen beta (FGB) and fibrinogen gamma (FGG) genes (Kamath & Lip, 2003; Lee *et al*., 1999; Uitte de Willige *et al*., 2005). These genes are clustered together in a 50 kb region on the long arm of chromosome 4q23-q32 (Kamath & Lip, 2003; Lee *et al*., 1999; Scott *et al*., 2004; Uitte de Willige *et al*., 2005). The FGA gene contains 6 exons and is oriented with the FGG gene, which contains 10 exons (Uitte de Willige *et al*., 2005). The FGA and FGG genes are transcribed in the direction opposite the FGB gene, which contains 8 exons and is located downstream of the FGA gene (Lee *et al*., 1999; Scott *et al*., 2004; Uitte de Willige *et al*., 2005). There are single copies of the genes, with the α gene in the middle, and the β and γ genes flanking either side of the α gene (Kamath & Lip, 2003; Scott *et al*., 2004).

Genetic variability accounts for 20−51% of variation in plasma fibrinogen concentrations and in twin studies it accounts for 40−50% of variation in plasma fibrinogen (De Lange *et al*., 2001; Freeman *et al.,* 2002; Kamath & Lip, 2003; Lane & Grant, 2000; Pearson *et al*., 1997; Scott *et al*., 2004). Fibrinogen is primarily synthesised in the liver and has a biological half-life of about three to four days (Kamath & Lip, 2003; Lee *et al*., 1999).

The usual plasma concentration of fibrinogen is between 1.5 and 4.5 g/L, but this concentration is far greater than the minimum concentration needed for haemostasis, which is 0.5−1 g/L (De Moerloose *et al.*, 2010; Kamath & Lip, 2003). In addition to the effect of the genetic make-up of fibrinogen on its concentrations, Barker *et al*. (1992) linked fibrinogen concentrations to growth in infancy. The control of the haemostatic balance in adult life is partly controlled by intrauterine and infant environments (Barker *et al*., 1992). Infants with low birth weight have reduced growth of the liver, which can lead to long-term alterations in fibrinogen metabolism (Barker *et al*., 1992).

While genetic variability accounts for about 50% of the variation in plasma fibrinogen concentration, the other 50% is influenced by environmental factors. It is important, therefore, to investigate also what effects the environmental factors have on the variation in plasma fibrinogen concentration, as well as the effects of possible gene−environment interactions. These environmental factors include ethnicity, age, gender, BMI, hypertension, smoking, diabetes mellitus, menopause in women, oral contraceptive use, HRT use, insulin concentrations, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, triglycerides (TG), leukocytes, CRP, inflammation, infection, alcohol intake, dietary intake, seasonal variations, urbanisation and physical inactivity (Ajjan & Grant, 2006; Danesh *et al.,* 2005; Feinbloom & Bauer, 2005; Kamath & Lip, 2003; Lee *et al*., 1999; Pearson *et al*., 1997; Pieters & Vorster, 2008; Scott *et al*., 2004).

2.2.2.2 Role of fibrinogen in cardiovascular diseases

Many studies have shown that elevated plasma fibrinogen is an independent risk factor for cardiovascular disease (Ajjan & Grant, 2006; Kakafika *et al*., 2007; Kamath & Lip, 2003; Kannel *et al.,* 1987; Lefevre *et al*., 2004). Kannel *et al.* (1987) reported that individuals with fibrinogen concentrations above 3.1 g/L experienced a higher incidence of coronary heart disease events. The various mechanisms through which fibrinogen can contribute to cardiovascular disease risk are discussed below.

One of the main purposes of fibrinogen is that it is the precursor protein for fibrin, which, upon activation by thrombin, forms a network that entraps platelets, red blood cells, proteins and other cells necessary to form a stable blood clot to prevent blood loss through the injured endothelium (Mosesson, 2005).

Upon binding of thrombin to fibrinogen, thrombin cleaves four short peptides from the N termini of the Aα and Bβ-chains, releasing fibrinopeptides A and B (Ajjan & Grant, 2006; Lord, 2011). The release of fibrinopeptides A and B enables the interaction between monomers to form a fibrin clot, as indicated in *Figure 2.3* (Ajjan & Grant, 2006; Lord, 2011; Mosesson *et al*., 2001).

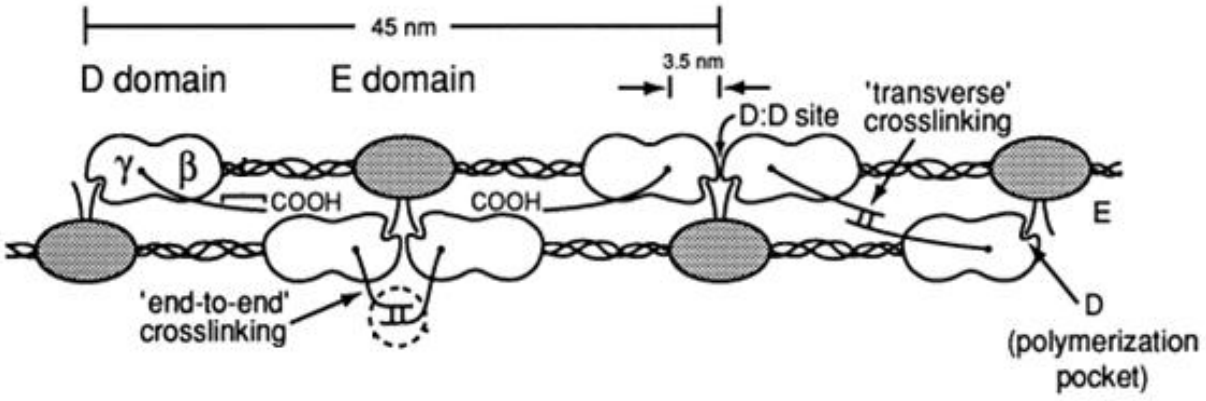

Figure 2.3: **Schematic diagram of fibrinogen, indicating the structural domains and the association sites that participate in fibrin polymerisation and cross-linking (adapted from Mosesson *et al*., 2001)**

In order to stabilise the fibrin clot, thrombin must activate factor XIII, which cross-links the fibrin fibres through the involvement of glutamine and lysine residues by a transglutaminase reaction (Ajjan & Grant, 2006; Lord, 2011; Mosesson *et al*., 2001). Formation of multiple cross-links within the γ and α-chains takes place and this results in the formation of a complex branched structure between the fibrin molecules, which forms a stable fibrin clot to protect it from premature fibrinolysis (Ajjan & Grant, 2006).

