

**FAMILIAL HYPERCHOLESTEROLAEMIA -
THE CAPE TOWN EXPERIENCE**

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DECLARATION

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Jean Catherine Firth

Date

DEDICATION

I dedicate this work to my 'girl-children' -
my daughter Cathy, my daughter-in-law Heather
and my grand-daughter Ella Grace -
with the message, that although there are prior claims
in a women's life, you are never too old to fulfil your dreams!

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ABSTRACT

This dissertation is a descriptive study of 1029 patients with familial hypercholesterolaemia attending a referral clinic for severe dyslipidaemia.

The author was involved in patient management and clinical research over a period of almost 20 years. Information for the thesis was extracted from patient records and expanded to include data obtained from a routine as well as a research lipid laboratory. Data were analysed using conventional statistical methods.

The thesis reviews familial hypercholesterolaemia from its original description to current opinions. A historical perspective, with particular emphasis on South African founder effects is given. Lipoprotein metabolism is briefly reviewed to enable a better understanding of the pathology and complications of the disease. Demographics of patients attending the clinic, their clinical signs, lipid profiles and complications are analysed in the context of different ethnic origins and genetic defects.

Lipoproteins, especially low density lipoprotein particle size, were examined in detail, with particular reference to the risk of ischaemic heart disease. Factors known to influence low density lipoprotein size in normals were examined. Body mass, age, gender and triglyceride concentration all had the expected effects. Carotid intima media thickness was examined to ascertain whether it could discriminate between early and late onset of heart disease in middle-aged subjects with familial hypercholesterolaemia. Patient management and response to treatment was analysed in the context of gender, lifestyle, ethnic origin, lipid profile and genotype.

Familial hypercholesterolaemia in Cape Town identifies high risk individuals with an average age of cardiovascular complications of 43 years in men and 49 years in women. Average age of death

in 1994 was 48 years, this improved to 55 years by 2001, presumably as a consequence of statin treatment. In the study cohort, 46% had an identified genotype, varying from 94% in the Asian population to 24% in patients with mixed ancestry. The first description of a geographic distribution of Afrikaners with familial hypercholesterolaemia is given. This regional variation might relate to migration of early settlers away from the Cape. Null defects of the low density lipoprotein receptor resulted in worse biochemical and clinical effects whilst variation in low density lipoprotein size did not appear to confer additional risk. Patients with very premature ischaemic heart disease tended to smoke more, had lower high density lipoprotein and higher low density lipoprotein, but did not have thicker intima media thickness.

ABBREVIATIONS

ACAT	acylCoA:cholesterol acyltransferase
Apo	apolipoprotein
CAD	coronary artery disease
CE	cholesterol ester
CETP	cholesterol ester transfer protein
CM	chylomicron
CMR	chylomicron remnants
CVD	cardiovascular disease
CTX	cerebro tendinous xanthomatosis
EGF	epidermal growth factor
FCH	familial combined hyperlipidaemia
FDB	familial defective apolipoprotein B
FH	familial hypercholesterolaemia
GSH	Groote Schuur Hospital
heFH	heterozygous familial hypercholesterolaemia
hoFH	homozygous familial hypercholesterolaemia
HL	hepatic lipase
HDL	high density lipoprotein
HDLC	high density lipoprotein cholesterol
HMGCoA	hydroxymethylglutaryl coenzyme A
ICA	internal carotid artery
IHD	ischaemic heart disease
IMT	intima media thickness
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
LDLC	low density lipoprotein cholesterol
LDLR	low density lipoprotein receptor
Lp(a)	lipoprotein small a
LPL	lipoprotein lipase
LPR LDL	receptor related protein
MTHFR	methylenetetrahydrofolate reductase
MTP	microsomal triacylglycerol transfer protein
NARC1	neural apoptosis regulated convertase 1
NEFA	non esterified fatty acids
PCR	polymerase chain reaction
PCSK9	proprotein convertase subtilisin/kexin 9
PL	phospholipid
SCAP	sterol cleavage activator protein
SREBP	sterol regulatory element binding protein
TC	total cholesterol
TG	triacylglycerol (triglyceride)
UCT	University of Cape Town
VLDL	very low density lipoprotein

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1.1 Historical review

Leonardo da Vinci has been credited with many brilliant discoveries and inventions. It is not commonly known that he was the first to document arterial wall thickening in the elderly and to suggest that this could be due to “excessive nourishment from the blood.” (1) (He also clearly documented the xanthelasma on the inner aspect of the left upper eyelid of the Mona Lisa!) Marchand (around 1860) first used the word “atherosclerosis” to describe the combination of atheroma (gruel-like) and sclerosis (hardening) which he observed in the aorta and large vessels at autopsy. (2)

Virchow gave the first accurate description of the process of atherosclerosis and proposed the “response to injury” theory in which he suggested that a mechanical cause was most likely. (3) This view was opposed by von Rokitansky (4) who proposed a “thrombogenic theory”. Both these theories recognised that the process of atherosclerosis involved lipid accumulation and were thus not mutually exclusive.

Despite these early observations it was only at the beginning of the twentieth century that the association between atheroma and raised serum cholesterol levels was made. In 1914 Schmidt concluded that an elevated cholesterol level was the cause of xanthomata and that there was a hereditary component to the problem. (5) At the same time Anitschkow found, that by feeding cholesterol to rabbits he could produce atherosclerosis similar to that found in humans. (6) The “lipid hypothesis” was thus postulated, implicating a raised serum cholesterol as the cause of atheroma.

The first observation of a possible link between xanthomata and cardiac disease was made by

Harbitz in 1925. He described patients with extensive tendon xanthomata who had sudden unexpected deaths and who were found to have atheromatous vessel changes at autopsy. What was even more striking, was the fact that this condition appeared to be hereditary. Muller described this as a clinical entity, publishing case studies linking tendon xanthomata, hypercholesterolaemia and ischaemic heart disease in family members. (7) He described familial hypercholesterolaemia as a genetic disorder of cholesterol metabolism. In 1951 these clinical observations were confirmed by both Aldersberg and Leys. (8;9)

As far back as 1897 Sir William Osler observed that genetic factors played a role in coronary heart disease. (10) Muller confirmed these observations but it was Khachadurian who first proposed that this disorder was an autosomal dominant condition and defined heterozygous and homozygous phenotypes. (11)

Goldstein and Brown confirmed the genetic basis of Familial Hypercholesterolaemia (FH). (12) This spurred on the quest to discover the biochemical basis for the disease, culminating in the award of the Nobel Prize for Medicine and Physiology to Goldstein and Brown in 1985 for the description of the defect in the LDL receptor. This defect results in inadequate cholesterol uptake from the blood by the liver, causing hyperlipidaemia. They then went on to describe the regulation of the cholesterol synthetic pathway. (13)

As early as 1977 Barndt and Blankenhorn indicated that regression of femoral artery atherosclerosis was possible by modifying plasma lipoprotein concentration with medication. (14) In 1988 Illingworth suggested that the rationale behind treating patients with hypercholesterolaemia was based on the premise that effective sustained lowering of these atherogenic particles would lead to slowing of the rate of progression of the atherosclerotic process, arresting the process completely and even causing regression of existing atherosclerosis. (15) With the discovery by Dr Akira Endo that a new class of drug, the

HMGCoA reductase inhibitors, could effectively lower cholesterol, the possibility of treating patients with FH became a reality. (16;17)

Although there was no doubt that the severe (genetic) dyslipidaemias required treatment, the lack of powerful and safe drugs to lower plasma cholesterol and the apparent marginal benefits in intervention studies lead to a reduction in enthusiasm for lowering cholesterol levels. (18)

The landmark Scandinavian Simvastatin Survival Study ("4S") published in 1994 conclusively established that cholesterol lowering was effective in reducing death and cardiac events in patients with hypercholesterolaemia. (19) This finally dismissed any doubt that cholesterol lowering was an essential elem in the strategy of reducing coronary artery disease. The advent of the HMGCoA reductase inhibitors, now known as "statins", has been hailed as one of the major medical advances of the past two decades.

1.2 Definition

Familial Hypercholesterolaemia (FH) is an autosomal dominant disorder characterised clinically by a family history of premature ischaemic heart disease, lipid deposits manifesting chiefly as tendon xanthomata and biochemically by elevated plasma LDL cholesterol level.

There are currently three different genes which account for the clinical phenotype. All three relate to impaired clearance of LDL and are inherited in an autosomal dominant fashion. The possibility exists that further genetic defects may be unveiled in the future.

The most commonly affected gene involves the LDL receptor gene located on chromosome 19 and is inherited as a dominant trait. It is one of the most commonly occurring single gene

defects amongst a host of inborn errors of metabolism, occurring at a worldwide frequency of 1/500. It causes hyperlipidaemia and premature atherosclerosis with resultant heart disease. (20) More than 840 different mutations in the LDL receptor gene have been described to date and new mutations are regularly being identified. (21)

The same phenotype is caused by two other biochemical defects. Familial Defective Binding of apoB₁₀₀ (FDB) is a condition in which the ligand on LDL, namely apoB, is not recognised by the LDL receptor, resulting in diminished clearance and an elevated LDL concentration. Mutations in the (22) apoB₁₀₀ gene alter binding capacities of apoB₁₀₀. The classical form of FDB is caused by a single base change in the apoB gene located on chromosome 2, resulting in an arginine to glutamine substitution at the codon for amino acid 3500 of apoB₁₀₀ (R3500Q). Other mutations have also been described - R3500W (23) and R3480W. In persons of Celtic origin the mutation R3531C has been reported. (24) A new but frequently occurring mutation of apoB₁₀₀, apoB His3543Tyr, has recently been described. (25) FDB may result in a milder phenotype than FH, especially in children (26), but the adult phenotype is generally regarded as indistinguishable from FH. (27;28) The estimated frequency in the United Kingdom, the Netherlands and Germany is 1/600-700 but in Switzerland and the Rhein-Main area of Germany the prevalence can be as high as 1:72 - 1:114. (29)

A third FH gene abnormality also resulting in the clinical picture of FH has recently been described on chromosome 1, now referred to as autosomal dominant FH3. This gene, pro-protein convertase subtilisin/kexin type 9 (PCSK9), encodes for neural apoptosis regulated convertase 1 (NARC1). The exact mechanism by which the LDL receptor becomes dysfunctional in this disorder is unknown. (30) Low LDLR activity appears to be a part of this phenotype but there is also evidence for over production of lipoproteins. (31)

This thesis will examine the experience with FH and does not include the genetic defects of FDB

or FH3. FDB patients were excluded by genotyping. Owing to the apparent rarity of FH3 and the cost of sequencing this gene in a large cohort of undefined LDLR gene status, specific identification of FH3 has not been undertaken by the Lipid laboratory.

1.3 FH and South Africa.

1.3.1 Historical perspective

The first white settlers arrived in South Africa in 1652 when the Dutch East India Company established a settlement to provide fresh farm produce for their trading vessels on the route to and from the East. The first Dutch settlement was soon enriched by settlers from France and Germany. In 1800 the total settler population numbered about 15,000, most having descended from a group of about 2,000 individuals of Dutch, French and German origin. (32)

This small gene pool expanded many times without much input of new genetic material. Bound into a fairly homogeneous group by the now rapidly developing language of Afrikaans, these settlers tended to remain separate from the newly arriving English immigrants. Common religious beliefs further bound them into a distinctive community which encouraged marriage within this restricted gene pool. This created the so called “founder gene effect” - resulting from a chance enrichment of a given gene during the expansion of a reproductively isolated population. (33) Torrington found a strong association between FH and Gereformeerde Kerk (Reformed Church) affiliation and has demonstrated two possible founder surnames, which were not subsequently published. (34)

In 1804 the Dutch East India company lost control of the Cape of Good Hope, with the British formally taking over governance in 1806. With the new rulers came religious tolerance,

encouraging immigration. Jews began to arrive from England and Germany. These Jews were living in poverty and oppression in the Russian Pole Settlement which became known as Lithuania, and the pogroms which followed the assassination of Tsar Alexander II in 1881 resulted in mass migration of Jews from Eastern Europe to the West. Two events in South Africa made this country a very attractive option: the discovery of diamonds in 1869 and gold in 1886. Spurred on by very favourable reports and by financial assistance from those already here, 40,000 Jews arrived in South Africa between 1881 and 1910, mainly from Lithuania. By 1936 the Jewish population numbered more than 90,000, representing 4.5% of the white population. As with the Afrikaners, the Jews were a distinct ethnic group, bound together by a unique religious expression, very strong traditional values and group identity. This encouraged marriage only within the group, sustaining the founder gene effect in this group.

The first Indian to set foot on South African soil, was Babu Naidoo, who arrived in 1855. The rapidly developing sugar industry in Natal required a steady supply of reliable and skilled labour. In 1860 the first indentured Indians arrived to work on the sugar cane farms. This proved to be a very successful scheme, attractive to both the farmers and the immigrants, and by 1872 the Indian population numbered 12,800. (35) As with both the Afrikaners and the Jews, the same group identity and common cultural and religious expression resulted in inter-marriage within the group and gene multiplication. The Muslim faith further united Indians immigrating from various regions in India with Indonesian refugees and local converts.

1.3.2 FH in South Africa

FH was first reported in South Africa in 1977. (33) At the 1986 Heart Foundation of Southern Africa Consensus Conference on FH it was concluded that more than 30,000 white Afrikaans-speaking South Africans could be affected by the gene defects responsible for FH. (36) The

problem of the unusually high prevalence of homozygous FH in South Africa was high-lighted in a publication in 1981 from Johannesburg, documenting 34 cases seen in the space of seven years. These were all Afrikaners from the then Transvaal Province. (37) From this observation a very high prevalence of the heterozygous FH state was predicted.

An Afrikaner could be described as a South African who comes from a family with Dutch, German and/or French ancestry, who has lived in this country for more than three generations and has a mother tongue of Afrikaans. Three distinct founder mutations have to date been identified in Afrikaners in this country. (38) Other mutations have also been identified in this community. The overall prevalence of FH as a result of all genotypes, is as high as 1/70, compared to a world wide prevalence of 1/500. The most commonly occurring defect, Afrikaner FH1 (D206E), has not been ascribed to any specific immigrant and the mutation has been reported from several countries in Europe.

The second most commonly occurring gene defect is Afrikaner FH 2 (V408M) This was identified in the Netherlands and subsequently also in patients of Dutch origin in Western Canada. (39) The origin of this defect has been traced to Andijk, a small village in the northwestern part of the Netherlands, the origin of the first settlers who came to South Africa in the 17th and 18th centuries.

Both these defects were first described in the Afrikaner population by Leitersdorf et al in 1989 (40) and they account for 80% of FH in South Africa. (41)

A third founder gene, Afrikaner 3, was identified in 1989 by Kotze et al. This was called Afrikaner FH3 (D154N). (38) This is a rarer defect than the more commonly occurring Afrikaner FH1 and FH2 and its origin is also unknown.

Other defects have been described in Afrikaners and in the White population of South Africa. Many mutations including those ascribed to the Afrikaners have been documented in the population of mixed ancestry. In this population only about a quarter of the genotypes is explained by the “founder genes”. (42) Founder effects may also be present in the Black population.

1.4 Lipid and lipoprotein metabolism

Lipid and lipoproteins will be reviewed briefly to simplify the discussion of the LDL studies presented in later chapters.

1.4.1 Lipoproteins

Genetic defects encountered in patients with FH are confined to the LDL receptor (LDLR), or its regulation by PCSK9 or the ligand of the LDLR, apoB. The consequences mainly involve LDL metabolism but other subtle metabolic changes in lipid and lipoprotein metabolism may be found. Lipoprotein particles are complex structures found in human plasma consisting of varying proportions of phospholipids, triglyceride, cholesterol, cholesterol ester (CE) and apoproteins. Phospholipids and cholesterol, though not highly water-soluble, are polar molecules, whereas triglycerides and cholesterol ester are non-polar molecules that are insoluble in aqueous media.

Phospholipids (PL) are integral components of all cell membranes, forming a bilayer in which their polar groups are orientated to the aqueous environment, both inside and outside the cell. The outer lipoprotein layer consists of one layer of phospholipid. Phospholipids consist of two main components, sphingomyelin and phosphatidyl choline (lecithin) and are synthesized mainly in the liver. They contain a phosphate group at one end and hydrophobic fatty acids at the other

end, directing this part of the molecule to the interior of the lipoprotein particle. These properties make phospholipids the ideal material to cover triglyceride and cholesterol ester, the other neutral lipid, for transport in the plasma. The unsaturated fatty acids with the PL may undergo oxidation, a process believed to be essential for atherosclerosis to proceed.

Triglycerides (TG) (or triacylglycerol) are free fatty acid esters of glycerol that are assembled in the small intestine and in the liver. Dietary triglycerides (or ingested fat) are absorbed into the enterocyte and incorporated into chylomicrons. Triglycerides are also synthesized *de novo* in the liver and are secreted into the plasma as very low density lipoprotein.(VLDL)

Cholesterol is a sterol which exists in either the esterified or free form. It is a crucial component of all cell membranes and is also involved in other metabolic processes such as hormone synthesis. All cells can synthesise cholesterol, but most production occurs in the hepatocytes and to a lesser degree, the gut and the central nervous system. It is transported in the circulation as part of a variety of lipoprotein particles in both its free and esterified forms. Cholesterol synthesis is a complex process, with one of the key and rate controlling steps being the conversion of 3-hydroxy-3-methylglutaryl CoA to mevalonic acid. The enzyme responsible for this step is HMG-CoA reductase, and drug therapy aimed at inhibiting this enzyme is extremely effective in lowering serum cholesterol. The HMG CoA reductase inhibitor drugs are collectively known as the “statins.”

Apoproteins are found on the surface of lipoprotein particles. Some are structural proteins, some determine cellular uptake of particles by binding to cell surface receptors whilst others activate or regulate enzymes such as lipoprotein lipase involved in triglyceride hydrolysis. The nature of the apoprotein also determines the ultimate fate of the particle. Apoproteins include apoA_I, apoA_{II}, apoA_{IV} and apoA_V, apoC_I, apoC_{II} and apoC_{III} and apoE. Whereas all these apoproteins are amphipathic and can exchange between lipoproteins, apoB is a very

hydrophobic apoprotein that is utilised for lipoprotein synthesis in the enterocyte and hepatocyte and remains with the lipoprotein particle for the duration of its existence.