Different fibrinogen concentrations influence the structure of the fibrin clot, resulting in different disease outcomes, as seen in *Figure 2.4*.

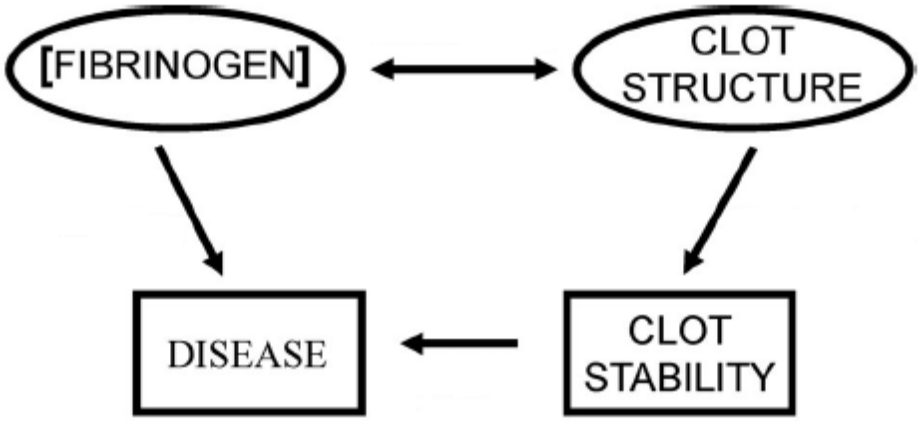

Figure 2.4: **Correlation between fibrinogen concentration, clot structure and disease (adapted from Lord, 2011)**

According to Dunn *et al*. (2004), fibrinogen concentrations correlate with fibrin clot pore size and fibre size, which are affected by an overlap between genetic and environmental factors. The fibrin clot found to be the cause of cardiovascular disease incidents is a platelet-rich fibrin mesh with thin fibres and a tight and rigid structure which is more resistant to fibrinolysis owing to small pores that restrict fibrinolytic enzymes from entering (Dunn *et al*., 2004). Elevated plasma fibrinogen concentrations cause a hypercoagulable state through the formation of this type of clot structure (Austin *et al*., 2000; Falls & Farrell, 1997; Kakafika *et al*., 2007; Mills *et al.,* 2002; Scott *et al*., 2004).

The rate of polymerisation of the fibrin clot is another factor that influences the clot structure. Polymerisation of fibrin begins when thrombin cleaves fibrinopeptide A from fibrinogen. The rate of the polymerisation is determined by, among other methods, the amount of fibrinogen (Mills *et al*., 2002). The higher the fibrinogen concentration, the faster the polymerisation rate, which is associated with denser and tighter fibrin networks (Mills *et al*., 2002).

Secondly, fibrinogen is also an acute-phase protein which is elevated during the inflammatory process; thus it plays a role in atherosclerosis, which is an inflammatory process (Folsom, 2001; Jensen *et al*., 2007; Kakafika *et al*., 2007; Kamath & Lip, 2003).

Fibrinogen plays a role in the development of atherosclerotic plaques by moving into the intimae of the injured vessel walls, where it forms cross-linked fibrin clots (Feinbloom & Bauer, 2005; Kakafika *et al*., 2007). When fibrin reaches the arterial intimae, it stimulates smooth muscle cell proliferation and migration, and fibrin then forms part of the atherosclerotic plaques in the vasculature (Folsom, 2001; Feinbloom & Bauer, 2005; Kakafika *et al*., 2007; Kamath & Lip, 2003). Inflammatory reactions also cause proliferation and migration of vascular smooth muscle cells (Ajjan & Grant, 2006). Atherosclerotic plaques have a fibrous cap and when this has a weak tensile strength it can rupture, resulting in coagulation factors being activated in the bloodstream, which can promote the formation of a thrombus (Aikawa & Libby, 2004; Ajjan & Grant, 2006; Libby, 2004). The weakening of the fibrous cap can be due to inflammatory cytokines and macrophages producing matrix metalloproteinases that degrade collagen, which is responsible for the strengthening and stabilisation of the fibrous cap (Ajjan & Grant, 2006; Libby, 2004). When a blood clot or thrombus forms inside a blood vessel it has the potential to obstruct the blood flow through the system and can result in ischaemia (Libby *et al.,* 2011).

Another mechanism through which elevated fibrinogen could potentially cause cardiovascular disease is through enhanced platelet activation, which creates a hypercoagulable state and increases plasma viscosity (De Moerloose *et al.,* 2010). Activation of platelets occurs at the site of vessel injuries, where it is stimulated by collagen and/or thrombin (Ajjan & Grant, 2006). This then results in surface exposure of procoagulant phospholipids, which causes structural changes in the platelets to form membrane blebs (Ajjan & Grant, 2006). Platelet aggregation is further facilitated by fibrinogen binding to the glycoprotein IIb-IIIa receptor on the platelet surface (Folsom, 2001; Ajjan & Grant, 2006; Kakafika *et al*., 2007; Kamath & Lip, 2003). The last 11 amino acids of the fibrinogen γ chain have an important role in platelet aggregation through the platelet-fibrinogen receptors (Ajjan & Grant, 2006). This increased red cell aggregation consequently increases plasma viscosity, limiting the fluidity of the blood, which could lead to thrombosis (De Moerloose *et al.,* 2010; Folsom, 2001; Kakafika *et al*., 2007; Késmárky *et al*., 2006). Additionally, since fibrinogen is the second most abundant protein in the blood, increased concentrations can increase plasma viscosity through its abundance (De Moerloose *et al.,* 2010; Folsom, 2001; Kakafika *et al*., 2007).

2.2.3 Fibrinogen γ'

2.2.3.1 Biochemistry of fibrinogen γ'

As previously mentioned, the polypeptide fibrinogen γ chain has two different forms, namely the γA chain and γ' chain (Lovely *et al*., 2007; Uitte de Willige *et al*., 2009a). The γA chain is the main form of fibrinogen and consists of 411 amino acids, composed of 10 exons and 9 introns in the FGG gene (Lovely *et al*., 2007; Uitte de Willige *et al*., 2009a). Polyadenylation [addition of a poly(A) tail at the end of a ribonucleic acid molecule] of this chain occurs at the polyadenylation signal downstream of exon 10 in the FGG gene, where intron 9 is spliced out; thus the polymerase translates exon 9, which is followed by exon 10 with four amino acids up to a stop codon (Cooper *et al*., 2003; Lovely *et al*., 2007; Uitte de Willige *et al*., 2009a).

Alternative polyadenylation of the γ' chain (as seen in *Figure 2.5*) occurs at the polyadenylation signal in intron 9 in the FGG gene, where intron 9 is not spliced out and the polymerase translates from exon 9 into intron 9. This results in the translation of a twenty amino acid extension at the carboxyl terminus with a stop codon present after twenty amino acids (Cooper *et al*., 2003; Lovely *et al*., 2007; Scott *et al*., 2004; Uitte de Willige *et al*., 2009a). These twenty amino acids (408-427) of the γ' chain are encoded by intron 9 and replace the four amino acids (408-411) of the γA chain of exon 10 (Cooper *et al*., 2003; Uitte de Willige *et al*., 2009a; van den Herik *et al*., 2011).

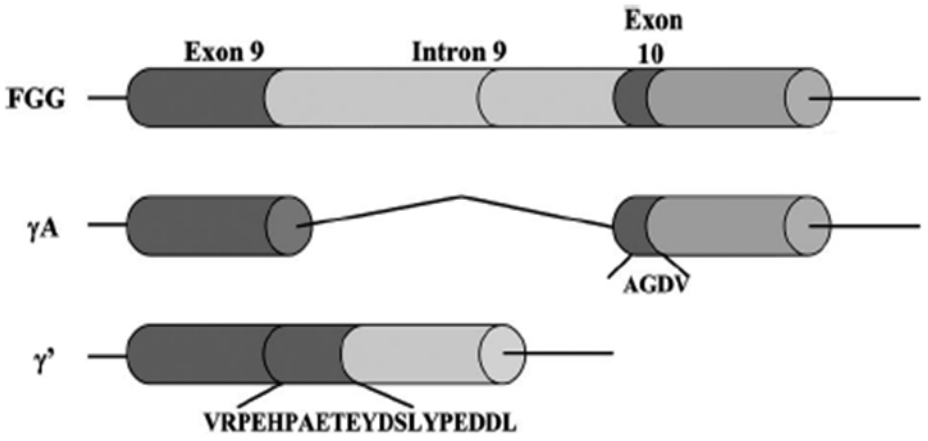

Figure 2.5: **Polyadenylation of the γA and γ' chain (adapted from Uitte de Willige *et al*., 2009a)**

The fibrinogen γ chain can be a homodimeric chain found as the γA/γA or γ'/γ' chain or a heterodimeric chain found as the γA/γ' chain (Uitte de Willige *et al*., 2009a). The total amount of the homodimeric γA and γ' variant in a healthy person is approximately 85–92%, and 0.5% of the total fibrinogen concentration, respectively (Wolfenstein-Todel & Mosesson, 1980). The total amount of the heterodimeric fibrinogen γ' variant in a healthy person is approximately 8−15% of the total fibrinogen concentration (Chung & Davie, 1984; Fornace *et al.,* 1984). The chain extension of fibrinogen γ' protrudes from the D region and can span up to 30−40 Å (Uitte de Willige *et al*., 2009a). The fibrinogen γ' chain may interact with the D-D interface during early polymerisation and possibly stretches to the E region of the neighbouring fibrinogen molecule in the D-E-D complex (Uitte de Willige *et al*., 2009a).

Fibrinogen γ' has several physiological effects, causing it to differ from fibrinogen molecules with γA chains. Firstly, fibrinogen γ' has the ability to interact with thrombin by acting as a reservoir for thrombin, possibly protecting it from inhibition by antithrombin (Scott *et al*., 2004; Siebenlist *et al.,* 1996; Uitte de Willige *et al*., 2009a). However, other studies indicate that thrombin binding to fibrinogen γ' inhibits thrombin activity (Uitte de Willige *et al*., 2009a). Thus it is still not clear whether or not thrombin is active when bound to fibrinogen γ'.

Secondly, fibrinogen γ' lacks a platelet-binding sequence, resulting in limited platelet aggregation, owing to a more anionic, carboxyl-terminal sequence than the γA chain; thus fewer platelets will be adhering to damaged blood vessels (Siebenlist *et al.,* 1996; Uitte de Willige *et al*., 2009a). Fibrinogen γ' causes a bridging action between factor XIII and activated platelets (Uitte de Willige *et al*., 2009a).

Thirdly, factor XIII can bind to fibrinogen γ', which also acts as a carrier of factor XIII, delivering factor XIII to its place of action (Falls & Farrell, 1997; Scott *et al*., 2004; Siebenlist *et al.,* 1996; Uitte de Willige *et al*., 2009a). This increases the concentration of factor XIII at the fibrin clot, resulting in more cross-linking than seen with fibrinogen γA (Falls & Farrell, 1997; Scott *et al*., 2004). Factor XIII stabilises the fibrin clot by catalysing the formation of covalent bonds between the α and γ chains of the fibrin monomers (reviewed by Voetsch & Loscalzo, 2004).