The four major types of lipoprotein particles found in the plasma are chylomicrons and VLDL, which are rich in triglycerides, and the two cholesterol rich lipoproteins, low density lipoprotein (LDL) and high density lipoprotein (HDL). Remnant lipoproteins of chylomicrons and VLDL are encountered in very low concentrations and, especially for VLDL remnants, fall into the IDL category.

Chylomicrons (CM) are the largest particles and contain mainly triglycerides. They are synthesised by the enterocyte after a fat-containing meal. After secretion into the lacteals, they reach the circulation via the thoracic duct and are usually cleared from the blood within twelve hours. Chylomicrons undergo lipolysis by lipoprotein lipase, resulting in the conversion of the triglyceride component to glycerol and fatty acids. The latter are taken up by the cells for energy or storage. Chylomicron remnants are then removed from the circulation by virtue of apoE, which is the ligand for LDL receptor-related protein (LRP), the LDLR and possibly other receptors. Chylomicrons are distinctive in containing one molecule of apo B₄₈ as their major apoprotein. Apo B₄₈ is thus found only on gut derived lipoproteins. It is generally thought that CM metabolism is not affected in patients with FH.

By contrast, **very low density lipoprotein (VLDL)** is secreted by the hepatocyte and contains apoB₁₀₀ as its apoprotein. Apo B₁₀₀ is made in the liver and is the major ligand for binding LDL with the LDLR. ApoE is an abundant apoprotein on VLDL and also mediates the clearance by LRP of chylomicron remnants, VLDL, small "remnant particles" and some LDL. C apolipoproteins are found on chylomicrons and VLDL. Apo C_{II} is a cofactor of lipoprotein lipase (LPL). Apo C_{III} delays triglyceride clearance through an uncertain mechanism. Apo A_I and apo A_{II} are also present on VLDL, transferring back and forth to high density lipoprotein (HDL) particles.

Hepatic VLDL synthesis is enhanced by increased flux of fatty acids to the liver and also by a high intake of carbohydrate, which increases the rate of synthesis of endogenous fatty acids. VLDL is hydrolysed by lipoprotein lipase, resulting in the formation of smaller particles such as intermediate density lipoprotein (IDL) and remnant particles and finally, low density lipoprotein (LDL), the final product in the lipolytic cascade.

Low density lipoprotein (LDL) contains very little triglyceride and only one molecule of apoB₁₀₀ which not only provides structural integrity, but is also essential for the clearance of these particles by LDL receptors in the liver and peripheral tissue. The role of LDL is to supply cholesterol to the tissues during growth or tissue repair and to the adrenal glands for the synthesis of steroid hormones. LDL particles can vary in size depending on their precursor state or modulation in plasma. Modulation of LDL particles and their role in FH will be discussed in chapter five.

High density lipoprotein (HDL) is synthesised in both the liver and the intestine. The particle consists of phospholipid, TG, cholesterol ester (CE), apoE, apoC, apoA_I and apoA_{II}. HDL receives excess cholesterol from the tissues which can then be transferred back to the liver or to other particles such as VLDL. The uptake of FC from the tissues is facilitated by lecithin cholesterol acyl transferase (LCAT) because it esterifies FC in the phospholipid shell. The CE moves to the hydrophobic core of the lipoprotein particle and creates a space for more FC to enter the HDL. The transfer of cholesterol ester from HDL to VLDL is mediated by cholesterol ester transfer protein (CETP).

Other particles include lipoprotein (a) (Lp(a)), intermediate density lipoprotein (IDL) and chylomicron remnants. **Lp(a)** is larger but more dense than LDL, because it consists of an LDL particle with a covalently linked protein, apo(a). The concentration of Lp(a), which can vary considerably in individuals, is largely determined genetically. It bears a close homology to

plasminogen, but the exact role of Lp(a) is still not clear. An increased level of Lp(a) has been independently associated with an increased risk of cardiovascular disease. It has been reported that the concentration of Lp(a) in FH is higher for a given isoform and that the survivors of FH have lower Lp(a) concentrations associated with the commensurate isoform. (43)

IDL and remnant particles are present in varying concentrations and in some circumstances where clearance is impaired, can be more atherogenic than VLDL or LDL.

1.4.2 Cellular cholesterol metabolism and LDL receptor regulation

Whilst FC has an important structural role in cell membranes, its excessive accumulation in membranes has toxic implications for the cell. As FC accumulates in a cell, regulatory responses occur to avoid excessive accumulation. FC can be converted to CE by acylCoA:cholesterol acyltransferase (ACAT), storing cholesterol in intracellular droplets that are especially prominent in foam cells. Through the same regulatory mechanism, de novo synthesis and LDL receptor expression are also down-regulated.

The cholesterol pool is regulated by the sterol regulatory element binding protein (SREBP). When the cell is depleted of cholesterol, the SREBP-SCAP (sterol cleavage activator protein) complex migrates to the Golgi apparatus where two proteases cleave SREBP. (44) The N-terminus of SREBP migrates to the nucleus to activate the genes for the de-novo synthesis of cholesterol. The expression of LDL receptors, and some additional genes involved in lipid metabolism are also regulated. This process is still poorly understood but may include the process in which PCSK9 affects LDL receptor expression.

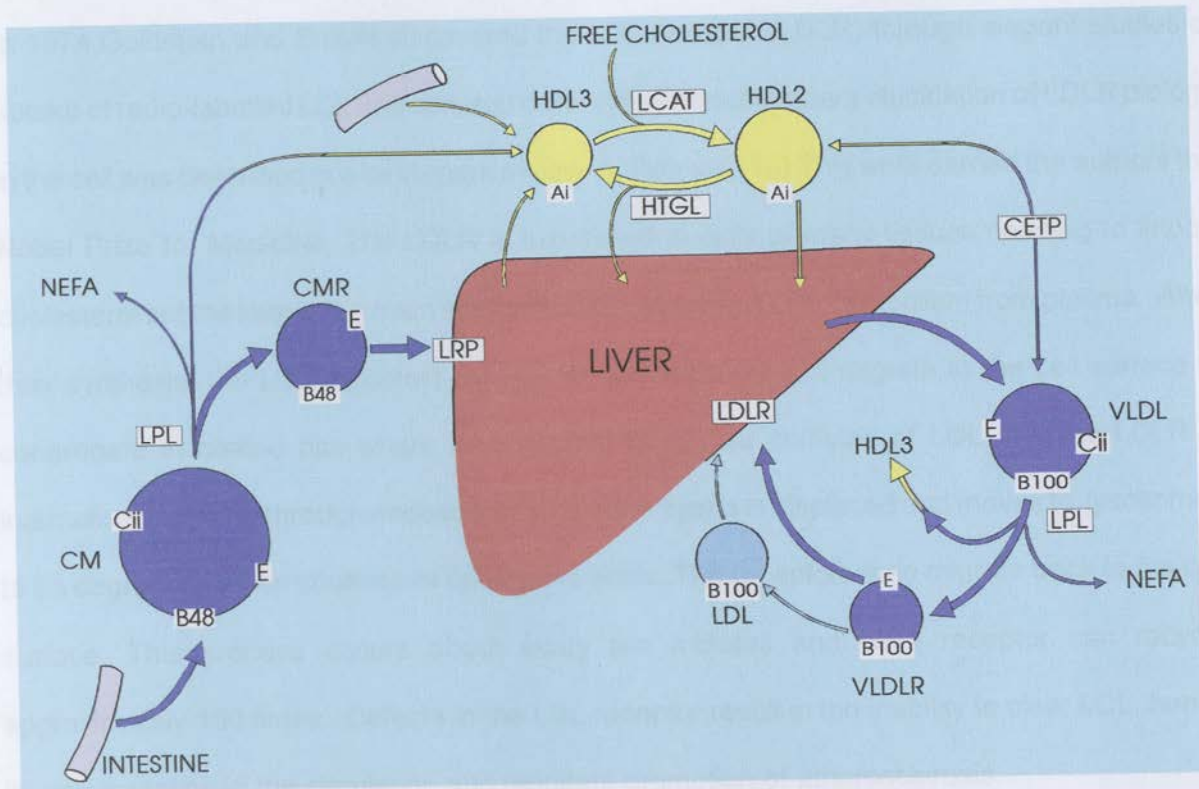


Figure 1.4.2 Metabolism of lipoprotein

(Taken from departmental teaching material)

A ₁	apo A ₁
B ₄₈	apoB ₄₈
B ₁₀₀	apoB ₁₀₀
CETP	cholesterol ester transfer protein
CM	chylomicron
CMR	chylomicron remnant
C ₁₁	apo C ₁₁
HDL	high density lipoprotein 2 and 3
HTGL	hepatic triglyceride lipase

LDL	low density lipoprotein
LDLR	low density lipoprotein receptor
LCAT	lecithin:cholesterol acyl transferase
LRP	LDL receptor-related protein
LPL	lipoprotein lipase
NEFA	non esterified fatty acids
VLDL	very low density lipoprotein
VLDLR	very low density lipoprotein remnants

1.4.3 LDL receptor defects

In 1974 Goldstein and Brown discovered the LDL receptor (LDLR) through elegant studies of uptake of radio-labelled LDL into cultured cells. (45) The subsequent elucidation of LDLR biology in the cell was described in a land-mark review in Science. (13) This work earned the authors the Nobel Prize for Medicine. The LDLR is expressed in cells of many tissues needing to import cholesterol but the liver is the main site of receptor-mediated LDL catabolism from plasma. After their synthesis, the LDL receptors mature by glycosylation and migrate to the cell surface to congregate in coated pits where they bind to LDL. The complex of LDL and the LDLR is internalised, moving through endosomes where the ligand is displaced and moves to lysosomes to be degraded to free cholesterol and amino acids. The receptors then migrate back to the cell surface. This process occurs about every ten minutes and each receptor can recycle approximately 150 times. Defects in the LDL receptor result in the inability to clear LDL, hence its accumulation in the circulation and resultant promotion of atherosclerosis.

Five functional defects of the LDL receptor have been well characterised - (1) failure to express receptor protein, (2) defective transport of the receptor from the endoplasmic reticulum to the cell surface, (3) impairment of receptor binding to the ligand, (4) impaired internalisation of the receptor- ligand complex and (5) defective receptor recycling. A possible sixth defect relates to cellular localisation. (46) Any of these defects will result in the clinical phenotype of FH. LDL receptor dysfunction can also be broadly grouped into receptor-defective and receptor-negative defects - the former retain some receptor function, <30% in the homozygous cell lines, whilst the latter have little or no function (<2%). A wide variety of mutations is now known to cause LDLR dysfunction with more than 840 currently having been documented. (47;21) The mutations include missense and nonsense mutations as well as insertion and deletion defects. New mutations are identified on a regular basis.

Metabolism of lipids and lipoproteins are complex, permitting monogenic abnormalities to present with variable phenotypes that could also be influenced by environmental and dietary factors. Notwithstanding these variations, two distinct phenotypes are recognised according to the degree of disruption of LDLR function. Heterozygous FH (heFH) is characterised by an elevated LDL cholesterol level of 5 to 12 mmol/L, tendon xanthomata and premature ischaemic heart disease, generally after the third decade. Homozygous FH (hoFH) has a more severe phenotype with the LDLC greater than 12 mmol/L and heart disease often by the second decade. The homozygous FH subjects classically also have cutaneous and gross tendon xanthomata. The hoFH phenotype can be due to a true genetically homozygous state or due to a compound heterozygous state for LDLR mutations. The clinical phenotype of a compound heterozygote, with two differing defective LDL mutations, is indistinguishable from classical homozygous FH mutations. Whereas heFH is present in approximately 1/500, hoFH is rare, occurring at a frequency of 1/1,000,000 in the general world wide population.

1.5 Physical signs and pathogenesis

Clinical evaluation of a patient depends on obtaining an accurate history and detecting physical signs resulting from pathological processes. Physical signs are not invariable in patients with FH nor are they exclusive to patients with LDL receptor gene defects. Xanthelasma can occur in patients with a normal lipid profile, arcus cornealis can occur in the elderly without signifying a lipid problem and tendon xanthoma can occur in other genetic lipid abnormalities as will be discussed in the differential diagnosis. All these signs are however useful in alerting the clinician to the possibility of an underlying defect in lipid metabolism.

1.5.1 Xanthelasma

Xanthelasma palpebrum is a common clinical finding that may be associated with dyslipidaemia. The importance of this clinical feature is that it should alert the clinician to the possibility of FH and the recognition of xanthelasma should certainly be followed by a full lipid analysis.

The lesions appear as single or multiple soft, velvety yellow-orange papules or plaques, often presenting initially at the medial canthus of the eye and then spreading laterally. The plaque lies embedded in the corium and the overlying epidermis is thin and may be wrinkled. The lesions occur on the upper and/or lower lids of the eye, but the lid margins are generally spared. Patients experience no symptoms, the only disability being the cosmetic effect.

The prevalence of xanthelasma increases with age and it is more commonly found in women than in men. A high association between xanthelasma and arcus cornealis has been found, especially in young people. In the large Lipid Research Clinics Program Prevalence Study (22) no association was found between xanthelasma and smoking, alcohol consumption, blood pressure,

obesity, sedentary lifestyle, family history of ischaemic heart disease, or glucose and uric acid plasma levels. Data indicate a correlation between xanthelasma or arcus cornealis with ischaemic heart disease, but there was also no independent correlation with peripheral vascular disease.

The pathogenesis of this condition is obscure. Cholesterol ester is the major lipid component in xanthelasma. (48) The LDL-derived cholesterol ester is taken up by the macrophages located in the interstitial spaces of the dermis and it is the accumulation of resultant foam cells that accounts for the typical raised lesions. (49) The expression of xanthelasma seems to be related to the serum lipoprotein profile. Patients with xanthelasma generally show elevated levels of plasma triglycerides and, by implication, of very low density lipoprotein (VLDL) and lower levels of high density lipoprotein (HDL). (50) About 50% of individuals with xanthelasma are broadly normolipidaemic but on detailed analysis may have triglyceride-enriched VLDL and low HDL cholesterol levels. (51)

A later study confirmed the association of low HDL cholesterol with the presence of xanthelasma - 94% of the cohort studied showed lower HDL levels than those of age matched population controls. There was however, no significant difference in total cholesterol, LDL cholesterol or triglyceride levels. (49)

Another study showed that patients with xanthelasma had higher levels of total cholesterol, LDL cholesterol and apoB, and lower levels of HDL2 cholesterol than the controls. The apoE3/4 phenotype was also found to be more common. By comparison to the control subjects the patients were also found to be more obese and to have a higher incidence of ischaemic heart disease in themselves and in their families. (52)

Although xanthelasmata cause no symptoms or discomfort, patients frequently request their removal for cosmetic reasons. On aggressive lipid lowering the xanthelasmata regress slowly.

The first notable change is the lessening of intensity of colour and flattening of the lesion. This is often accompanied by an itchiness of the affected area. After about two years many of the lesions will resolve completely leaving no scarring.

Surgical, chemical, physical and laser therapies are also used in the treatment of xanthelasma. These methods of treatment should not be undertaken before the lipid profile is normalised. Surgery is carried out under local anaesthesia. The skin and the "tumour" are raised together like a flap and incised just above the orbicularis muscle. The plaque is peeled out by micro surgical technique. After the enucleation the skin is sutured back leaving minimal scarring. (53)

Chemical treatment of the lesions is also a recognised form of treatment. Careful application of 50% - 100% trichloroacetic acid is considered a simple yet highly effective form of therapy. Liquid nitrogen has been used in a similar fashion. (54)

The most recent and to date most successful form of therapy is ultra-pulsed CO₂ laser treatment of the lesions. This appears to be better than previous laser therapy methods with argon, pulsed dye laser and CO₂ laser, as the lesions could be removed completely with a single ultra pulsed laser treatment. The only side effects were transient pigmentary changes but there was no visible scarring. (55)

Unfortunately recurrence is common with all methods of removal of xanthelasmata. The above series (ultra pulsed CO₂ laser) had a 13% recurrence after 10 months of follow-up. It is essential that lipid profiles are kept optimal after any such removal.