Cross-linking and factor XIII activation are also normal processes in the γA variant, but the effect seems to be enhanced in the presence of fibrinogen γ' (Siebenlist *et al*., 2005; Uitte de Willige *et al*., 2009a).

Fourthly, fibrinogen γ' concentrations can affect the fibrin network structure (Allan *et al*., 2012; Collet *et al*., 2004; Cooper *et al*., 2003; Gersh *et al*., 2009; Mannila *et al*., 2007a; Siebenlist *et al*., 2005). To date, however, only a limited number of studies have been done on fibrinogen γ' and its effect on fibrin clot structure and a great deal of controversy exists on the effects of fibrinogen γ' on the fibrin clot structure, as various studies have found different results (Allan *et al.,* 2012; Collet *et al.,* 2004; Cooper *et al.,* 2003; Gersh *et al.,* 2009; Mannila *et al.,* 2007a; Siebenlist *et al.,* 2005).

Some studies have indicated that increased fibrinogen γ' can cause the formation of thinner fibres, forming a denser fibrin network (Allan *et al.,* 2012; Cooper *et al.,* 2003; Gersh *et al.* 2009; Siebenlist *et al.,* 2005). In contrast, a study by Collet *et al.* (2004) indicates that fibrin clots formed by fibrinogen γ' are less compact than fibrin clots formed by fibrinogen γA, resulting in a decreased fibrin clot fibre density and an increased fibre diameter, while Mannila *et al*. (2007a) indicated that fibrinogen γ' concentration did not affect fibrin clot permeability.

Differences in cross-linking and branching have also been observed in the studies, as some reported an increase (Collet *et al.,* 2004; Cooper *et al.,* 2003; Gersh et al., 2009), some a decrease (Siebenlist et al., 2005) and some did not report the effect of fibrinogen γ' on cross-linking and branching (Allan et al., 2012; Mannila et al., 2007a). What the studies were in agreement on was that the fibrin clots formed by fibrinogen γ' had a longer lysing time than fibrin clots formed by fibrinogen γA (Allan *et al.,* 2012; Collet *et al.,* 2004; Cooper *et al.,* 2003; Gersh *et al.,* 2009; Mannila *et al.,* 2007a; Siebenlist *et al.,* 2005). Differences between the different studies could be due to different experimental study designs.

For example, the different studies used different fibrinogen γ' preparations, as some used heterozygous (heterodimeric) fibrinogen γA/γ' (Allan *et al*., 2012; Cooper *et al.,* 2003; Mannila *et al*., 2007a; Siebenlist *et al*., 2005), another used homozygous (homodimeric) fibrinogen γ'/γ' (Collet *et al.,* 2004) and another used both heterozygous and homozygous fibrinogen γ' (Gersh *et al*., 2009) for the different experimental studies. The fibrinogen γ' used in the different studies was also different in that some studies used fibrinogen γ' purified from pooled plasma (Allan et al., 2012; Cooper *et al.,* 2003; Siebenlist et al., 2005), fibrinogen γ' purified from plasma of different subjects (Mannila *et al*., 2007a) and others used recombinant fibrinogen γ' (Collet *et al.,* 2004; Gersh *et al*., 2009). In the studies that used fibrinogen purified from plasma, factor XIII concentrations may have varied significantly, depending on the degree to which it co-purified with fibrinogen and fibrinogen γ' (Allan *et al*., 2012; Cooper *et al*., 2003; Mannila *et al*., 2007a; Siebenlist *et al*., 2005).

Factor XIII may co-purify to a different extent with each of the different types of fibrinogen, leading to significant confounding of results. Currently there is not enough evidence and there are too many contradictory results for clear statements to be made regarding the effect of fibrinogen γ' on fibrin clot structure. Additionally, it has been found that clots containing fibrinogen γ' were non-homogeneously arranged into tight interconnecting bundles with smaller pores with bundled fibres and large open pores in other areas of the clot (Allan *et al*., 2012; Collet *et al*., 2004; Gersh *et al*., 2009).

While only a limited number of studies investigating factors influencing fibrinogen γ' concentration have been conducted to date, various factors have been identified that could potentially influence fibrinogen γ' concentration, such as genetic polymorphisms (e.g. FGG 10034 C>T, FGG 9340 T>C, FGA 2224 G>A), age, smoking, diabetes mellitus, glucose concentration, triglycerides, HDL-cholesterol, BMI, total fibrinogen concentration, insulin and gender (Lovely *et al.,* 2010; Mannila *et al*., 2007a; Uitte de Willige *et al*., 2009a). Associations between environmental or demographic factors and fibrinogen γ' have indicated that fibrinogen γ' increases as age, BMI, tobacco use, diabetes mellitus, glucose concentrations and triglycerides increase, and HDL-cholesterol decreases (Lovely *et al.,* 2010; Mannila *et al*., 2007a).

As reported by Lovely *et al.* (2010), in contrast to total fibrinogen, fibrinogen γ' does not have any association with systolic blood pressure and total cholesterol.

2.2.3.2 Role of fibrinogen γ' in cardiovascular diseases

The relationship between fibrinogen γ' and thrombosis seems to be dependent on the type of vascular disease. Increased fibrinogen γ' has been found in arterial disease such as peripheral arterial disease (Drouet *et al.*, 1999), ischaemic stroke (Drouet *et al*., 1999; Cheung *et al.*, 2008; Van den Herik *et al.,* 2011), myocardial infarction (Drouet *et al*., 1999; Mannila *et al.,* 2007a) and coronary artery disease (Lovely *et al.,* 2002).

In contrast, patients with venous disease seem to have decreased fibrinogen γ' concentrations. Uitte de Willige *et al.* (2005) found decreased fibrinogen γ' concentrations in deep venous thrombosis patients compared with controls. Mosesson *et al.* (2007) found decreased fibrinogen γ' concentrations in patients with thrombotic microangiopathy.

It seems, therefore, that fibrinogen γ' associates with prothrombotic risk in arterial disease, but with an antithrombotic effect in venous disease. A possible explanation for this is that the prothrombotic mechanisms of the fibrinogen γ' chain, such as the altered fibrin structure and elevated factor XIII activity, may prevail in arterial disease, whereas the antithrombotic mechanisms such as reduced thrombin generation and platelet activation may be more prominent in venous disease.

This notion is, however, somewhat counterintuitive as platelets are considered to play a larger role in arterial than in venous disease. To date there does not seem to be a clear explanation for the effects of fibrinogen γ' on thrombotic risk and further investigation is needed.

2.3 GENETIC SINGLE NUCLEOTIDE POLYMORPHISMS THAT INFLUENCE THE CONCENTRATION OF FIBRINOGEN AND FIBRINOGEN γ'

According to the literature, there are numerous genetic single nucleotide polymorphisms (SNPs) found that affect fibrinogen and fibrinogen γ' concentrations and their function (Ajjan *et al*., 2008; Austin *et al*., 2000; De Maat, 2001; Jacquemin *et al*., 2008; Lane & Grant, 2000; Lim *et al*., 2003; Lovely *et al.,* 2011; Mannila *et al*., 2006; Mannila *et al*., 2007a; Schmidt *et al*., 1998; Scott *et al*., 2004; Uitte de Willige *et al*., 2005; Voetsch & Loscalzo, 2004). Genome-wide association (GWA) studies further found 73 SNPs that exceeded the threshold of genome-wide significance with fibrinogen concentration (Dehghan *et al.,* 2009) and 54 SNPs that exceeded the threshold of genome-wide significance with fibrinogen γ' (Lovely *et al*., 2011).

Six GWA studies analysed 2 661 766 SNPs and found four loci that showed significant effects related to fibrinogen (De Moerloose *et al*., 2010). These loci included SNPs on the FGB gene and three SNPs outside the fibrinogen genes: the interferon regulatory factor 1 (IRF1), propionyl coenzyme A carboxylase (PCCB) and nucleotide-binding leucine rich family pyrin domain containing 3 isoforms (NLRP3) genes (De Moerloose *et al*., 2010). These genes encode proteins which are involved in inflammation (De Moerloose *et al*., 2010). Thus GWA studies are certainly needed to expand our knowledge regarding the genetic mechanisms of fibrinogen, as well as of fibrinogen γ'.

For the purpose of this study, only the SNPs mentioned in *Table 2.1* will be discussed in detail as these have been reported to have the most abundant effects on fibrinogen and fibrinogen γ' concentrations. SNPs can be missense mutations within a coding region which result in either a change of the nucleotide without affecting the amino acid, or in a nucleotide change that result in the coding of an alternative amino acid (Roche & Mensik, 2003). There are two types of missense mutations, namely a transition or transversion. A transition results when one pyrimidine (cytosine or guanine) is replaced by another pyrimidine, or one purine (adenine or thymine) is replaced by another purine (Roche & Mensik, 2003). A transversion results when one purine is substituted for a pyrimidine or *vice versa* (Roche & Mensik, 2003).

When a genetic variation is within an untranslated (non-coding) region of the gene it could change gene expression, but not the amino acid or protein as this region is not translated (Roche & Mensik, 2003). Genetic variations within the promoter region (includes untranslated sequences responsible for the proper initiation of transcription) and terminator region (contains an untranslated signal for addition of a sequence of adenosine residues at the end of transcription) might influence the normal expression of the gene (Nussbaum *et al.* 2004).

Table 2.1: **Genetic single nucleotide polymorphisms of fibrinogen and fibrinogen γ'**

Alteration name	Mutation	Rs number	Effect of SNPs on fibrinogen γ' concentration		Effect of SNPs on total fibrinogen concentration		Studies that reported no effect on fibrinogen and/or fibrinogen γ' concentration
			Increase	Decrease	Increase	Decrease	
FGA 2224 G>A	UTR-5*	rs2070011	Mannila *et al*. (2007a)	Lovely *et al*. (2011)	Mannila *et al*. (2006) Mannila *et al*. (2007a)	Carty *et al*. (2008) Jacquemin *et al*. (2008) Ken-Dror *et al.* (2012)	Mannila *et al*. (2007b)
FGA 6534 A>G Or FGA Thr312Ala	Missense mutation – transversion*	rs6050	None	Lovely *et al*. (2011)	Lim *et al*. (2003) Reviewed by Voetsch & Loscalzo (2004) Scott *et al*. (2004) Uitte de Willige *et al*. (2005)	Carty *et al*. (2008)	Jacquemin *et al*. (2008) Titov *et al.* (2012)

Table 2.1 **(continued)**

Alteration name	Mutation	Rs number	Effect of SNPs on fibrinogen γ' concentration		Effect of SNPs on total fibrinogen concentration		Studies that reported no effect on fibrinogen and/or fibrinogen γ' concentration
			Increase	Decrease	Increase	Decrease	
FGB Arg448Lys (A>G)	Missense mutation – transversion*	rs4220	Not determined	Not determined	Jacquemin *et al*. (2008) Ken-Dror *et al.* (2012) Leung Ong *et al*. (2010)	Dehghan *et al.* (2009)	Lim *et al*. (2003) Scott *et al*. (2004)
FGB -148 C>T	nearGene-5 Untranslated region*	rs1800787	Not determined	Not determined	Cook *et al*. (2001) Reviewed by Grant & Humphries (1999) Titov *et al.* (2012) Wong *et al.* (2008) Wypasek *et al.* (2012)	None	Schmidt *et al*. (1998)