Figure 1.5.1.1 Superior and inferior xanthelasma in FH patient



Figure 1.5.1.2 Xanthelasma in FH patient

1.5.2 Arcus cornealis

The term arcus cornealis has been given to the visible partial or completely opacified circle that develops at the periphery of the cornea in both eyes. It generally first appears superiorly, then inferiorly and then becomes circumferential. The superior and inferior limbal areas are the warmest regions of the cornea owing to increased vascularity, and here increased capillary flow may lead to preferential lipoprotein deposition. The prevalence varies between 20% and 35% in the general population and can vary with ethnicity, with black males having the highest frequency. (56) Arcus cornealis may occur with ageing in normal individuals and Friedlaender reported that its occurrence approached 100% in people over the age of 80. (57) Winder also commented that the development of arcus cornealis correlates with age rather than concentration of LDL cholesterol, making it a time dependent rather than a dose dependent factor. Arcus does not appear to be related to HDL levels. (58) However, its presence under the age of 40 years is frequently associated with hypercholesterolaemia. Unilateral arcus may develop secondary to corneal damage and then occurs predominantly at the site of injury, apparently related to abnormal vascularity at the site. Double arcus cornealis has also been reported as a rare finding. (59)

Cholesterol, predominantly in esterified form, accumulates in the extracellular space adjacent to the limbus (the junction of the corneal and the conjunctival stroma). These deposits are similar to lipid particles found in atherosclerotic lesions but occur in the absence of foam cells. Ultra structural studies showed that extracellular solid spherical lipid particles were enmeshed between collagen fibres, in which 75% of the cholesterol is in the esterified form. Immunostaining showed significant apoE and apoA₁, but very little apoB in the peripheral cornea. (560) The peripheral concentration of the lipid deposit is presumed to be due to trapping of LDL by glycosaminoglycan binding and tight stromal packing, whilst the more central areas of the cornea are less dense and are cleared by HDL. (60;61) The larger the size of the lipid particle the greater

particle the greater the light scatter, thus the amounts of large LDL particles may determine whether the cornea will show an arcus. (60)

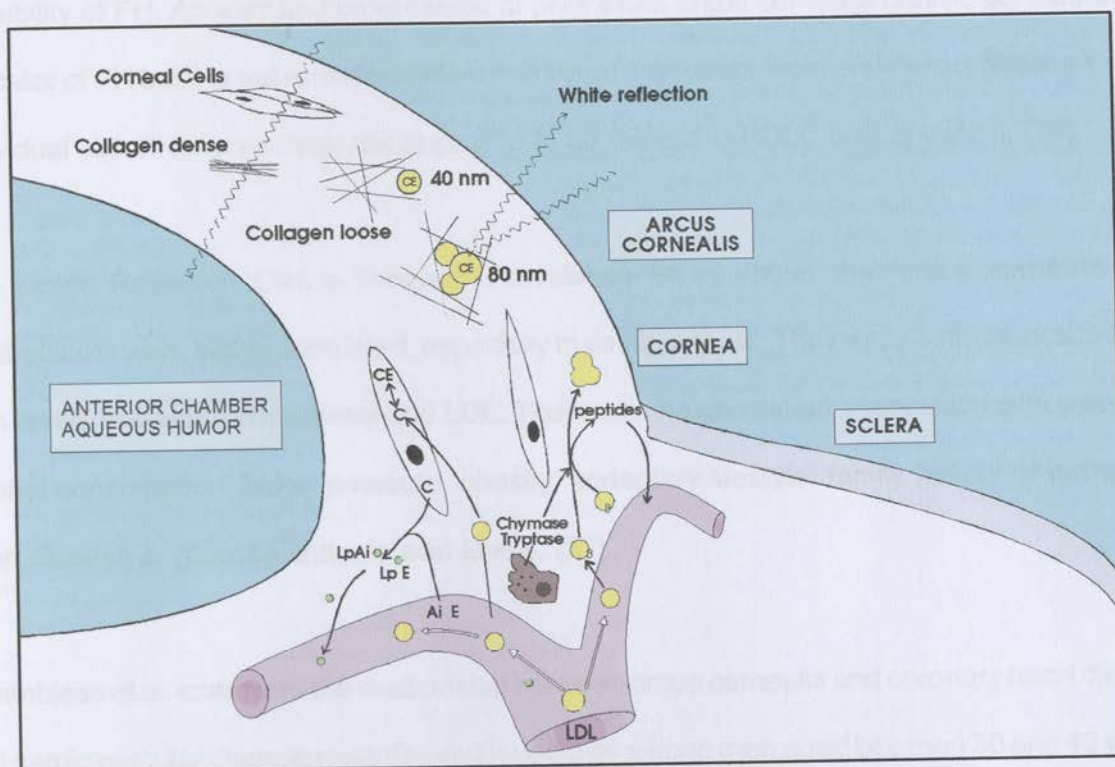


Figure 1.5.2.1 Schematic diagram to illustrate lipid deposition in the cornea in the development of arcus cornealis
(From departmental teaching material)

In FH patients, some degree of arcus cornealis has been found in up to 50% of patients between the ages of 31 and 35 and by age 50, 50% of FH patients had a circumferential arcus.

(62) In a control group of the same age, the incidence was 5%. Circumferential arcus at age 50 in both men and women and partial arcus at ages 30 - 35, must alert the observer to the possibility of FH. Accelerated progression of premature arcus cornealis can be considered an indicator of FH but it is not an independent marker of premature ischaemic heart disease for the individual with FH. Arcus does not appear to be related to HDLC concentration. (58)

The Lipids Research Clinics Program Prevalence Study found that arcus cornealis and xanthelasma were highly correlated, especially in young people. They were both associated with high levels of plasma cholesterol and LDL. There was no consistent association with smoking, alcohol consumption, blood pressure, obesity, sedentary lifestyle, family history of ischaemic heart disease or glucose and uric acid levels. (22)

Chambless et al. examined the relationship between arcus cornealis and coronary heart disease and cardiovascular disease mortality and found that among men aged between 30 and 49 years, it appears to be a prognostic factor for cardiovascular disease independent of its association with hyperlipidaemia. (63) This association was only found in men without FH. Another group looked at the correlation between arcus cornealis and some of the risk factors for coronary artery disease and found that arcus is more common in males, and that the frequency and size of the arcus (as measured by a digitizer) were positively associated with age. In males there was a positive correlation between the size of the arcus and the levels of total cholesterol and LDL cholesterol. (64)



Figure 1.5.2.2 Circumferential arcus cornealis in FH

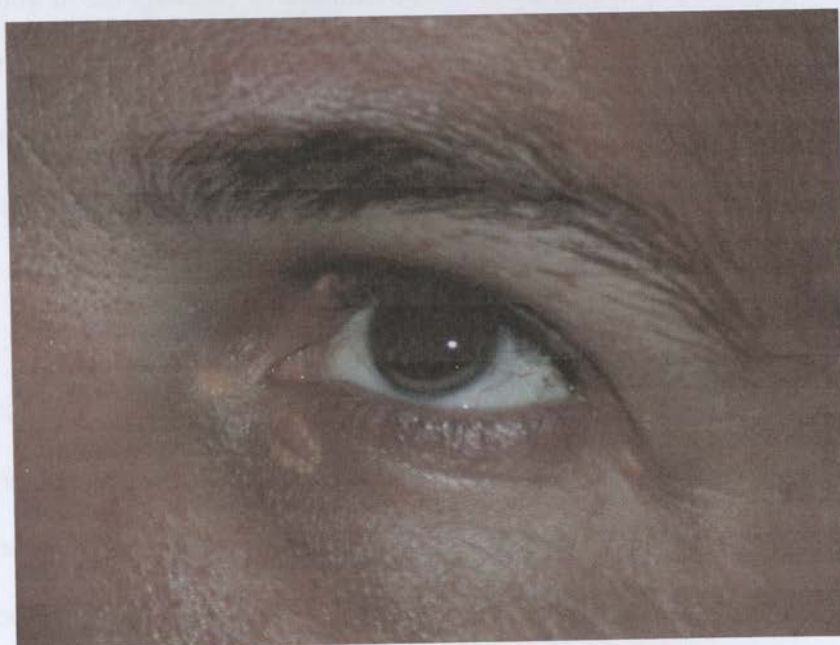


Figure 1.5.2.3 Inferior arcus cornealis and xanthelasma

1.5.3 Xanthomata

Xanthomata are localised infiltrates consisting almost exclusively of lipid-containing foam cells that are usually found within the dermis or in tendons. The foam cells are thought to be tissue macrophages which have phagocytosed lipoproteins that have been deposited in tissues from the circulation. Lipids found in foam cells consist primarily of free and esterified cholesterol but occasionally other sterols and triglycerides accumulate in significant amounts.

In familial hypercholesterolaemia, xanthomata can be cutaneous, tuberous or planar, or most commonly, tendinous. Tuberous xanthomata are large nodular lesions which extend beyond the dermis into the sub-cutaneous tissues and generally appear on the elbows, knees and buttocks. They are not common in heterozygous FH but are often seen in homozygous FH.

Planar xanthomata appear as diffuse or well circumscribed, non-inflammatory dermal macules or papules. They occur typically in the interdigital webs of the fingers where they are virtually diagnostic of homozygous FH. They may also occur in other areas such as the axillae and the ante-cubital and popliteal fossae. Eruptive xanthomata are typical of hypertriglyceridaemia and are thus not seen in FH. Xanthelasma, a planar xanthoma of the eyelid, has already been discussed.

Tendon xanthomata are firm subcutaneous nodules which are attached to or form an integral part of the underlying tendon, fascia or ligament, covered by normal-looking skin which can easily be moved over the nodule. They often involve the Achilles tendon and the extensor tendons of digits. They may also occur as periosteal xanthomata that occur on tibial tuberosities and on the elbows. These xanthomata develop very slowly over years and are probably the result of infiltration of LDL into the tendons. Non-esterified cholesterol accumulates in the tendon, predominantly in extracellular spaces. (65)

Achilles tendon xanthomata in the presence of a raised cholesterol level with normal triglycerides are virtually diagnostic of FH. Other well-recognised causes of tendon xanthomata can be distinguished relatively easily: In Cerebrotendinous xanthomatosis and Sitosterolaemia the cholesterol level should be normal and in dysbetalipoproteinaemia (Fredrickson type III hyperlipidaemia), triglycerides should be raised.

In 1978 a Japanese group measured Achilles tendon thickness radiographically in FH patients and found that this was the earliest sign of FH, being present in 85% of the group studied. (66) This method of measurement has been superseded by ultrasonography. Tendon xanthomata are demonstrated by the presence of hypo-echoic nodules or an increase in the anterior-posterior diameter of the tendon. In recent studies it has been shown that sonography reveals Achilles tendon xanthomata in patients with FH before these are detected clinically and could therefore be a useful tool in the diagnosis of the condition. (67;68)

In a study done in Belgium (68) in order to establish Achilles tendon thickness as a diagnostic tool for FH, the antero-posterior thickness cut-off was set at 5.8 mm. This was found to be the most useful measurement (compared to width, section or roundness) and gave a sensitivity of 75% and a specificity of 85%. It was felt that this method of diagnosis of FH yielded a higher specificity than serum cholesterol. Achilles tendon thickening occurred later in women than in men and there was a trend to finding a greater proportion of Achilles tendon thickening in men before the age of 45. This group concluded that measurement of tendon thickening was a more accurate and reproducible diagnostic method than assessing hypo-echoic areas, as it is less prone to observer error. (69)



Figure 1.5.3.2 Dorsal view of an individual with xanthomata in FH



Figures 1.5.3.1 Extensor tendon xanthomata on the hands in FH



Figure 1.5.3.2 Lateral view of an Achilles tendon xanthoma in FH



Figure 1.5.3.3 Posterior view of Achilles tendons xanthomata in FH

1.6 Complications

In FH the most serious consequence of raised LDL cholesterol is premature ischaemic heart disease and early death.

The first study to document the greatly increased risk of coronary heart disease in a cohort of FH was published by Slack. (70) She found that in England the mean age of onset of coronary heart disease was 43.3 years for men and 53.3 years for women in heterozygous FH. In men the risk of a myocardial infarction was 5% by age 30, 51% by age 50 and 85% by 60 years of age.

In 1974 Stone published a study assessing cardiovascular disease in FH subjects compared with matched non-affected relatives. This was the largest study to date and concluded that in the FH group 29.5% had CAD (coronary artery disease) compared to 10.5% in normal relatives. The cumulative probability of fatal or non-fatal CAD in men by the age of 60 was 62%, compared to 12.7% in non-affected matched relatives. In women with FH aged 60 the risk was 32.8%, compared to 9.1% in matched relatives. (71)

Beaumont found that although levels of serum cholesterol and clinical features were similar in men and women, the incidence of CAD was markedly different. (72) The incidence was much lower in women and occurred about nine years later, the mean age of onset in men being 44 and in women 54 years. Interestingly the age of onset of CAD in smoking women was the same as in men.

Combining the findings of Slack (70), Stone (71) and Beaumont (72) one gets a clear picture of the risk of cardiovascular disease and death resulting from untreated FH. These results are given in table 1.6.1

AGE (yrs)	CHD incidence (%)		CHD mortality (%)	
	Men	Women	Men	Women
<30	5	0	0	0
30 - 39	22	2	7	0
40 - 49	48	7	25	1
50 - 59	80	51	52	15
60 - 69	100	75	78	23

Table 1.6.1 CHD incidence and mortality associated with untreated heterozygous FH
 CHD = coronary heart disease
 Compilation of studies in the UK (Slack), the USA (Stone) and France (Beaumont)
 Durrington. (73)

By contrast, Mabuchi looked at the development of CAD in Japanese patients with FH and reported myocardial infarction first occurring in the third decade in men and the fourth in women. The mean age of death was 54 in males and 69 years in women, which was considerably older than that reported in the Western population groups. (74) The healthy dietary habits of the Japanese probably provide some measure of protection against FH and the harmful effects of their heavy smoking habits.

In the UK accurate documentation and analysis has been made possible by a register of FH patients. Without effective treatment, the cumulative risk of a coronary event is 50% in men over the age of 60, and 30% in women for the same age. Despite treatment, the relative risk of a fatal coronary event in women aged 20 - 39 is increased 125-fold and for men in this age bracket, 48-fold. (75) The relative risk for coronary death decreased with age, so that by the age of 60 to 79 years men had no excess mortality from CAD and the risk in women was just greater than 2-fold. A recent study conducted in six lipid clinics in the UK on the incidence of coronary artery disease

(CAD) in treated patients with FH found that the mean age of onset in men was 43.1 years and 46.5 years in women. (76) As raised cholesterol leads to atherosclerosis, it is reasonable to suspect that there would also be an increase in the incidence of stroke and peripheral vascular disease in patients with FH. In settings other than FH, the association between stroke and cholesterol level is controversial and the results of several recent large scale cohort studies suggest that cholesterol levels are not associated with ischaemic stroke events. (77;78) Earlier studies, examining vascular disease other than coronary artery disease in FH, were inconclusive in that two showed an increase in the incidence of stroke, and another two showed no increased risk. A recent report on a prospective study in FH patients treated with lipid-lowering therapy strongly suggests that there is no increase in the risk of a fatal stroke. (79) However, in the Heart Protection Study (80), which was undertaken in a different context of dyslipidaemia, a reduction of about one third in the incidence of stroke was achieved in patients taking statin therapy compared to those on placebo. (81) It has been speculated that the mechanism underlying this reduction of cerebrovascular accidents might be the result of a cholesterol independent mechanism. (80)

Slack, Stone and Beaumont all reported that peripheral arterial disease as indicated by intermittent claudication was increased by 8-16 percent in FH heterozygotes. By use of the echo-Doppler method to assess arterial disease and diminished blood flow, two studies identified peripheral arterial disease in 30-45 percent in FH patients. The severity increased with age. (82;83) Several other studies have confirmed the same results. (83-85) In 1995 studies done in the Netherlands found the incidence of peripheral vascular disease (PVD) in FH compared to age, sex, weight, smoking and hypertension-matched controls to be increased 10-fold. The onset of PVD could occur as young as 30 years of age and the age of onset was the same in males and females. (86) It would appear that PVD is increased from 5- to 10-fold in FH subjects compared with non-FH controls. (87)

Intima media thickness (IMT) of the carotid and/or femoral artery is a surrogate marker of arterial disease and large epidemiological studies have shown an association between increased IMT and future development of coronary artery disease and stroke. Studies have also shown an increase in IMT in FH subjects when compared to both matched normolipidaemic and hyperlipidaemic non-FH controls. (87) De Groot demonstrated that on average, healthy controls reached a mean IMT measurement of 0.8 mm by age 80 years whereas FH subjects reached this value around the age of 40 years. (88) Tonstad reported that the carotid bulb IMT in FH children was 0.04 mm thicker than in normal controls and she also noted the presence of plaque in 10% of FH adolescents studied. (89) IMT is considered a useful predictor of cardiovascular disease in FH, with the IMT of the carotid and femoral arteries in FH subjects with CVD, being greater than the IMT (of the same arteries) in those without CVD. The mean carotid IMT measurements were respectively 0.86 (0.21) and 0.80 (0.15), $P=0.03$. (90) Regression of IMT is also found with aggressive lowering of LDL compared to less aggressive treatment in FH. (91) In this study, regression also occurred at higher LDLC concentrations than is the target for secondary prevention. The impact of reduction of IMT on cardiovascular risk has not yet been shown.

The clinical associations of hypercholesterolaemia, namely arcus cornealis, xanthelasma and tendon xanthomata seldom produce complications. Apart from possible cosmetic sensitivity, arcus cornealis has no complications. Xanthelasmata may be disfiguring but this can be corrected. (see 1.5.2) Tendon xanthomata can also be cosmetically disfiguring, especially on the hands, and large tuberous xanthomata on the elbows are not attractive. These can be removed surgically, but this practice is not encouraged. The sheer size of xanthomata on the Achilles tendons can make the wearing of shoes very uncomfortable. (92) Achilles tendon xanthomata do not appear to predispose to tendon rupture. Xanthomata on the buttocks as seen in homozygous FH can be extremely uncomfortable and may need to be excised. Protruding Achilles tendon xanthomata can show marked regression on lipid lowering therapy, as can tuberous and extensor tendon xanthomata. (92)

There has been a report of a rare occurrence of a xanthoma in the floor of the anterior cranial fossa involving the frontal bone and orbit. (93) It is not clear from the description whether the patient had FH. With the elevated TG levels he could also have had dysbetalipoproteinaemia. Such "ectopic" xanthomata appear to be extremely rare.

Achilles tendinitis or tenosynovitis can occur in some patients and can be very painful and debilitating. The pathological process involves inflammation within the tendon where the deposition of cholesterol occurs, or possibly inflammation in adjacent structures. It is not known why it occurs in some patients and not in others. It does not seem to bear any relationship to the level of cholesterol or the genetic defect. It seems to be more common in teenagers, but again appears to have no relationship to rate of growth or physical activity. It responds well to anti-inflammatory treatment. A more generalised tenosynovitis may also occur in FH and is most commonly seen when cholesterol is rapidly lowered by statin therapy and should not be confused with a direct side effect of drug. This is thought to be due to the mobilisation of cholesterol which is deposited throughout the tendon and peri-articular tissues. (73) In addition to Achilles tendinitis, other articular manifestations of FH have been reported. (94) These can manifest as oligoarthritis or polyarticular rheumatic fever-like arthritis, but rarely seen.

Carrying a severe risk factor may have a profound psychological impact on a person. If the knowledge of such risk seriously affects the well being and happiness of the individual, the cost-benefit of such knowledge becomes less favourable. However, such knowledge did not appear to cause long term distress. (95) There is not much information about the psychological effect of the diagnosis of the disease on FH patients apart from studies done in Scandinavia. A small number of patients regretted that the diagnosis had been made, whilst a few developed symptoms or had worsening symptoms once they became aware of having FH. Almost half of the cohort felt anxious about the diagnosis. (96) Tonstad looked at the psychological impact of such a diagnosis on children. Compared to their peers, there was no difference in psychological functioning between

these children and the controls, although children with FH tended to worry more that their parents would get sick and die. Usual activities were not affected and these children did not worry excessively about their health.(97;98)

1.7 Diagnosis

The diagnosis of FH is made in the time-honoured way of taking a detailed history, executing a careful physical examination of the patient and then doing laboratory tests before making a final decision. The combination of a family or personal history of premature coronary artery disease, a tendon xanthoma and (LDL) hypercholesterolaemia clinches the diagnosis of the FH phenotype. While genetic tests should be regarded as the final confirmation, they are not always practicable: binding defective B₁₀₀ can be excluded by testing for a few mutations, but there are numerous mutations in the LDLR and such investigation is only cost-effective in founder populations.

1.7.1 History

The history should include all the presenting complaints with specific enquiry into conditions that may cause secondary hyperlipidaemia such as pregnancy, hypothyroidism, diabetes, anorexia nervosa, renal or liver disease or gout. A history of medication use may reveal causes of secondary dyslipidaemia. Drugs such as beta blockers, hormone replacement therapy, thiazide diuretics, retinoids, steroid hormones and microsomal enzyme-inducing agents generally only increase triglyceride levels. Anti-retroviral therapy has now also become an important cause of secondary hyperlipidaemia. Achilles tendinitis as a teenager may also provide a pointer to tendon xanthomata. Because of the high risk of coronary artery disease (CAD), specific enquiry should be directed to symptoms and even non specific complaints should be taken seriously. A dietary

history will give an indication of the intake of cholesterol and saturated fat and will assist in giving better targeted dietary advice. If there is a suspicion of FH a detailed family history is very informative and drawing a family tree is a practical and graphic way of storing the information.

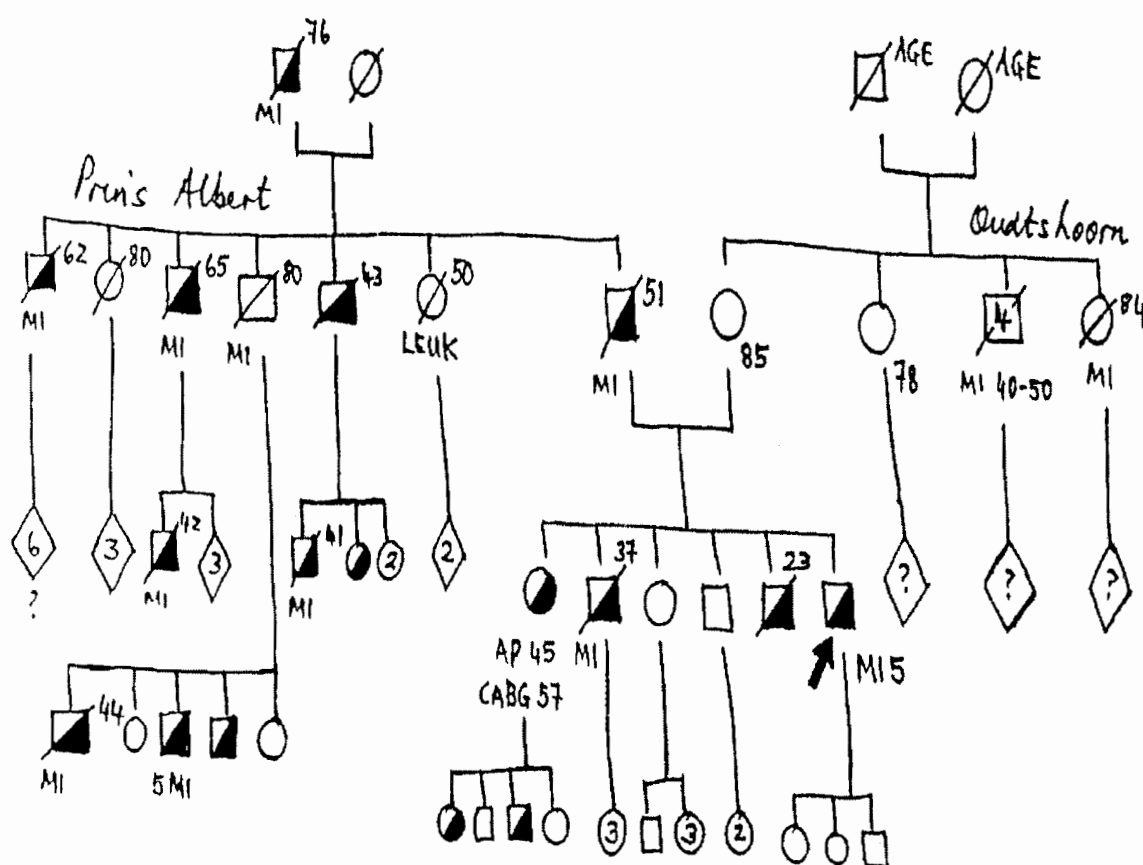


Figure 1.7.1 Family tree drawn at consultation in the Lipid Clinic

○ = female □ = male ◇ = children (number) ↗ = index case

Half shaded symbols indicate affected individuals.

A line drawn through the symbol indicates death, with age given in years

1.7.2 Clinical examination

Physical examination should assist in exclusion of conditions that are associated with secondary hyperlipidaemia. More specific examination for evidence of FH should include examination of the eye, looking for the presence of xanthelasma on the upper and lower eye lids and for arcus cornealis. Xanthelasmata may be obvious as a soft, raised, palpable plaque with yellow/orange pigmentation, or fairly subtle as a thin pigmented line. Arcus cornealis may occur inferiorly, looking like a crescent moon, bilaterally, superiorly or circumferentially. It is important to retract the upper lid when examining for arcus as a superior arcus can be missed if not looked for in this way. Arcus cornealis is almost always bilateral.



Figure 1.7.2.1 Elevation of the upper eyelid to expose superior arcus cornealis which could otherwise be missed. Also superior, inferior and lateral canthus xanthelasma

Although cutaneous xanthomata are rare in heFH, the skin needs to be carefully examined. Planar xanthomata occur particularly in homozygous FH (hoFH) and interdigital xanthomata are pathognomonic for the disease. In hoFH planar xanthomata can also occur in the natal cleft, gluteal fold, ante-cubital and popliteal fossae and at the wrists and heels. Tuberous xanthomata usually occur on the elbows and on the knees in FH but may also be found on the buttocks in hoFH.

Tendon xanthomata occur classically in the Achilles tendon but may also be found in the extensor tendons of the hand and foot, and also in the plantar fascia of the foot. The Achilles tendon xanthomata can be subtle and careful palpation may be required. The tendon is best examined with the foot at 90 degrees and palpating the edges of the tendon from the calcaneus to the muscle junction. Initially the tendon may just feel broadened or rounded in the antero-posterior dimension as cholesterol deposition changes the dimensions. Later there may be a roughening of the normally smooth edges or the development of a fusiform swelling or a frank nodule. In some patients the xanthomata can be so gross that they are easily visible and may make wearing of normal shoes is difficult.

The extensor tendons are best seen when the hand is made into a claw, which makes the tendons prominent. There may also only be a roughening of the edge of the extensor tendon or a nodule may be palpated. These lesions move with the tendon. Occasionally there may be large xanthomata, especially near the knuckles.

These clinical signs seldom occur before the age of 25 years in heterozygous FH and regress on treatment.



Figure 1.7.2.2 The claw position of the hand gives the best exposure of extensor tendons to display xanthomata

1.7.3 Laboratory investigation

A random cholesterol level may give one a strong suspicion of FH but a full fasting lipid profile comprising a triglyceride, total cholesterol, HDLC and LDLC concentration is essential for the confirmation of FH, as hypertriglyceridaemia and hyper-alpha lipoproteinaemia may also give high total cholesterol values.

Further biochemical tests are necessary to exclude secondary hyperlipidaemia. An assay of thyroid stimulating hormone (TSH) is needed to exclude hypothyroidism. Plasma urea and creatinine are recommended to exclude impaired renal function. Serum albumin will detect nephrotic syndrome

or the very rare condition of analbuminaemia as a cause of LDL hypercholesterolaemia. Plasma bilirubin concentrations are useful to exclude cholestatic liver disease, whilst liver enzyme studies are recommended if statin therapy is considered. A fasting blood sugar is needed to exclude diabetes mellitus and uric acid to exclude gout as these are commonly encountered illnesses that may also affect patients with FH.

In FH the total cholesterol level is generally above 7.2 mmol/L. In children the level will already be raised at birth and is roughly twice the level of normal controls. The level rises in the first year of life and then remains static until the early teenage years and is the same in boys and girls. A level of >6.5 mmol/L will identify >95% of FH children. (73)

A confident diagnosis of the FH phenotype can thus be made with the presence of a family history of premature ischaemic heart disease, the physical finding of a tendon xanthoma (in the patient or a first degree relative) and an elevated cholesterol level. Further genetic confirmation of an LDL receptor defect is useful when there are no physical signs or when there is doubt about the aetiology of the hyperlipidaemia.

1.8 Differential diagnosis

1.8.1 Secondary hyperlipidaemia

In establishing the diagnosis of FH it is essential to exclude secondary hyperlipidaemia such as some common causes tabled below.

CONDITION	VLDL	LDL	HDL
Type 1 diabetes	↑	- or ↓	- or ↓
Type 2 diabetes	↑↑	↑	↓
Hypothyroidism	↑	↑↑	↑
Pregnancy	↑	↑	↑
Obesity	↑	- or ↑	↓
Alcohol	↑	- or ↑	↑
Nephrotic syndrome	↑	↑↑	- or ↓
Chronic renal failure	↑	-	↓
Cholestasis	-	↑↑(LpX)	↓
Hepatocellular disease	↑(IDL)	-	↓
Hyperuricaemia	↑	-	↓

Table 1.8.1 Causes of secondary hyperlipidaemia

LpX = lipoprotein X

(73)

1.8.2 Primary hypercholesterolaemia

Once it has been established that one is dealing with primary hypercholesterolaemia, other conditions to consider are indicated in the table below. A positive family history of premature CAD need not be specific to FH nor is an elevated cholesterol level or the presence of a tendon xanthoma.

	FH PHENOTYPE			OTHER DYSLIPOPROTEINAEMIA		
	LDLR	FDB	PCSK9	FCH	POLYGENIC	DYSBETA
Fam hist	Y	Y	Y	Y	N	N or Y
Ach xma	Y	Y	Y	N	N	Y
LDLC	↑	↑	↑	↑	↑	↓
Triglyceride	N	N	N	N or ↑	N or ↑	↑
Mutations	>700	3	3	?	Multiple	19
Prevalence	1/70-1/500	1/210-1/700	?	1/100	Unknown	1/10,000
Founder report	Gujerat Lebanon Fr Can Lithuania ZA Afrikaner	Swiss German		Ubiquitous	-	Neth

Table 1.8.2 Differential diagnosis of primary dyslipidaemia
(The physical signs are not in variate)

FH	familial hypercholesterolaemia
LDLR	low density lipoprotein receptor
FDB	Familial defective binding apoB ₁₀₀
NARC1	neural apoptosis regulated convertase 1
FCH	Familial combined hyperlipidaemia
Dysbeta	dysbetalipoproteinaemia or Type 111
Fam hist	family history
Ach xma	Achilles xanthomata
Fr Can	French Canadian
ZA	Zuid Afrikaans = South African

To definitively distinguish between FH and FDB and PCSK9 one would need a genetic diagnosis or a functional test, but the latter may not be discriminatory and in the case of NARC1 is not available.

In clinical practice the differential diagnosis between FH and dys- β lipoproteinaemia is important. The presence of a raised triglyceride level, particularly if there is a 2:1 ratio of cholesterol to triglyceride level, or if the TG levels are raised even more, should suggest the possibility of dys- β lipoproteinaemia. In this condition the hypercholesterolaemia often does not manifest until the mid twenties and in FCH it can be as late as early middle age. The presence of an apoE2/2 genotype and plasma ultracentrifugation would confirm the diagnosis of dys- β lipoproteinaemia. Rarer genetic causes of dys- β lipoproteinaemia may be autosomal dominant, eg. R145C and K146Q. Should these sophisticated tests not be available, a simple method using the apoB/TC ratio as a diagnostic test can be used. (99) A ratio of <0.15 will identify dysbetalipoproteinaemia in almost 90% of cases. The clinical relevance is that dys- β lipoproteinaemia responds particularly well to fibrate therapy, although statins may also be effective.

1.8.3 Xanthomatous disease

Other causes of xanthomatous hypercholesterolaemia are extremely rare but should be considered in the presence of xanthomata and the absence of a family history of premature ischaemic heart disease. These are tabulated below in table 1.8.3.1. In phytosterolaemia the cholesterol levels are initially normal to moderately raised, but may also be elevated to the levels seen in FH.

	FH	PHYTOSTEROLAEMIA	CTX
Tendon xanth	+	+	+++
Family history of premature CAD	+	-	-
Elevated Chol	++	-/+	-
CAD by middle age	+	+	-
Cataracts	-	-	+
CNS involvement	-	-	+

Table 1.8.3 Differential diagnosis of tendinous xanthomatous diseases

FH	Familial hypercholesterolaemia
CTX	Cerebro-tendinous xanthomatosis
Xanth	Xanthomata
CAD	Coronary artery disease
Chol	Cholesterol
CNS	Central nervous system

1.9 Founder effects

A founder effect is a phenomenon causing a higher than anticipated prevalence of a mutant gene in a population. It generally results from the introduction of the gene into a population in a confined or new geographical location or cultural isolation by the migration of relatively small numbers. The prevalence is maintained if the group remains isolated from other communities. In the case of the Afrikaner population isolation resulted in a more than 1000-fold increase in this gene restricted pool over a period of 300 years. (37)

Three different founder mutations, Afrikaner FH1 (D206E), Afrikaner FH2 (V408M) and Afrikaner FH3 (D154N) have been identified in this country. As discussed in chapter 1.3.2, this accounts for the incidence of FH in certain areas in South Africa being as high as 1/72 compared to the world wide incidence of 1/500. (100)

FH Lithuania (G197del), previously known as FH - Piscataway, the most prevalent LDL receptor mutation causing FH amongst Ashkenazi Jews, is also commonly found in South African Jews with FH. This defect dates back to the 14th century and corresponds with the establishment of the Jewish community of Lithuania in AD1338. Thereafter there was a great demographic expansion of Ashkenazi Jews in eastern Europe. (101) At the turn of the 19th century and particularly with the build-up of anti-Semitism during the following decades, massive emigration saw Jews of Lithuanian origin settling in Israel, North America and South Africa, where this is the most common LDL receptor defect in persons of Jewish origin. Resulting from the admixture of genes that has occurred in this country, this defect has also been found in the mixed race or so-called "Coloured" population group. (102)

FH Gujerat (P664L) has been identified in the Indian population in South Africa who can trace their origins to state of Gujerat. (103) At the turn of the 19th century, under the auspices of the British, a large number of Indian immigrants were brought to this country from the west of India as traders and to work on the sugar cane farms. In this population group living in South Africa this gene mutation has also been identified and contributes to the commonly occurring genetic defects seen at the lipid clinic at UCT and Groote Schuur Hospital (GSH).

The "Lebanese allele" (C660X), first described in 1987 as being responsible for the extraordinarily high incidence of FH in Christians in Lebanon (104), has also been found in Lebanese populations around the world, including South Africa.

FH in French Canadians in North Eastern Quebec occurs at a frequency of 1 in 154. Between 1608 and 1759, 8500 French settlers colonised “Nouvelle France”. Their linguistic and relative geographic isolation gave rise to a number of founder effects in 22 Mendelian diseases. (105) Eleven mutations in the LDLR have been found in this population and account for more than 90% of cases of FH. (106) The E207K defect (also known as the Acadian mutation) has also been identified in patients at the GSH lipid clinic (107), but haplotype analysis has not been done to ascertain whether these are recurrent mutations or share a common ancestry.

The differences in the expression of the FH phenotype has been widely described. (108;109;110;11;112) These differences occur between countries, between people of the same ethnic origin living in the same country and even between members of the same family.

1.10 Management

The management of FH, as with all other dyslipidaemias, requires a comprehensive approach. The severe LDL hypercholesterolaemia is the major target for treatment but all the risk factors involved in the causation of ischaemic heart disease need to be addressed. The involvement of other health professionals can enhance the quality of the management and the additional expense incurred is justified by the high risk and anticipated benefit of this approach.

1.10.1 Lifestyle modification.

1.10.1.1 Dietary modification.

As far back as 1939, Muller recommended eliminating “egg yolks, butter, cream, fat milk and animal fat in general”. (113) These recommendations have now been substantiated by much

scientific evidence. Prospective with-in population studies have shown that the dietary intake of cholesterol is significantly related to long term CHD risk, independent of and in addition to serum cholesterol, blood pressure and smoking. Conversely, lower intakes of cholesterol were associated with significantly lower risks of CHD and of all cause mortality. (114)

Brown and Goldstein elegantly describe how dietary cholesterol suppresses LDLR activity in the liver, leading to hypercholesterolaemia. (13) The aim of dietary modification is to lower the LDL concentration by means of up-regulating the functional LDL receptors of FH heterozygotes to enhance clearance of LDL. Both cholesterol and saturated fat down-regulate the LDLR thus diminishing clearance of LDL from the plasma. In addition the effect of saturated fat on the down-regulation of the LDLR is enhanced when cholesterol is concurrently present in the diet. The additive effects of cholesterol and saturated fats have been combined in the so-called Cholesterol-Saturated Fat Index (CSI). The cholesterol in milligrams and the saturated fat in grams are given in a single number to denote the potential of a foodstuff to elevate the plasma LDLC. (115) Other factors affecting LDL synthesis such as obesity, which enhances VLDL production, are also important. Conversely, ω -3 fatty acids depress the synthesis of VLDL and LDL. ω -3 fatty acids affect vascular function favourably and in secondary prevention of CVD were shown to reduce coronary mortality. (116)

The Indo-Mediterranean diet, in which a diet rich in fruit, vegetables, legumes, walnuts and almonds was consumed with the intake of α -linolenic acid being twice that of the control group, resulted in a significant reduction of fatal and non-fatal myocardial infarction. (117) In the elderly prospective cohort study (EPIC) the modified Mediterranean diet (in which polyunsaturated fats were substituted for monounsaturated fats) was associated with a lower overall mortality. (118) It is likely that FH subjects will also have beneficial outcomes on these interventions.