Table 2.1 **(continued)**

Alteration name	Mutation	Rs number	Effect of SNPs on fibrinogen γ' concentration		Effect of SNPs on total fibrinogen concentration		Studies with no effect on fibrinogen and/or fibrinogen γ' concentration
			Increase	Decrease	Increase	Decrease	
FGG 10034 C>T	nearGene-5 Untranslated region*	rs2066865	None	Carty *et al*. (2008) Grünbacher *et al*. (2007) Lovely *et al*. (2011) Uitte de Willige *et al*. (2005) Uitte de Willige *et al*. (2007) Uitte de Willige *et al*. (2009b)	None	Carty *et al*. (2008)	Jacquemin *et al*. (2008)
FGG 9340 T>C	UTR-3*	rs1049636	Lovely *et al*. (2011) Mannila *et al*. (2007a) Uitte de Willige *et al*. (2009b)	None	Lovely *et al*. (2011)	None	Jacquemin *et al*. (2008) Mannila *et al*. (2006) Mannila *et al*. (2007b) Uitte de Willige *et al*. (2005)

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

Apart from the six SNPs identified from the literature, four additional SNPs were also investigated (*Table 2.2*). These four SNPs were identified by sequencing the promoter area of the fibrinogen β-gene, being the rate limiting factor in the gene transcription (Humphries *et al.,* 1997), of thirty participants of the PURE study to determine which of the SNPs are prevalent in the African population. A number of SNPs were identified and with Haplotype analysis [molecular genetic testing which identifies closely linked segments of deoxyribonucleic acid (Nussbaum *et al.* 2004)], these four tagging SNPs (FGB 1038 G>A, FGB 1643 C>T, FGB 40 A>G and FGB 749 A>G) were identified.

Table 2.2: **Four tagging SNPs identified by Haplotype analysis**

Alteration name	Mutation	Rs number	Studies related to SNPs
FGB 1038 G>A	nearGene-5 Untranslated region*	rs1800791	Carty *et al*. (2008) – no effect on total fibrinogen Jacquemin *et al*. (2008) – no effect on fibrinogen Mannila *et al*. (2006) – no effect on total fibrinogen Uitte de Willige *et al*. (2005) – no effect on total fibrinogen
FGB 1643 C>T	nearGene-5 Untranslated region*	rs1800788	Jacquemin *et al*. (2008) – no effect on total fibrinogen Ken-Dror *et al.* (2012) – decrease total fibrinogen concentrations Titov *et al.* (2012) – no effect on total fibrinogen Uitte de Willige *et al*. (2005) – no effect on total fibrinogen Lovely *et al*. (2011) – decrease fibrinogen γ' concentrations

Table 2.2 **(continued)**

Alteration name	Mutation	Rs number	Studies related to SNPs
FGB 40 A>G	nearGene-5 Untranslated region*	rs2227385	None
FGB 749 A>G	nearGene-5 Untranslated region*	rs2227388	None

** = dbSNP, 2012; A = adenine; Ala = alanine; C = cytosine; FGB = fibrinogen β; G = guanine; nearGene-5 = Includes the upstream promoter region and untranslated 5' mRNA; T = thymine*

Below in *Table 2.3*, more information is given regarding the different studies presented in *Table 2.1* and *2.2* in terms of study population, age and gender. Differences in the study design and population may explain the different results observed in the different studies.

Table 2.3: **Variables of study populations in studies mentioned in *Table 2.1* and *2.2***

Reference	Study population	Mean age	Gender
Schmidt *et al*. (1998)	399 white European (Austria) individuals with history of cerebrovascular risk factors and carotid atherosclerosis	60.1 years	195 men and 204 women
Cook *et al*. (2001)	453 white, 459 South Asian and 479 black individuals, of South Asian, West African and Afro-Caribbean ethnicity. Fifty-five had a history of MI and 163 had a history of diabetes.	White 49.4 years, South Asian 48.9 years and Black 50.5 years	762 women and 629 men
Lim *et al*. (2003)	125 white European (United Kingdom) patients with clinical diagnosis of acute stroke	69 years	65 men and 60 women

Table 2.3 **(continued)**

Reference	Study population	Mean age	Gender
Uitte de Willige *et al*. (2005)	474 European (Netherlands) patients with DVT and 474 European (Netherlands) controls	45 years for patients and controls	For each group 272 women and 202 men
Mannila *et al*. (2006)	60 European (Sweden) patients with a first MI	54 years	Not mentioned
Grünbacher *et al*. (2007)	358 DVT patients, 354 in-house controls and 429 population-based controls (European - Austria)	DVT patients 53.3 years, in-house controls 54 years and population-based controls 57 years	DVT patients, men 154 and women 204; in-house controls, men 146 and women 208; population-based controls, men 210 and women 219
Mannila *et al*. (2007a)	387 European (Sweden) patients with prior MI and 387 controls	Patients 52.5 years and controls 53 years	Patients (women) 18, patients (men) 82, controls (women) 18 and controls (men) 82
Mannila *et al*. (2007b)	1213 European (Sweden) patients with MI and 1561 controls	Patients (men) 58.3 years, controls (men) 58.8 years, patients (women) 61.6 years and controls (women) 62 years	Patients (men) 852, patients (women) 361, controls (men) 1054, controls (women) 507
Uitte de Willige *et al*. (2007)	*In vitro* study		

Table 2.3 **(continued)**

Reference	Study population	Mean age	Gender
Carty *et al*. (2008)	3969 American adults of European descent and 719 of African descent with no prior MI or stroke	73 years	2359 European American women, 1610 European American men, 463 African American women and 655 African American men
Jacquemin *et al*. (2008)	895 European (Germany, Spain, Finland, Italy and Sweden) patients with history of MI, but not less than 3 months prior to study	63.1 years	694 men and 201 women
Wong *et al.* (2008)	265 Southern Chinese subjects	Men: 47.3 years and women: 45.9 years	140 men and 125 women
Dehghan *et al.* (2009)	GWA study composed of 6 population based studies with subjects of European decent	Ranged from 46.6 years to 73.2 years	Men and women included in all studies of equal proportions
Uitte de Willige *et al*. (2009b)	African American: 537 DVT and/or PE patients and 586 controls. Caucasian: 557 DVT and/or PE patients and 678 controls.	African American: DVT and/or PE patients – 46.8 years and controls – 47.9 years. Caucasian: DVT and/or PE patients – 50.3 years and controls – 50.2 years.	African American: DVT and/or PE patients (227 men and 310 women) and controls (238 men and 348 women). Caucasian: DVT and/or PE patients (322 men and 235 women) and controls (384 men and 294 women).

***Table 2.3* (continued)**

Reference	Study population	Mean age	Gender
Leung Ong *et al*. (2010)	1294 Hypertensive Chinese subjects	42.3 – 56.9 years at baseline and 48.6 – 59.5 years at follow-up	664 women and 630 men at baseline, 664 women and 630 men at follow-up
Lovely *et al*. (2011)	3042 American subjects with CVD risk factors	61 years	1629 women and 1413 men
Ken-Dror *et al.* (2012)	2778 subjects of NPHS-II study and 3705 subjects of WH-II study. All are European subjects from the United Kingdom.	NPHS-II study: 58.5 years WH-II study: 52 years	NPHS-II study: All were men. WH-II study: 70% men and 30% women at beginning of recruitment, but no indication of how many men and women at the end of study.
Titov *et al.* (2012)	200 Russian ischaemic stroke patients and 140 Russian controls	Ischaemic stroke patients: 64.1 years Controls: 61.8 years	Ischaemic stroke patients: 123 men and 77 women Controls: 78 men and 62 women
Wypasek *et al.* (2012)	243 Polish white patients with stable angina	64.6 years	185 men and 58 women

CVD = Cardiovascular disease; DVT = Deep vein thrombosis; GWA = Genome-wide association; MI = Myocardial infarction; NPHS-II = Second Northwick Park Heart Study; PE = Pulmonary embolism; WH-II = Whitehall-II study

As seen from *Table 2.3*, most of the studies were done among Caucasian individuals, mostly of 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. The reason, therefore, for the different results in the studies of Cook *et al*. (2001), Schmidt *et al*. (1998) and Titov *et al.* (2012), as well as for the difference in results of Leung Ong *et al*. (2010) and Lim *et al*. (2003), could be the different ethnic groups studied.

The different results found in the studies of Ken-Dror *et al.* (2012) and Titov *et al.* (2012) could also be due to the different ethnic groups studied. The study of Uitte de Willige *et al*. (2007) was an *in vitro* study, but did not show different results from the majority of studies reported in *Table 2.3*. None of the studies were underpowered and most studied subjects were between forty and seventy years of age. Most studies included subjects representative of both genders in the same proportions, except for the studies of Carty *et al*. (2008), Jacquemin *et al*. (2008), Mannila *et al*. (2007a) and Mannila *et al*. (2007b), which could be a reason for the different results obtained by the studies, as gender differences have been shown to influence fibrinogen and fibrinogen γ' concentrations (Kamath & Lip, 2003; Lovely *et al.,* 2010). Currently, more GWA studies are needed as these studies can detect unsuspected genetic associations with phenotypes across millions of loci (De Moerloose *et al*., 2010).

2.4 GENE–ENVIRONMENT INTERACTIONS

Both genetic background and environmental factors play a role in the variability of fibrinogen concentrations between individuals (De Maat, 2001; Jacquemin *et al*., 2008); however, gene–environment interactions (phenotypic effect of interactions between genes and the environment) also exist. In studying gene–environment interactions, information about both genetics and the environment is required (Hunter, 2005). Thus many studies collect both genetic and environmental data in order to examine the interaction between the two (Hunter, 2005). For example, fibrinogen is an acute-phase protein and its plasma level rises during infection or injury, but it is possible that the genotype of some individuals has a greater response to the inflammatory environmental factors than that of other individuals and that, therefore, the mean increase in their plasma fibrinogen concentrations in response to moderate environmental stimuli is greater than in the individuals with the lower response to inflammatory environmental factors (Humphries *et al*. 1999). Thus in some individuals who have a particular lifestyle or environment, the genotype will have a more significant effect than in other individuals, where it will make no essential contribution (Humphries *et al*., 1999). For example, Grant (1997) reported that fibrinogen has genotype-specific regulation by both exercise and smoking. As reviewed by Voetsch and Loscalzo (2004), the results of several genetic polymorphisms (FGB Arg448Lys and factor XIII fibrinogen gene) are enhanced in smokers in comparison with non-smokers (Lim *et al.,* 2003; reviewed by Voetsch & Loscalzo, 2004).