The Lyon Diet Heart Study found that when survivors of a first MI were placed on a

Mediterranean-type diet (in which mono-unsaturated fats, as in olive oil, predominated, an α -linolenic acid enriched margarine was supplied and which included fruit, vegetables and complex carbohydrates and fibre) for a mean of 46 months and were compared to those who followed a Western diet, there was a significantly lower risk of cardiac death or recurrent MI. (14 vs 44 events, $P < 0.00$. (119) This reduction in the recurrence rate of cardiac events of up to 70% is greater than any achieved in published drug intervention studies. This impressive finding in non-FH subjects needs confirmatory studies and may not necessarily apply in the severe dyslipidaemia found in FH.

In 1988 the National Cholesterol Education Program established dietary guidelines, endorsed by the American Heart Association (AHA), which became known as step one, step two and step three diets. (120) A study was published by Connor in which these dietary modifications were implemented. (115) See table 1.10.1.1.

	Total fat (% of cal)	Saturated fat (% of cal)	Polyunsat. fat (% of cal)	P/S ratio	Cholest. (mg/day)	Predicted total change in plasma cholesterol from Western diet
Western diet	40	15	6	0.4	500	
Low cholesterol, low fat, high carbohydrate diet						
Phase 1	35	14	9	0.6	350	- 6%
Phase 2	25	8	8	1.0	200	- 13%
Phase 3	20	5	8	1.3	100	- 19%

Table 1.10.1.1 Predicted plasma cholesterol lowering from the three phases of the low cholesterol, low saturated fat, high carbohydrate diet
% of cal = percentage of total daily calorie intake

It is clear that reduction of dietary cholesterol and saturated fat intake has a marked effect on

plasma cholesterol levels and should be implemented as the foundation of treatment. (121;122) More than 30 years ago the effects of dietary modification in secondary prevention were investigated by the Oslo Diet-Heart study in which MI survivors were given a low-cholesterol, low fat diet that included oily fish and anti-smoking measures were implemented. Compared to a usual diet, total cholesterol was reduced by 18% at 5 years and the recurrence rate of fatal or non-fatal MI was reduced by 37%. (123) In many dietary trials the decrease in the CHD incidence was shown to be greater than would have been anticipated by the LDL lowering effect alone. (73)

In FH diet alone will not get patients to target. The high risk of CVD necessitates intervention from a young age and dietary management over a long period may reduce risk and may also reduce the doses of medication required for the control of the dyslipidaemia. While drug treatment is thus required for all FH subjects, the time of commencement of treatment has not been universally agreed upon.

1.10.1.2 Cessation of smoking

Cigarette smoking is well-recognised as an independent major risk factor for cardiovascular disease (124) and the risks of smoking and heart disease are well known to the medical profession as well as to the public. Nevertheless many smokers do not believe that smoking is harmful to them. In one study over 60% of current smokers, even those smoking more than 40 cigarettes a day, did not believe that they were at an increased risk of CVD. (125) The risk of myocardial infarction is increased six-fold in women and three-fold in men who smoke 20 cigarettes a day compared to non-smokers. (126) Patients who continue to smoke in the presence of ischaemic heart disease have a greater risk of re-infarction and of death, including sudden cardiac death. (127;128) In addition to increasing cardiovascular mortality, cigarette

smoking also increases all-cause mortality. (129) In those subjects who stopped smoking, there was no increased risk of death. It is also estimated that in the USA passive smoking may cause almost 40,000 deaths from cardiovascular disease each year. (130) Passive smoking increases the risk of CVD in non-smokers by 20%. (131)

The direct effect of smoking on the development of atherosclerosis was assessed in the ARIC (Atherosclerosis Risk in Communities) study. (132) Intimal-media thickness (IMT) was measured in more than 10,000 patients showing a 50% increase in IMT in smokers compared to non-smokers over a three-year period. The rate of progression of current and past smokers was no different, suggesting that some of the adverse effects of smoking may be cumulative and irreversible.

Despite the clear relationship between smoking and CVD, the mechanism by which complications occur is not completely understood, possibly because multiple factors are involved. LDLC and TG are raised, and HDLC is reduced. (133) and the oxidation of LDLC is promoted. Smoking also enhances the prothrombotic state via inhibition of tissue plasminogen activator release, (134) elevation of fibrinogen (135), increasing platelet activity and expression of tissue factor. (136;137) It has also been associated with raised C-reactive protein levels, (138) increased concentrations of soluble adhesion molecules and raised serum homocysteine. (139) Nicotine in cigarette smoke plays a major role in transient smoking-related increases in cardiac output, heart rate and blood pressure but it is not clear if it has a direct role in atherosclerosis, apart from a variable effect on nitric oxide. (140).

Despite being a major risk factor for CVD, smoking is also a reversible risk factor. The benefits of smoking cessation are firmly established. In patients without CHD reduction in cardiac event rate is almost 50%, and this reduction in risk occurs relatively soon after quitting and continues to do so. This benefit also extended to those who had established IHD. (141)

1.10.1.3 Regular exercise and maintenance of ideal body weight.

Regular physical activity is important in both primary and secondary prevention of CVD. Inadequate physical activity has been recognised as an independent risk factor in the development of premature ischaemic heart disease and in the USA about 12% of all mortality is related to lack of regular physical activity. (142) The lack of physical activity is associated with at least a twofold in the risk of coronary events. Conversely, physically active individuals have a lower incidence of CHD and cardiorespiratory fitness is associated with a lower incidence of mortality from cardiovascular disease and from all causes of death. In secondary prevention, exercise rehabilitation reduces cardiovascular and total mortality by 31 and 27 percent respectively. (143)

However, the benefit of regular exercise on the risk of CVD is difficult to determine as this is usually associated with other lifestyle improvements such as a healthy diet and cessation of smoking as well as seeking medical attention for the management of hypertension and dyslipidaemia. Despite this the improvement in cardiovascular and general fitness, the enhancement in the quality of life, and the benefit of weight control and other cardiac risk factors fully justify the merits of regular exercise. Although the mechanisms by which physical activity protects against CVD are not fully understood, the benefits may in part be due to the favourable impact of exercise on the lipid profile. These factors are also expected to apply to FH.

Early studies comparing middle-aged male runners to sedentary men showed that runners had lower TG, TC, LDLC and VLDL and higher HDLC concentrations. (144) In a later study with a more detailed protocol, the benefit of exercise extended to LDL particle size. In the high intensity exercise group LDL particle size increased. The beneficial effects on the lipid profile occurred independently from weight loss. (145) The mechanisms by which exercise impacts favourably on the lipid profile are not fully understood. Exercise increases lipoprotein lipase (LPL) and

reduces hepatic lipase (HL) activity. (144) This suggests that increased lipolysis of TG-rich lipoproteins may lead to elevated HDLC. Exercise is also associated with a reduction in cholesterol ester transfer protein (CETP) (146) and an elevation of serum lecithin cholesterol acyl transferase (LCAT) concentrations. (147)

Exercise also influences haemostatic factors. Platelet adhesion and aggregation was shown to be reduced after eight weeks of moderate exercise in a study done on men. (148) In physically active women VO₂ max was higher and PAI-I activity and t-PA antigen were lower when compared to inactive controls. (149) Similar benefits could be expected in patients with FH.

A study in FH patients looking at exercise capacity and heart rate recovery as predictors of coronary heart disease, found that decreased exercise capacity, a delayed decrease in heart rate during the first minute after cessation of graded exercise and increased peak pulse pressure were strong predictors of coronary events. The study concluded that physical exercise should be strongly recommended in patients with FH. (150)

1.10.2 Lipid modifying drug therapy.

As discussed in 1.1, the landmark “4S” study showed conclusively that lipid lowering reduced cardiac events and overall mortality in moderate hyperlipidaemia. (19) The FATS study was the first angiographically controlled study to show that marked LDL lowering by drug therapy resulted in a significantly reduced rate of progression of coronary artery lesions. (151) The publication of the results of the intensive lipid lowering study with atorvastatin (152) (also known as the TNT study) showed that intensive lipid-lowering therapy with 80 mg atorvastatin in patients with stable IHD provided significant clinical benefit beyond that afforded by treatment with 10mg of atorvastatin per day. Despite the difference in the LDLC being only 0.6 mmol/L

there was a 22% reduction in relative risk. These results would indicate that “lower is better” and encourage more intensive lipid lowering.

These targets are however difficult to obtain in FH. The ASAP study (91) compared IMT progression in FH subjects treated with 80mg atorvastatin and 40mg simvastatin. On this drug regimen, 27% of patients taking atorvastatin reached the goal of 3 mmol/L and only 7% taking simvastatin achieved this target. There was significant regression in the atorvastatin group whereas IMT in the simvastatin group increased. The study concluded that although target was not reached, a 45% lowering in LDLC in FH patients is required to achieve regression. Even in the simvastatin group, the recorded progression of IMT was slower than the anticipated progression.(91)

Whilst “statins” have been shown to have the most effective LDLC lowering effect they are not always tolerated and other drug therapy may be required as an alternative or in addition to statin therapy to achieve ideal levels.

1.10.2.1 HMG -CoA reductase inhibitors or statins.

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) (statins) reductase inhibitors have been shown to reduce cardiovascular events. (19) The statins have become the first line of treatment for FH because they are the most powerful agents to reduce LDLC concentration. The advent of this group of drugs holds promise that the prognosis of FH can be reversed to the background risk of the population.

Statins inhibit HMG - CoA reductase, a rate limiting enzyme in cholesterol biosynthesis. Their main effect is the reduction of LDLC although there is some reduction of triglyceride (mainly in

proportion to its untreated level) and some elevation of HDLC. Statins increase the removal of IDL and LDL from the plasma by increased expression of LDLR in the liver in response to the reduction of the free cholesterol pool. Additionally the statins may also limit the production of VLDL and LDL. (153)

The statins are generally well-tolerated with the most commonly encountered clinical side effect being myalgia, which occurs in about 0.5% of patients. Other general side effects include headache, nausea, tiredness, insomnia and constipation. Elevation of hepatic transaminases may occur and serum creatine phosphokinase (CPK) may be elevated, with or without accompanying muscle stiffness or pain. Rhabdomyolysis, defined as a rise in CPK greater than 10 times the upper limit of normal, is infrequent and is usually as a result of unusually high dosage and in combination with other drug therapy.

The individual dose and potency of each statin is variable and responses also vary from patient to patient. Many clinical trials have shown that varying doses of different statins result in a reduction of total cholesterol by 17% - 35% and LDLC by 24% - 49%. TG is reduced by 10% - 17% and there is an increase in HDLC from 6% -12% in patients with hypercholesterolaemia. Fixed doses of 40 mg simvastatin, 10 mg atorvastatin or 5 and 10 mg rosuvastatin reduced LDLC by 30% and more. (154) Despite these significant drug-induced changes many hyperlipidaemic patients do not reach target, as was shown by the Lipid Treatment Assessment Program in the USA. (155) 4888 patients were studied over a two-year period and overall only 38% reached NCEP-specified LDLC target levels.

1.10.2.2 Bile acid sequestrants or resins.

These agents, which were developed more than four decades ago, are non-absorbable anion

exchange resins that bind bile salts irreversibly, resulting in depletion of the bile-acid pool. This leads to greater cholesterol breakdown to form bile acids which in turn up-regulates the LDL receptors to maintain cholesterol levels within the liver, thus lowering plasma cholesterol levels.

The older agents are cholestyramine and colestipol, which are difficult to take, with frequent side effects. The newer compound, colesevelam, is a polymeric, high potency, selectively engineered water-absorbing hydrogel. This unique formulation has substantially reduced the gastrointestinal side effects of this class.

1.10.2.3 Nicotinic acid or Niacin

Nicotinic acid is a B vitamin which in high doses (more than 900mg /day) reduces triglyceride, reduces LDLC and raises HDLC. It is also the only lipid modifying agent that has been shown to reduce Lp(a). Its mechanism of action is not clear but it appears to reduce VLDL secretion by the liver, perhaps in response to reduced fatty acid secretion by the adipocytes. Nicotinic acid is also cheap and would thus be the ideal lipid lowering drug were it not for the considerable side effects. These include flushing which is invariable at commencement of therapy and may persist, dyspepsia, gastritis, aggravation of gastric ulceration, pruritus and dry skin. Nicotinic acid may also cause hepatitis, gout and hyperglycaemia. The large numbers of tablets that are required to be taken, three or four times a day are also very inconvenient. Where 500 mg tablets are available this may be easier, and the slow release formulation is also reported to produce less flushing.

1.10.2.4 Ezetimibe

Ezetimibe is an azetidinone derivative that acts as an inhibitor of intestinal absorption of cholesterol. It inhibits the absorption of cholesterol and plant sterols by inhibiting the passage of dietary and biliary cholesterol across the intestinal wall by interference with uptake by the Niemann Pick C1-like protein. (156) Ezetimibe localizes at the brush border of the small intestine and decreases cholesterol uptake into the enterocytes. This apparently does not affect absorption of other soluble food nutrients and related compounds, such as triglycerides, fatty acids, bile acids, progesterone, ethynyl estradiol and soluble vitamins.

Ezetimibe will not suffice as monotherapy with an expected LDLC reduction of 18% with the registered dose of 10 mg. However, as additional therapy where a target has not been reached by statin therapy alone or where higher doses are not tolerated, this drug may be useful. Ezetimibe 10 mg per day administered as monotherapy or in combination with statins or fenofibrate lowered LDLC in hypercholesterolaemic patients by 15% - 20% while increasing HDLC by 2.5% - 5%. (157) A variable response in LDL lowering has been demonstrated, which could possibly be genetically determined. (158)

1.10.2.5 Fibric acid derivatives or fibrates.

The mechanism of action of fibrates is through peroxisomal proliferator activator receptor alpha (PPAR α) which acts on the promoter of several genes involved in lipid and lipoprotein metabolism. (159) Their impact is greatest on VLDL triglyceride but LDLC decreases moderately and HDLC is generally increased. LDL particle size also increases. This class of drug is generally well tolerated, but there may be side effects such as headache, diarrhoea, pruritus, rashes or hair loss. Bile acid lithogenicity may be increased and they should not be used in

patients with gall stones. Warfarin may be also be potentiated and the use of fibrates in patients with impaired renal function should be monitored carefully.

The fibrates are not the first line of treatment in FH. They are the drugs of choice in type V hyperlipidaemia and in dysbetalipoproteinaemia and may be very effective in mixed hyperlipidaemia.

1.10.3 Non drug therapies

1.10.3.1 Plant sterol enriched margarines

Margarines enriched with plant sterols have become available as an alternative lipid lowering strategy when a small reduction in LDLC would be beneficial, when medication is not tolerated or as an addition to medication when treatment goals are not achieved. These margarines contain large quantities of plant sterols or stanols. These steroids are similar to cholesterol in their chemical structure, differing in their side chain configuration, and are thought to inhibit cholesterol absorption in the gut. The average reduction found in trials using 1 - 3 g per day is 10% reduction in total cholesterol and 13% reduction in LDLC with no significant reduction in triglyceride or HDLC. (160) Studies in children showed greater reductions. (161)

1.10.3.2 Ileal bypass surgery

Partial ileal bypass surgery was developed by Buchwald as a surgical means of lowering plasma cholesterol, with the first results published in 1974. (162) The effect of ileal bypass surgery on mortality and morbidity from coronary heart disease was examined in the POSCH study. (163)

In this study 421 hypercholesterolaemic patients who had survived myocardial infarction had partial ileal bypass surgery. The surgical group had a 38% LDLC lowering compared to the control group. Overall mortality and mortality due to coronary heart disease were reduced in those who had the surgery but not significantly so. There was a significant (35%) reduction in the combined end point of death due to coronary heart disease and confirmed nonfatal myocardial infarction. Up to ten years following surgery there was less progression of atherosclerosis in the surgical group. These results provided strong evidence for the beneficial effects of lipid lowering in the reduction of atherosclerosis progression but this method of treatment has been totally superseded by the advent of effective drug therapy in patients with FH.

1.10.3.3 Plasmapheresis

This technique was first described by de Gennes in Paris in 1967 and used successfully in a homozygous patient. (164) Thompson in London instituted the therapy on a regular basis in 1974. (165) This method of cholesterol removal is very successful in hoFH, improving well-being, reducing xanthomata, impacting positively on the complications of severe hyperlipidaemia and prolonging life expectancy. In heFH, this form of therapy as well as selective LDL adsorption or heparin assisted extracorporeal lipoprotein precipitation (HELP) has been surpassed by the very effective lipid lowering action of the statins.

1.10.3.4 Liver transplantation

This form of therapy has been reported to successfully reduce LDLC in hoFH (166;167) but has apparently not been used in heFH.

1.10.3.4 Gene therapy

Gene therapy may have additional benefits to statin therapy where a very low level of LDLC is desired. One approach is to correct or replace the dysfunctional receptor in the liver. This has been tried experimentally in patients with hoFH with mixed success. (168) It is probably more feasible to inhibit LDL production by limiting the production of lipoprotein containing apoB₁₀₀. This may be possible through anti-sense mRNA but careful studies need to be undertaken to ascertain whether fatty liver may become a problem.

2.1 Clinical documentation

The Lipid Clinic is located in Groote Schuur Hospital, which forms part of the tertiary and teaching hospital group serving a large part of the Western Cape. Whilst the Clinic sees mainly indigent patients, patients referred from the private medical sector are also assessed.

In 1990 new data capture sheets were designed for use in the clinic to document initial data, including the demographics, physical findings, clinical tests and laboratory investigations. Thereafter all new patient information is entered onto the database (Paradox/Corel). A 'follow-up' sheet is used to document all subsequent visits to the clinic. All the forms are filled out in duplicate, a carbon copy is kept in the patients hospital file and the originals are stored at the Lipid Laboratory at the Health Science Faculty. These files are taken to the clinic for each clinic visit to update clinical findings and laboratory results, which are copied into the patients hospital folder. Not only does this approach facilitate access for research but it also allows for rapid access on enquiries and serves as a source document for missing notes.

Once informed consent, approved by the University Research Ethics Committee is obtained, DNA is prepared from each patient for subsequent analysis according to the availability of research funds and specific projects.

Admission Form 1 summarises identification, symptoms, past medical history and current medication, as well as dietary and lifestyle habits. On the reverse side of the admission form 1, a detailed family tree is drawn at the first visit, and is often added to at subsequent visits as the patient brings in new information about the family. If large family cohorts are identified, all the family information is pooled at a later date to compile a comprehensive family tree.

The second sheet (Form 2) contains the physical findings and routine chemistry. Admission form 3 contains the clinical diagnoses and management. The first follow-up sheet summarises lipid profiles and medication for five visits. The second follow-up sheet summarises the text for each of these five visits.

These computerised details of the records of patients visiting the Lipid Clinic were searched for candidates with FH for inclusion into this study. A database was set up using Paradox (Corel). Some of these records dated back to the early 1970's, with some of these patients or family members still attending the clinic.

Initial criteria for including patients in this study included a presenting untreated TC level of >7.5 mmol/L, the presence of tendon xanthomata in the patient or a first degree relative and a family history of premature ischaemic heart disease. Subjects lacking tendon xanthomata but who had an apparent dominant inheritance of LDL hypercholesterolaemia together with premature heart disease and who were considered to have probable FH, were included if genotyping for locally prevalent mutations were identified. More than 5,000 files of patient data were perused to verify the data and to identify other patients not captured by the electronic search. All the relevant information was entered into a Paradox database for analysis. Many patients were contacted telephonically to obtain missing data and some were re-examined at the clinic to confirm physical findings. Once the database was as complete as possible and all the current patients were entered, it was locked for analysis.

PD1165

GROOTE SCHUUR HOSPITAL LIPID CLINIC (ADMISSION FORM)

DATE _____	PATIENT _____
SEEN BY _____	NUMBER _____ RACE _____
	BIRTH _____ SEX _____
REFERRED BY: _____	PATIENT ADDRESS _____
_____	_____
_____	_____
PHONE No: _____	(H) _____ (W) _____
REASON _____	

EFFORT & ANGINA

CLAUDICATION/TIA

OTHER CVS

RESPIRATORY

DIGESTION

OTHER SYMPTOMS

PAST

AP

MI

DM

HYPERTENSION

DRUGS

TOBACCO: Start:

Quit

Current

Avg/D:

ETHANOL

g/week of

daily

wknd

☐

binge

☐

EXERCISE

DIET

Breakfast

Lunch

Supper

In - between

High fat foods

Schooling:

SOCIAL: S

☐

M

☐

D

☐

W

☐

OCCUPATION

Figure 2.1.1 Admission data sheet 1 for Lipid Clinic

(The reverse side is blank for pedigree)

GSH LIPID CLINIC ADMISSION FORM (2)

PD 1141

PATIENT:

HEIGHT __,m __ MASS __,kg __ BMI __

Waist __ Hip __ WHR __

Arcus cornealis

Xanthelasma

Tendon xanthoma

Cutaneous xanthoma

GENERAL EXAMINATION

PULSE

BP Erect

Supine

Site	Radial	brachial	femoral	poplit	dors. ped	post tib	carotid	
R								

N=Normal
R=Reduced
A=Absent
B=Bruit
E=Ecstatic

AORTA

RENAL

JVP

APEX

AUSCULT

ECG (DATE _____)

RESPIRATORY

ABDOMINAL

NEUROLOGIC

URINALYSIS

Lipid tests	1	DATE		2	DATE		3	DATE	
1 = mM/L		TG			Apo A			Origin	
2 = m		T Chol			Apo B			Beta	
3 = %		HDL - C			Apo (a)			pre - R	
		LDL - C						Alpha	

APPEARANCE

CHEMISTRY	Glucose		Alk Ph		Urate	
	Protein			GGT		Urea
	Albumin			ALT		Creat
	Bili			AST		

Previous investigation:

DATE								

Figure 2.1.2 Admission data sheet 2 for Lipid Clinic.

PD 1142

GSH LIPID CLINIC ADMISSION FORM (3)

PATIENT

RISKS ASSESSMENT

TRIGLYCERIDE		FAMILY HIST	HYPERTENSION		
TOTAL CHOL		SMOKING	DIABETES MELL		
LDL-CHOL		EXERCISE	RENAL DISEASE		
HDL - CHOL		DIET	HYPERURICAEMIA		
Apo (a)		OBESITY			

CLINICAL ASSESSMENT

LIPID DISORDER

FREDRICKSON TYPE:

OTHER CLINICAL PROBLEMS

1

2

3

4

5

6

7

8

MANAGEMENT

FAMILY STUDIES

DIET

OTHER

DRUGS

FOLLOW - UP

COMPUTERISED

DICTATED

Figure 2.1.3 Admission data sheet 3 for Lipid Clinic

Figure 2.1.4 DNA Consent form for Lipid Clinic.



**THE LIPID CLINIC AND LIPID LABORATORY AT THE
UNIVERSITY OF CAPE TOWN & GROOTE SCHUUR HOSPITAL
CONSENT FOR DIAGNOSTIC RESEARCH INVESTIGATION**

Please read carefully and feel free to ask any questions you may have. If you agree, place a check (✓) in the relevant block; if you disagree, delete the relevant clause.

I, ... hereby consent
to the removal of blood ☐, skin ☐, or other tissue (specify)
..... from myself and/or
..... to whom I am the (relationship)
.....

The purpose of the investigation is to perform

- ☐ diagnostic tests for dyslipidaemia and/or other metabolic disease that may predispose to atherosclerosis
- ☐ research investigations currently and in future, subject to the conditions of the institutions above and the ethics review that govern the research at these institutions. This may mean the investigation for alternative inherited defects to explain my condition, or other factors which may have a bearing on the outcome of my condition, or for testing of any other gene that may be under research.

The result(s) of the analysis carried out on the sample(s) will be made known to me, via my doctor, in accordance with the relevant protocol if and when available. The doctor(s) to be informed:

.....

Additionally, I authorise that the results be made known to the following family member(s):

.....

After due explanation by, I understand that

- (1) Conventional procedures and techniques are employed by trained staff to obtain and process the samples and that consequently the risk to my health is minimal.
- (2) Confidentiality and anonymity of the sample(s) is(are) respected by coding the samples with numbers. My written permission is required for release of identifiable information to any other party.
- (3) Precise diagnoses may not always be possible because of limited knowledge about the cause(s) of the condition(s). Even under the best conditions, current understanding and technology are imperfect. The research may thus be of no direct benefit to me but the samples may provide general insight into disease and health.
- (4) The cost involved for me will be declared and no tests for which payment is required will be undertaken without prior consultation. Costs may be determined according to scheduled tariffs, or borne by research budgets, and that
- (5) No commercial claims can be made on developments from these investigations by myself or family.
- (6) Permission to investigate may be withdrawn at any time by me, without prejudice to my future medical care.

Signed Date.....

Witnessed by (Signatures)

Printed Names & Dates

PD 1140

GSH LIPID CLINIC FOLLOW -Up (1)		PATIENT		
		FOLDER SEX		
		BIRTH RACE		
		Lp(a)	GGE	TYPE

LIPID CHEMISTRY					
DATE/ASSAY					
TRIGLYCERIDE					
TOTAL CHOL					
HDL - CHOL					
LDL - CHOL					
CLINICAL & DRUGS (PTO FOR NOTES)					
DATE OF VISIT					
MASS/HEIGHT					
BP AND PULSE					
OTHER CHEMISTRY					
Glucose/URATE					
UREA/CREAT.					
AST/ALT.					
GGT/ALK.P.					
CPK/LDH					

Figure 2.1.5 Follow-up chemistry and drug sheet

PD 1143	
GSH LIPID CLINIC FOLLOW - UP (2) BRIEF CLINICAL NOTES TO INCLUDE COMMENT ON EXERCISE, DIET, AND SIDE EFFECTS.	PATIENT FOLDER SEX BIRTH RACE
DATE _____ SEEN BY _____	
DATE _____ SEEN BY _____	
DATE _____ SEEN BY _____	
DATE _____ SEEN BY _____	
DATE _____ SEEN BY _____	

Figure 2.1.6 Follow up form for clinical notes

2.2 Routine chemistry

The first visit of a new patient to the Lipid Clinic is for venesection only, for which the patient fasts for 12 hours. The nursing sister takes blood which is sent to the local laboratory for routine chemistry and to the lipid laboratory for the more specialised lipid tests.

Routine chemistry included -

Lipid profile - TG, TC, HDLC and LDLC by Friedewald calculation

ApoA, apoB, Lp(a).

Liver function - Bilirubin, AST, ALT, GGT, ALP, albumin

Renal function - urea, creatinine

Endocrine - TSH, T4, uric acid and glucose

For these tests conventional assays are used in the routine Chemical Pathology Laboratory on autoanalysers with appropriate quality control.

Detailed methods used in the Lipid Laboratory are given in the appendix. Brief descriptions of these methods are given in this chapter.

2.3 Gradient gel electrophoresis

LDL subclasses may be described by ultracentrifugation, electrophoresis or by means of proton nuclear magnetic resonance. For use in the UCT Lipid laboratory a gradient gel electrophoresis method was developed. Lipoproteins are separated by size using polyacrylamide gel electrophoresis. A 2 - 8% polyacrylamide gradient gel is poured with a 3% acrylamide stacking gel. Serum or EDTA plasma samples are pre-stained with a Sudan Black

in ethylene glycol solution and run overnight in a refrigerator at 120V. The gel is then examined to identify the LDL particle sizes.

This method has been published (169) as a means of diagnosing dysbetalipoproteinaemia so that this diagnosis which can occasionally present as an autosomal dominant condition with premature IHD and tendon xanthomata is also ruled out.

2.4 Lipoprotein ultracentrifugation

A detailed ultracentrifugation study is not part of a regular work-up for FH but was done once to compare several lipoproteins. This was done according to the method of Lindgren. (170) A plasma sample was adjusted to a high density with sodium bromide and was over-layered with successively less dense saline and sodium bromide solutions to yield VLDL1, VLDL2, IDL and LDL.

2.5 Extraction of DNA

The rapid method according to Parzer was used for extracting genomic DNA from whole blood. (171) This has a good yield (about 250 µg from 5 ml whole blood) and good absorbance ratios at 260 nm and 280 nm to indicate relatively pure DNA.

2.6 Polymerase chain reaction methodology

2.6.1 LDL receptor genotyping

2.6.1.1 Heteroduplex assay for LDLR mutations in the 3' portion of exon 4

This method was introduced to screen for several mutations in the 3' end of exon 4 in the LDL receptor gene. The mutations are: FH Afrikaner 3 (D154N), FH Lithuania (delG197), FH Padova (D200G), GSH4 (D203A), FH Afrikaner 1 (D206E), French Canadian 3 (E207K). All of these are added as controls. Multiple loadings of the gel after intervals permits several runs of PCR to be analysed on the same gel. Heteroduplexes of different sizes are formed according to the mutation and are better analysed over a UV light with a Polaroid picture than by exposure in the gel documentation apparatus. The different mutations are confirmed by restriction enzyme digest analysis. Novel heteroduplex patterns found on this technique may be followed by sequencing.

2.6.1.2 PCR assay for LDLR mutation R329X (FH Fossum) in exon 7

The R329X mutation in the LDLR was originally described by Solberg. (172) It was subsequently identified in Afrikaner families by Kotze at the University of Stellenbosch and also at the University of Cape Town (through Academisch Medisch Centrum, Amsterdam). The mutation is in exon 7 and it may have originated in Scandinavia. (172)

The assay involves the amplification of a 234 bp fragment of DNA in exon 7, with the mutation changing the Arg at codon 329 to the TGA stop codon. This mutation creates an extra cutting site with restriction enzyme BsrI.

2.6.1.3 Exon 3 amplification for heteroduplex analysis (R57C, R60C)

The mutations R57C and R60C were discovered in patients with familial hypercholesterolaemia at the lipid clinic of Groote Schuur Hospital by heteroduplex assay and subsequent sequencing. They were named FH GSH1 and GSH2 respectively. Other mutations may also be discovered in this region of the LDLR gene as it is part of the binding domain. Originally the work-up was done in collaboration with Dr M J Kotze at Human Genetics based in Obstetrics and Gynaecology at the University of Stellenbosch.

2.6.1.4 LDLR exon 7,8 deletion diagnosis by PCR - FH Cape Town 2

Exon 7 and 8 deletions have been described as the cause of familial hypercholesterolaemia (FH) from the Netherlands and South Africa. In the former case the deletions have been reported as 3 and 4kb, and in the latter there is a 2.5kb deletion. The South African deletion appears to be particularly prevalent in the mixed ancestry (Coloured) group in which there are 2 unrelated families with homozygous FH children in the clinic and several other heterozygotes have also been identified.

This method supercedes the Southern blot method originally described for the lesion. The long range PCR assay is the amplification of a 6.5 kb normal fragment while the 2.5kb deletion as the mutant allele renders a 4kb fragment. The amplification is from 5' end of exon 6 to the middle of exon 9.

2.6.1.5 Detection of FH Afrikaner 2 and Familial Defective ApoB100 (ARMS ASSAY - Amplification Refractory Mutation System)

This assay determines whether the DNA contains the exon 9 mutation V408M (Afrikaner FH2) in the LDL receptor, and/or whether there is binding defective B₁₀₀ from the mutation R3500W or R3500Q, and possibly R3531C. The Afrikaner 2 mutation constitutes about 20% of FH in Afrikaners and confers a biochemically worse disease, while familial binding defective B₁₀₀ is responsible for about 1% of the xanthomatous hypercholesterolaemia phenotype at the UCT Lipid Clinic.

The ARMS PCR method is used to detect the FH Afrikaner-2 mutation while the same reaction vial also contains oligonucleotides that amplify the apo B gene which serves as an internal control for the PCR. Additionally, a normal allele can be amplified as indicated below for 9.6 (G87) instead of the mutant primer 9.7 (G88) to differentiate a homozygote from a heterozygote.

2.6.1.6 PCR assay: Exon 14 FH Gujarati

The mutation P664L has been described from several regions of the world, including Norway and Zambia. The Zambian epithet came from investigating an immigrant Gujarati Indian family in Britain. Subsequently it has become known as FH Gujarati. It has been found frequently in South African Indians hailing originally from Gujarati and mainly confined to the Muslim faith. One family of mixed ancestry has the mutation. DNA in exon 14 is amplified according to Soutar et al. and run on a 1% cross linked gel. The mutation forms a heteroduplex and can be confirmed with restriction enzyme digest as it creates a Pst1 cutting site.

2.6.1.7 PCR assay for 5'- Exon 4 mutation

A mutation was found in a FH family at the Groote Schuur Hospital Lipid Clinic and was termed FH GSH3. The mutation is a 2bp deletion T2951 and C2952, resulting in a frame shift. The heteroduplex that this mutation forms can be seen clearly on a 10% PAGE as well as the long gel.

2.6.1.8 PCR assay for mutation N384K

This mutation in exon 9 abolishes one of the two cutting sites with restriction enzyme Msp1. This analysis was done in the laboratory of Dr M.J.Kotze.

2.6.2 Apo E genotyping

The genotyping is done by amplifying the segment spanning the aminoacids 92 to 176 which includes the domain which binds to the LDL receptor. It is then cut with the restriction enzyme HhaI which will allow discrimination of the 3 common isoforms ApoE2, E3 and E4 - done according to the Hixson and Vernier method. (173)

The commonest setting for type III hyperlipidaemia is the occurrence of apoE2 homozygosity. However, there are other mutations in apoE that can cause autosomal dominant type III hyperlipidaemia and they appear to occur in the ligand binding domain. One of these mutations, R145C, which can be detected by an Fnu4HI, IatI or Bbr1 restriction analysis, is remarkably common at the GSH Lipid Clinic.

2.6.3 MTHFR polymorphism

The method is that according to Frosst. (174) Oligonucleotide primers were used to amplify a fragment of 198 bp that straddle the mutation. A C - T substitution at nucleotide 677 changes an alanine to valine residue. After amplification the PCR product was digested with a restriction enzyme (Hinf 1) and analysed on agarose gel electrophoresis. The mutation causes a cutting site Hinf 1. (This assay was done in collaboration with Dr Riva Rubenstein in the laboratories of the department of Haematology).

2.7 B-mode ultrasonography method

B-mode ultrasound images were done of the far walls of three arterial wall segments of each carotid artery. The three segments are: the common carotid artery, the carotid bulb and the internal carotid artery. The carotid arterial walls were scanned from a lateral angle and the sonographer selected the best images for analysis. Images were captured and stored in joint photographic expert graphic (jpeg) format (DICOM compatible). Analysis was done off-line using Matlab software.