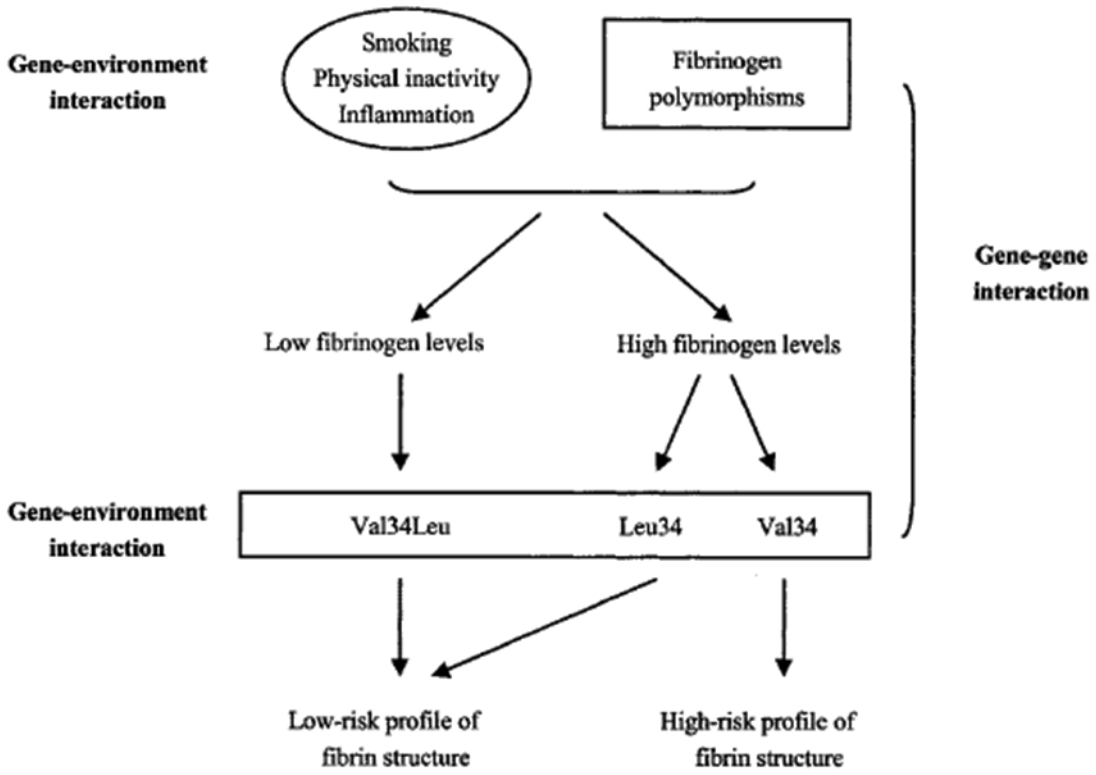

Figure 2.6: **Gene–environment interactions (taken from Voetsch & Loscalzo, 2004)**

Figure 2.6 is an example of gene–environment interactions that can influence the fibrin clot structure. Environmental risk factors, like smoking, physical inactivity and inflammation, interact with fibrinogen polymorphisms, which then determine the fibrinogen concentrations (Lim *et al*., 2003; reviewed by Voetsch & Loscalzo, 2004). The fibrinogen concentrations then interact with the factor XIII polymorphisms which influence the fibrin clot structure (Lim *et al*., 2003). This effect is caused by complex gene–environment interactions as well as gene–gene (factor XIII and fibrinogen polymorphism) interactions (Lim *et al*., 2003). According to Lovely *et al.* (2011), fibrinogen γ' concentrations are higher in individuals with cardiovascular disease than individuals without cardiovascular disease as fibrinogen γ' is an acute-phase reactant which increases during inflammation. Therefore, in this case, where cardiovascular disease is considered an inflammatory disease, environmental factors may be more prominent than genetic factors, which may not play a major role in this association (Lovely *et al.,* 2011).

There is a scarcity of studies aiming to detect gene–environmental interactions between the polymorphisms affecting fibrinogen and fibrinogen γ'. Therefore, one of the novel contributions of this research will be the exploration of possible gene-environment interactions between the polymorphisms and environmental factors that were determined in this study.

2.5 CHANGE OVER TIME

Fibrinogen concentration is known to increase with age (Kamath & Lip, 2003), but the underlying causes remain to be identified. Friedlander *et al*. (1995) determined that the phenotypic variation in fibrinogen concentration increases with age, as at the age of 20 and 80 years, environmental factors accounted for 10% and 60% of fibrinogen variation respectively. As reviewed by Danesh and colleagues (2005), when a cardiovascular disease event occurs, the strength of the association of fibrinogen declined as age increased. It was determined that between the ages of 60 and 69 years the usual fibrinogen concentrations were associated with an approximately two-fold increased risk of cardiovascular disease before the cardiovascular disease event occurred, but between the ages of 40 and 59 years the risk of cardiovascular disease was about 50% higher after a cardiovascular disease event occurred when compared with the older age groups (Danesh *et al*., 2005). The effect of genetic contribution to haemostasis with regard to cardiovascular diseases at older ages is not strong (Bladbjerg *et al*., 2006). Thus while the effect that genetic variation has on haemostatic variables in elderly individuals, including fibrinogen, is important, environmental factors are of equal importance (Bladbjerg *et al*., 2006). It is likely that the effect of genetic factors decreases with increasing age, as the effect of inflammatory factors (a major biological stimulus of fibrinogen production) increases owing to the presence of cardiovascular disease and other age-related diseases (Bladbjerg *et al*., 2006; De Maat *et al*., 2004). The environmental factors that influence haemostatic variables also modulate the risk of cardiovascular diseases through life-long interactions with multiple genes (Bladbjerg *et al*., 2006).

Currently, there is limited literature regarding the change of total fibrinogen and fibrinogen γ' over time, and the change over time in individuals harbouring specific genotypes. More studies are needed to investigate this effect as it could influence public health strategies that address cardiovascular disease risk over the lifespan. The main aim of the current study will address this hypothesis.

2.6 CONCLUSION AND RECOMMENDATIONS

From this review it is clear that fibrinogen and fibrinogen γ' have significant associations with cardiovascular disease, although research on fibrinogen and fibrinogen γ' concentrations in black South Africans is necessary as not many studies regarding fibrinogen and fibrinogen γ' concentrations have been conducted in this population. Furthermore, studies that investigate the influence of genetic polymorphisms on fibrinogen and fibrinogen γ' concentrations are needed, especially in African populations, which are known to be the most genetically diverse groups (Chen *et al*., 1995). Studies that investigate both genetics and the environmental factors are necessary to increase our knowledge regarding possible gene-environment interactions. It would also be valuable to study the change of fibrinogen concentrations over time and the determining factors involved in this change, as such a study could provide us with treatment modalities that might decrease the risk of cardiovascular disease in individuals harbouring certain SNPs.