Figure 2.7.1 illustrates the segments scanned for estimation of the intima media thickness of the carotid arteries. (88)

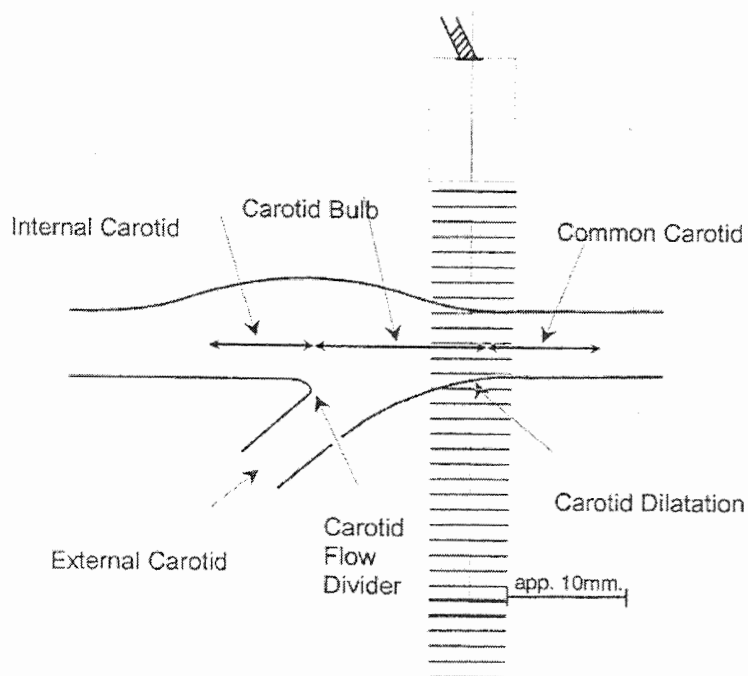


Figure 2.7.1 Diagram of the carotid arteries and site used for ultrasonography

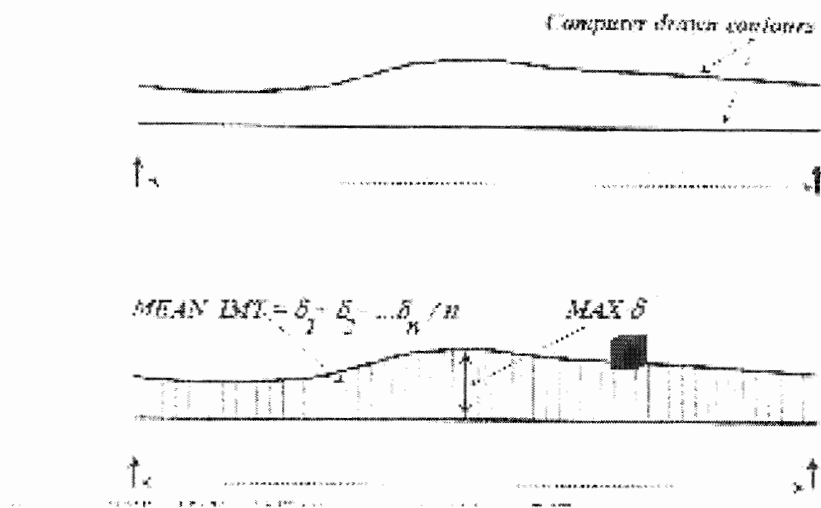


Figure 2.7.2 Diagram of the common carotid artery indicating areas of measurement for calculation of intima thickness
(de Groot, Jukema, et al.) (175)

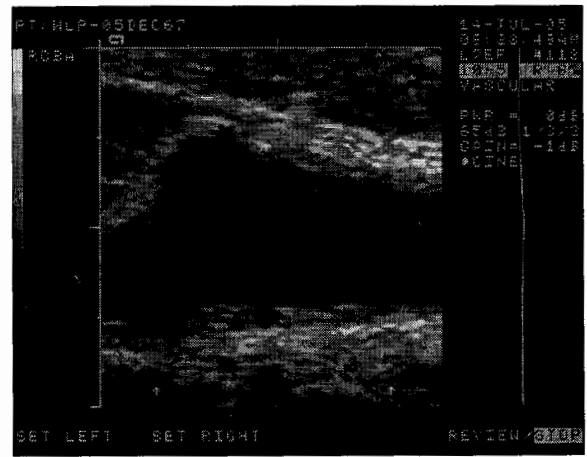
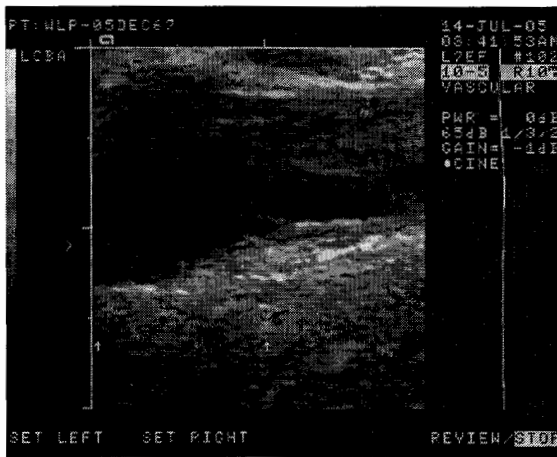


Figure 2.7.3 Ultrasound images of the left and right carotid artery bulb in a FH patient

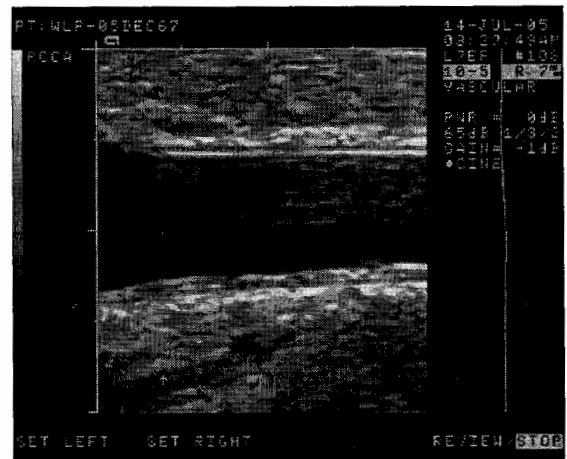
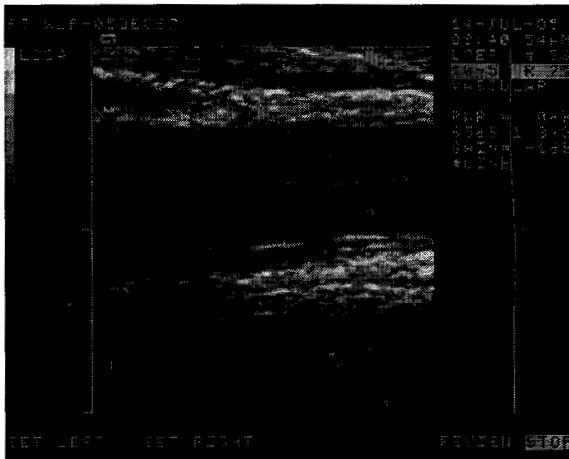


Figure 2.7.4 Ultrasound images of the left and right common carotid artery in the same patient

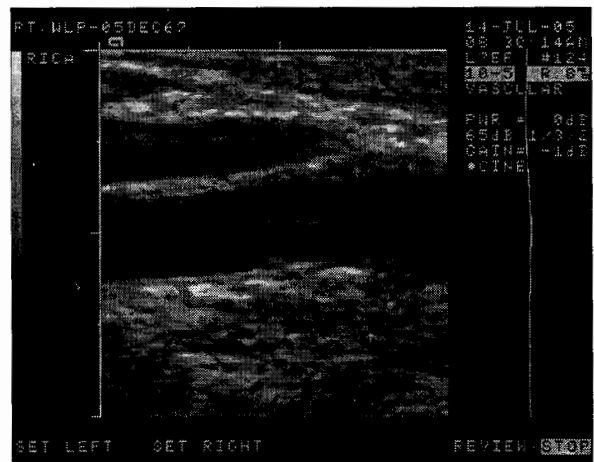
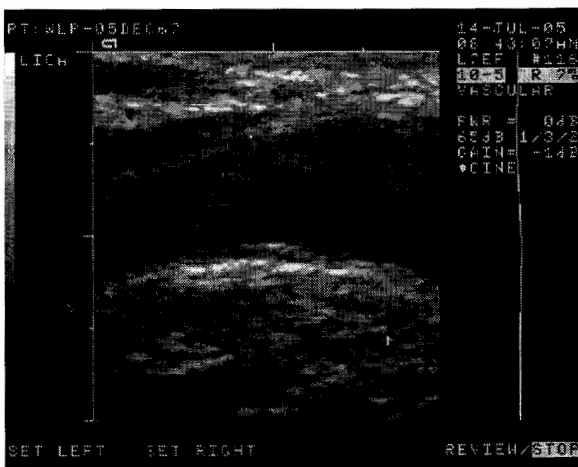


Figure 2.7.5 Ultrasound images of the left and right internal carotid arteries in the same patient

2.8 Statistics

The database was managed by Paradox (Corel) and calculations were done in Quattro Pro Spreadsheets. Data thus prepared were transferred to other software for statistical analyses. For statistical analysis standard commercially available software, Graph Pad InStat and Graph Pad Prism, was used. Numeric data were analysed parametrically or non-parametrically where appropriate. In certain instances data were transformed by logarithmic adjustment. Categorical data were analysed in contingency tables. The individual analysis methods are indicated in the text.

3.1 Introduction

In order to set up the database, 4494 Lipid Clinic patient records were perused to identify those who met the identification criteria for the FH phenotype for inclusion in this study. The inclusion criteria were - a known genotype, or an untreated cholesterol level of more than 7.5 mmol/L, a tendon xanthoma in the patient or a first degree relative and a family history of premature ischaemic heart disease. Additionally probable clinically diagnosed FH subjects were later included once an LDLR mutation was confirmed. Children with FH are generally seen at the Children's Hospital, but some over the age of twelve came with their family and were thus included in the cohort. Patients with Homozygous FH and FDB were not included in the analysis. There were 11 index cases with FDB, making this diagnosis in approximately 1.1% of the FH phenotypes.

By mid 2001, 1029 patients were identified and the information on these patients was entered in a Paradox database. The following data were entered - name, folder number, date of birth, sex, ethnic group, geographical origin of parents or grandparents, smoking habits, the presence of angina pectoris, myocardial infarction, diabetes, hypertension, vascular complications or re-vascularization and family history of IHD. Height, mass, body mass index (BMI), waist measurement, hip measurement, waist /hip ratio (WHR), the presence of xanthelasma, arcus cornealis or tendon xanthomata, date of presentation, triglyceride level, total cholesterol, HDLC, LDLC, apo A₁, apo B and Lp(a) levels, Fredrickson type, gradient gel electrophoretogram (GGE) result of LDL species, date of death, DNA result, kindred, ECG findings, apoE and methylenetetrahydrofolate reductase (MTHFR) polymorphism identity (where these were done for a sub-study).

New patients were admitted to the clinic by only a few highly experienced doctors who documented the history, pedigree and physical findings in detail. Of nine Lipid Clinic doctors, one admitted more than 90% of the cases. In the event of uncertainty about a physical finding, a consensus was reached by consultation with other clinicians. Missing data was retrieved either by contacting the patients telephonically, asking them to come in for re-evaluation and in some cases it was necessary to visit patients at home to verify data.

3.2 Demographics

The demographics of the study cohort are displayed in table 3.2.1. The total number of new patients seen at the clinic during the study period was 4494, with slightly more women than men (53% vs 47%). FH was diagnosed in 23% of patients. In the cohort of FH, Whites represent 55% of new patients with FH, persons of mixed ancestry or so-called Coloureds 43%, Asians less than 2% and Blacks less than 1%. This contrasts with the demographics of the clinic as a whole, where over the same time period the number of Whites presenting was only 40% of new patients, whereas Coloureds represented 56%, Asians and Blacks each accounted for 2%.

The incidence of FH amongst the whites presenting to the clinic is significantly higher than in the coloured group - 55% vs.43% ($P < 0.0001$, Chi-squared test) There may be several reasons for this. Whilst it may simply reflect the high FH prevalence due to the founder effect in the Whites, it may also reflect a greater awareness of this disorder by their medical practitioners or even a bias against FH in other racial groups.

Race/sex	Whole clinic	% of clinic	Without FH	With FH	% of FH cohort
Total number	4494		3469(77%)	1029(23%)	
Males	2109(47%)		1626(47%)	488(47%)	
Females	2384(53%)		1843(53%)	541(53%)	
RS 1	923(21%)	] 40	641(70%)	282(30%)	] 55
RS 2	848(19%)		560(66%)	288(34%)	
RS 3	1060(24%)	] 56	873(82%)	187(18%)	] 43
RS 4	1474(33%)		1224(83%)	250(17%)	
RS 5	60(1.3%)	] 2	47(78%)	13(22%)	] 1.6
RS 6	26(0.6%)		22(85%)	4(15%)	
RS 7	66(1.5%)	] 2	63(94%)	3 (6%)	] 0.6
RS 8	36(0.8%)		34(95%)	2 (5%)	

Table 3.2.1 Demographics of the clinic and 1029 patients with FH

RS is the hospital clerical code for race/sex

1 = white male 2 = white female 3 = coloured male
 4 = coloured female 5 = Indian/Asian male 6 = Indian/Asian female
 7 = black male 8 = black female

In the FH cohort, 80% were type IIa and 20% were IIb, according to the Fredrickson classification.

With the prevalence of FH being crudely estimated as 1/100 in Whites, 1/200 in Asians and 1/500 in Blacks and Coloureds it would appear, as judged by those presenting to the Lipid Clinic,

that FH is remarkably under-diagnosed. As estimated in the 1991 Census, the area within one hour's drive from Groote Schuur Hospital had a population of 574,200 whites, 23,000 Asians, 527,400 blacks and 1,156,000 coloureds. The expected number of FH subjects would thus be about 5,742 in Whites, 115 in Asians, 1,055 in Blacks and 2,312 in Coloureds. Although the region has two tertiary hospitals, the referral of severe dyslipidaemia is predominantly to the GSH Lipid Clinic as this clinic is a metabolic clinic with a dedicated laboratory. Accepting that a smaller proportion of referrals would have been seen at the other tertiary hospital, it is still obvious that only a small percentage of adults with FH had been referred.

The age of presentation to the clinic is given in table 3.2.2 in decades, with the numbers in the racial groups given as a percentage. It is interesting to note that both white males and females present earlier to the clinic than those of mixed ancestry of either sex. (males $P=0.0002$, females $P<0.0001$, Chi-squared test) The younger presentation is likely due to a greater awareness of FH in the white population, whether due to the medical practitioner or the patient. In contrast, Blacks appear to present the latest, often only following a myocardial infarction.

In the whole group, males present earlier than females, with 72% presenting before the age of 50 years, compared to 57 % of females, which is highly significant. ($P<0.0001$, Fisher's exact test.)

Age groups in years		12-19	20-29	30-39	40-49	50-59	60-69	>70
Males	White	74%	59%	71%	53%	48%	42%	86%
	Coloured	22%	37%	25%	43%	51%	58%	14%
	Indian	4%	4%	4%	2%	0%	0%	0%
	Black	0%	0%	0%	2%	1%	0%	0%
	Total n = 489	23	68	130	131	91	38	7
% of whole cohort		3	14	27	27	19	8	1
Females	White	73%	75%	61%	50%	42%	38%	59%
	Coloured	27%	23%	38%	49%	55%	62%	41%
	Indian	0%	2%	1%	1%	1%	0%	0%
	Black	0%	0%	0%	0%	2%	0%	0%
	Total n = 541	26	57	105	119	131	81	22
% of whole cohort		5	11	19	22	24	15	4

Table 3.2.2 Presentation age of racial groups with FH at the Groote Schuur Hospital Lipid Clinic

3.3 Lipid profiles

Table 3.3.1 summarises the untreated lipid profiles at presentation, in decades of age groups.

Triglyceride levels increase with age in both males and females. (ANOVA $P < 0.0001$)

This phenomenon is also observed in the general population. (176)

Age groups	12-19	20-29	30-39	40-49	50-59	60-69	>70
MALES							
n = 488	23	68	130	131	91	38	7
Trig	1.0(0.5)	1.4(0.8)	1.9(1.1)	2.0(1.0)	1.9(0.8)	2.1(0.9)	1.7(1.0)
T Chol	8.2(1.1)	9.8(1.1)	9.3(1.9)	9.1(1.6)	8.6(1.4)	8.3(1.2)	9.5(1.5)
HDLC	1.1(0.3)	1.0(0.4)	1.0(0.3)	1.1(0.4)	1.0(0.3)	1.1(0.4)	1.2(0.4)
LDLC	6.7(1.2)	8.1(2.0)	7.5(1.7)	7.4(1.7)	6.7(1.5)	6.4(1.4)	7.5(1.8)
Apo A1	92(21)	102(25)	100(24)	107(26)	108(27)	114(29)	112(21)
Apo B	154(51)	164(40)	153(38)	152(41)	155(36)	148(35)	157(59)
Lp(a)	31(26)	35(26)	29(24)	40(29)	36(26)	31(26)	32(31)
FEMALES							
n = 541	26	57	105	119	131	81	22
Trig	1.1(0.7)	1.1(0.5)	1.3(0.6)	1.6(0.9)	2.0(1.2)	2.0(0.9)	1.9(0.9)
T Chol	9.3(1.5)	9.1(1.8)	9.6(1.6)	9.1(1.7)	9.3(1.7)	9.2(1.8)	9.6(1.6)
HDLC	1.2(0.3)	1.3(0.6)	1.3(0.4)	1.2(0.4)	1.3(0.5)	1.3(0.4)	1.4(0.5)
LDLC	7.5(1.4)	7.4(1.6)	7.7(1.6)	7.1(1.8)	7.1(1.8)	7.0(1.8)	7.4(1.6)
Apo A1	109(23)	109(30)	118(38)	116(27)	126(37)	124(34)	142(44)
Apo B	173(54)	149(30)	155(43)	152(41)	159(38)	159(46)	176(45)
Lp(a)	32(25)	24(21)	36(26)	30(25)	39(29)	37(29)	20(19)

Table 3.3.1 Plasma lipid, lipoprotein and apoprotein values at age of presentation of patients with FH

Triglyceride, total, HDL and LDL cholesterol values are given in mmol/L.

Apo A1, apoB and Lp(a) values are given in mg/dl. Mean (SD) n = number

TG and Lp(a) were log-transformed

3.4 Lipoprotein ultracentrifugation

A small study was undertaken to evaluate several lipoprotein fractions in normal subjects, patients with dysbetalipoproteinaemia and FH subjects. The ultracentrifugation derived values for VLDL1C, VLDL2C, IDLC and LDLC as well as HDLC according to the method of Lindgren.(158) As shown in table 3.4.1, there was considerably more IDL in the FH subjects when compared to normal subjects ($P<0.0001$, unpaired t test). As expected the FH subjects also had more TC and LDLC than the normal subjects. It is not always appreciated that the Friedewald LDLC concentration (177), based on studies by the betaquant method, incorporated IDL and Lp(a) with LDL. This study demonstrates that VLDL1 and VLDL2 metabolism are not affected by the LDLR mutation but that IDL and LDL are increased.

Group	TG g/L	TC g/L	VLDL1C g/L	VLLDL2C g/L	IDLC g/L	LDLC g/L	HDLC g/L
Normal n = 12	1.069 (0.545)	1.938 (0.371)	0.126 (0.088)	0.250 (0.207)	0.576 (0.171)	1.438 (0.722)	0.557 (0.159)
FH n = 11	1.187 (0.514)	2.516 (0.671)	0.156 (0.097)	0.535 (0.387)	1.537 (0.674)	3.014 (0.850)	0.448 (0.216)
P value	0.6	0.02	0.5	0.04	<0.0001	<0.0001	0.18

Table 3.4.1 Ultracentrifugation of lipoprotein particles in FH patients and normal subjects
() = standard deviation

3.5 Physical signs

Age groups in years		12 - 19	20-29	30-39	40-49	50-59	60-69	>70
Males	Xanthelasma	0%	7%	9%	9%	9%	8%	0%
	Arcus cornealis	0%	21%	35%	50%	45%	58%	86%
	Tendon xanthoma	13%	65%	85%	89%	86%	87%	86%
	Total number = 488	23	68	130	131	91	38	7
Females	Xanthelasma	0%	12%	16%	24%	20%	19%	32%
	Arcus cornealis	4%	12%	15%	36%	34%	48%	82%
	Tendon xanthoma	8%	61%	76%	90%	90%	90%	91%
	Total number = 541	26	57	105	119	131	81	22

Table 3.5.1 Physical signs at presentation of 1029 patients with FH
Prevalence of abnormal sign at presentation (%)

In the FH cohort 14% had xanthelasmata, 36% had arcus cornealis and 80% had tendon xanthomata. In those with tendon xanthomata, 1% had only extensor tendon xanthomata without the presence of xanthomata in the Achilles tendons. The occurrence of arcus cornealis and tendon xanthoma in young subjects cannot be judged accurately against all FH subjects as there may be a selection bias where signs favour referral and because of small numbers. In males, xanthelasma shows no increase in prevalence after the age of 30 years, whereas in females there seems to be ongoing development of this sign. Arcus cornealis increases with age in both sexes, as would be expected. Given that the ageing process may be responsible for this physical sign, the sign may only be helpful before the age of 50 years. Although there may be some disagreement about the exact cut-off age, most authors agree in principle. In both sexes tendon xanthomata are well established by the age of 40 years and are present in the majority

by their third decade. The prevalence in males remains static, but in females increases progressively with age from 76% to 91% by the age of 70 years.

Comparing the age of occurrence of physical signs in increasing decades of life in males and females, there was no difference in xanthelasma presentation ($P=0.3$), whereas arcus occurred significantly earlier in males ($P=0.0001$), as did tendon xanthomata ($P<0.0001$). (Chi-squared test)

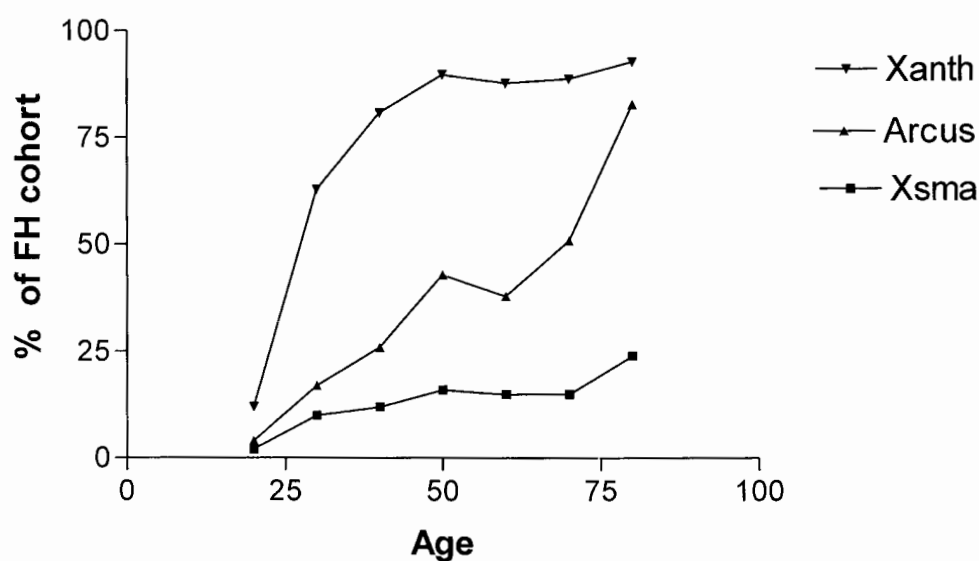


Figure 3.5.1 Cumulative incidence of physical signs in the whole FH cohort at age of presentation to the clinic

Xsma = xanthelasma

Arcus = arcus cornealis

Xanth = tendon xanthomata

Age	Arcus	Xanthelasma	Xanthoma
12-19	3	2	11
20-29	17	10	63
30-39	26	12	81
40-49	43	16	90
50-59	38	15	88
60-69	51	15	89
70-79	83	24	93

Table 3.5.2 Percentage of patients with physical signs in each age group of FH subjects as displayed in figure 3.5.2

Figure 3.5.1 displays the cumulative incidence of physical signs in the whole cohort at age of presentation. The graph reveals interesting trends. There is a low prevalence of the signs before the age of 25 years. Xanthelasma increases with age but less than 25% of the cohort will ever develop this physical sign. Arcus cornealis is present in about 25% by the age of 40 years, about 45% by the age of 50 years and may have an acceleration after this age to involve more than three quarters of the cohort by age 75 years. This latter portion may be due to senescence (arcus senilis).

The most interesting observation is the rapid rise of the prevalence of tendon xanthoma from 12% before the age of 25 years to 75% by the age of 40 years and about 90% by the age of 50 years, with very little increase after the age of 50 years. Since the same criteria were used for defining FH in the absence of tendon xanthoma at any age, this finding suggests that a very high proportion of subjects with the rigid criteria of FH will be diagnosed by this physical sign. It is possible that such rigid criteria may miss a significant number of individuals with FH. This problem can only be evaluated by a systematic analysis of large pedigrees with a known mutation. There was no systematic evaluation of the tendon xanthomata in the affected individuals.

In order to determine whether concentrations of lipid levels or Lp(a) or LDL particle size influence the presence of physical signs, the lipid profiles of those with a physical sign were compared with those without this physical sign and these results are given in table 3.5.3. The actual figures for triglyceride and Lp(a) are given in the tables but for the statistical comparison the log values were used, as these parameters do not have a Gaussian distribution. All the following P values were derived by the unpaired t test, unless stated otherwise.

Patients with FH who have xanthelasma have higher triglyceride ($P=0.04$), total ($P=0.002$) and LDL cholesterol ($P=0.003$) values than those without xanthelasma. Those with arcus cornealis,

have higher total ($P=0.0006$) and LDL cholesterol ($P<0.0001$) values than those without an arcus, and significantly more large LDL species ($P=0.02$). (Chi-squared test) In patients who had tendon xanthomata, triglyceride ($P<0.0001$), total ($P=0.006$), and LDL cholesterol ($P=0.015$) levels were higher than in those without tendon xanthomata, as was Lp(a) ($P<0.0001$); HDL cholesterol was significantly lower ($P<0.012$). They also had significantly more small dense LDL species ($p<0.007$). (Chi-squared test)

Whole cohort	Trig	Tchol	HDLC	LDLC	Lp(a)	LDL species
With xsma.	1.8 (0.8)	9.6 (2.0)	1.2 (0.4)	7.6 (2.0)	40 (44)	47:43:33
Without xsma.	1.7 (1.0)	9.1 (1.7)	1.2 (0.4)	7.1 (1.7)	40 (43)	311:267:194
p value	0.04	0.002	0.09	0.003	0.17	0.89
With arcus	1.7 (0.9)	9.4 (1.8)	1.14 (0.4)	7.5 (1.8)	42 (42)	126:126:75
Without arcus	1.7 (1.0)	9.0 (1.6)	1.19 (0.4)	7.0 (1.7)	39 (43)	143:188:143
p value	0.51	0.001	0.046	0.0001	0.21	0.02
With xanthoma	1.8 (0.9)	9.2 (1.8)	1.15 (0.4)	7.3 (1.8)	48 (42)	266:252:198
Without xanth	1.5 (1.0)	8.9 (1.5)	1.2 (0.4)	7.0 (1.5)	41 (37)	81:62:29
p value	0.0001	0.006	0.012	0.015	0.0001	0.007

Table 3.5.3 Lipid and lipoprotein studies in patients with and without physical signs of hyperlipidaemia in the cohort with FH

Mean (SD) Xsma = xanthelasma Xanth = tendon xanthoma

Arcus = arcus cornealis LDL Species = A+A/I:I/I/B+B (see Ch 5)

As the presence of a tendon xanthoma was a selection criterion in the absence of a known genotype, the significant lipid values may well reflect a selection bias. In order to verify the highly significant finding in the LDL species correlation, patients with xanthomata were matched for age

and sex with patients without xanthomata as it is known that LDL species changes to small dense LDL with increasing age. The significance was lost ($P=0.12$) when the impact of age was removed. This is shown in table 3.5.4.

	A	A/I	I	I/B	B	P value
With tendon xanthoma	97	35	60	17	13	] 0.12
Without tendon xanthoma	56	21	36	11	10	

Table 3.5.4 LDL particle size in FH patients with and without tendon xanthomata matched for age and sex

The correlation of a physical sign with the risk factors of male sex and smoking and the complications of angina pectoris and myocardial infarction (MI) are shown in table 3.5.5.

In the FH cohort, the physical sign of xanthelasma was significantly more common in females ($P<0.0001$), whereas arcus cornealis was more commonly observed in males ($P=0.0009$). Tendon xanthomata were present equally in both sexes ($P=0.69$). In smokers, arcus cornealis and tendon xanthomata occurred more commonly ($P<0.0001$, $P<0.0001$) than in non-smokers, but the occurrence of xanthelasma was not significantly different between the groups ($P=0.78$). Patients with angina were more likely to have an arcus ($P<0.0001$) and a tendon xanthoma ($P<0.0001$), with a tendency to more xanthelasma, but this did not reach statistical significance ($P=0.09$). Patients who had suffered a myocardial infarction were more likely to have a tendon xanthoma ($P=0.0003$), but did not have xanthelasma nor corneal arcus more frequently than those who had not had a myocardial infarction. (All these P values were derived by the Fisher's exact test) Selection bias cannot be excluded as an Achilles xanthoma in the presence of IHD is more likely to draw attention to the possibility of FH, whereas in the absence of a xanthoma a

high cholesterol alone may not prompt consideration of FH and subsequent referral. It is interesting to speculate that smoking may aggravate or promote the development of arcus cornealis and tendon xanthomata, possibly by oxidative stress on lipoproteins, increasing the vascular permeability or modifying tissue responses.

	Xanthelasma	Arcus	Xanthoma
Males n = 488	8% (39)	42% (202)	80% (388)
Females n = 541	18% (100)	31% (170)	81% (436)
P value	0.0001	0.0009	0.69
Smokers n = 541	14% (75)	43% (234)	85% (463)
Non - smokers n = 484	13% (64)	29% (138)	75% (361)
P value	0.78	0.0001	0.0001
With angina n = 375	16% (60)	46% (172)	91% (340)
Without angina n = 654	12% (79)	31% (200)	74% (485)
P value	0.09	0.0001	0.0001
With MI n = 237	11% (27)	39% (69)	88% (208)
Without MI n = 792	14% (112)	37% (303)	78% (616)
P value	0.33	0.42	0.0003

Table 3.5.5 Correlation of physical signs with risk factors of male sex and smoking and complications of angina pectoris and MI in FH

() = actual number with physical sign

n = number within the FH cohort

Smoking status of 4 patients unknown

An interesting clinical observation was made in patient CVR who presented to the clinic. At the time of presentation she was 30 years old. She was of mixed race and the genotype was not identified. On examination she had xanthelasmata both superiorly and inferiorly, and a clear tendon xanthoma on her right Achilles tendon. Her left leg was under-developed as a result of surgery as a child for Tuberculous bone disease, ankylosing the ankle. The Achilles tendon of this leg was normal, suggesting that xanthoma development may be dependent on physical activity.

The only musculo-skeletal complaints that were reported by the patients with FH in the clinic related to Achilles tendinitis. In less than 1% in whom it typically occurred, reported the occurrence as a teenager or young adult and it was often related to increased physical activity.

3.6 Genotype results

An attempt was made to identify a known genotype in all patients with the FH phenotype. The family tree or area of origin often gave a hint of the most probable genotype, or else mutations in exon 4 were sought for first as most of the genotypes identified in the clinic are located on this exon.

Mutation		White	Mixed	Asian	Black	Total	Percentage
Unknown		217 (38%)	332 (76%)	1 (6%)	5	555	54
Known		353 (62%)	105 (24%)	16 (94%)	0	474	46
Afr 1	D206E	190 (156)	32 (20)	0	0	222	21.4
Afr 2	V408M	98 (61)	17 (95)	0	0	115	11.2
Afr 3	D154N	26 (23)	6 (2)	0	0	32	3.1
Lith	Del197	21 (14)	8 (2)	0	0	29	2.8
Gujerat	P664L	0	2 (2)	9 (1)	0	11	1.1
CT 2	DelEx7.8	0	12 (8)	0	0	12	1.2
GSH1	R57C	0	3 (2)	0	0	3	0.3
GSH 2	R60C	0	4 (2)	0	0	4	0.4
GSH 3	2bpdel	0	5 (1)	0	0	5	0.5
GSH 4	D203A	0	4 (1)	0	0	4	0.4
GSH 5	N384K	0	0	4 (1)	0	4	0.4
Fr Can 3	E207K	2 (1)	5 (2)	3 (2)	0	10	1.0
Fossum	R329X	10 (2)	0	0	0	10	1.0
Padova	D200G	3 (2)	7 (4)	0	0	10	0.6
Lebanese	C660X	1	0	0	0	1	0.1
Amsterdam	S285I	1	0	0	0	1	0.1
Lancashire	E80K	1	0	0	0	1	0.1

Table 3.6.1 Genotyping in cohort of 1029 patients with FH

Percentages of known genotypes are calculated from the whole group.

() = no. of kindred % = percentage within each racial group

With a limited diagnostic strategy orientated towards previously established mutations, approximately 46% of genotypes could be determined. The founder effects permitted 94% identification in Indians, 62% in whites in general. In Whites who were identified as Afrikaners 76% could be identified by the three founder genes. Only 24% of FH could be identified in mixed ancestry populations. In Whites D206E, V608M, D154N, del197, E207K, R329X, D200G, C660X, S285I and E80K made up 62%. Del 197 was practically confined to Jews and C660X to a Lebanese immigrant.

3.7 Complications

The whole cohort of 1029 patients was analysed for complications, which have been grouped as ischaemic heart disease (IHD), comprising angina pectoris and myocardial infarction, cerebrovascular disease, including stroke and transient ischaemic attacks (TIA), peripheral vascular disease (PVD) and death from cardiovascular disease. The results are given in table 3.7.1. Percentages are given for the whole cohort and for the occurrence in each of the sexes.

The majority of complications occurred more frequently in males, IHD ($P < 0.0001$), (P=0.0005) MI ($P < 0.0001$) and death ($P < 0.0001$). PVD occurred equally in both sexes. (All P values derived by the Fisher's exact test) Cerebrovascular disease, especially TIA's were very infrequent. The detailed analysis is given in table 3.7.2.1.

FH cohort	IHD	Angina	MI	Stroke	TIA	PVD	Death
All patients n = 1029	438	375	237	16	10	38	107
% of cohort	43	36	23	1.5	1.3	3.7	10
Males n = 488	251	205	161	10	4	19	76
% of males	52	42	33	2	0.6	3.9	16
Females n = 541	186	170	76	6	6	19	31
% of females	34	31	14	1	1	3.5	6
Males vs females P value	<0.0001	0.0005	<0.0001	NSS	NSS	NSS	<0.0001

Table 3.7.1 Complications in cohort of 1029 patient with FH

n = number

NSS = not statistically significant

3.7.1 Ischaemic heart disease

Only 43% of the FH cohort had ischaemic heart disease, with males predominating. (57% vs 43%, $P=0.0005$). Sixty eight percent of those developing ischaemic heart disease smoked, with a higher percentage of smokers in males (78% vs. 55%, $P<0.0001$). Hypertension was present in 24% with IHD, with more female hypertensives (33% vs. 16%, $P<0.0001$). (All P values derived by the Fisher's exact test) Diabetes was uncommon, present in only 5%, and occurring similarly in males and females.

IHD	Whole group	Males	Females
Total with IHD	437 (43% of FH)	251 (57%)	186 (43%)
Total with AP	375 (36% of FH)	205 (55%)	170 (45%)
Age of onset	46	43yrs (10) Range 23 - 70	49yrs (11) Range 21 -75
Total with MI	237 (23% of FH)	161 (68%)	76 (32%)
Age of onset	46	43yrs (10) Range 21 - 70	49yrs (11) Range 25 - 70
No. who smoked	299 (68% of IHD)	197 (78%)*	102 (55%)*
No. with diabetes	22 (5%)	14 (6%)*	8 (4%)*
No. with hypertension	102 (24%)	40 (16%)*	62 (33%)*
No. with DM and hypt	29 (7%)	10 (4%)*	19 (10%)*
No. who had CABG	144 (33%)	97 (38%)*	47 (25%)*

Table 3.7.1.1 Ischaemic heart disease in the cohort with FH

* = % of total males or females

3.7.2 Neurological complications

Stroke occurred in only 1.6% of patients with FH. All but one of the patients who had a stroke smoked. Fifty six percent of both males and females were hypertensive, with a greater number of female hypertensives (83% vs 40%). Diabetes and hypertension occurred in 50% of the females, whilst only one male had diabetes. Stroke was not seen in the first month following a coronary event to suggest that endomyocardial thrombi could have resulted in an embolic stroke. TIA was even less common. Females predominated (6 vs.4), presented later (age 64

vs. 47), smoked less (66% vs 100%) but were equally as hypertensive as the males.

STROKE	Whole group	Males	Females
Number	16	10	6
Average age	49	50	48
Age range	29 - 73	29 - 73	40 - 56
Smokers	15 (94%)	10 (100%)	5 (83%)
With hypertension	9 (56%)	4 (40%)	5 (83%)
With diabetes	1 (6%)	1 (10%)	0
With DM and Hypt	3 (19%)	0	3 (50%)

TRANSIENT ISCHAEMIC ATTACK

Number	10	4	6
Average age	58	48	64
Age range	36 - 77	36 - 54	48 - 77
Smokers	7	3	4
With hypertension	5	2	3
With diabetes	1	1	0
With DM and Hypt	0	0	0

Table 3.7.2.1 Stroke and TIA in patients with FH
DM = diabetes mellitus Hypt = hypertension

There was a remarkably low prevalence of stroke in the FH cohort (1.6%). Ninety four percent of the patients with stroke smoked and the average amount was 25 pack years. The smoking

status reached a statistical significance of $P=0.0006$ when analysed for its prevalence between patients who developed or did not develop neurological complications. Hypertension was also a significant factor as 63% of the patients with stroke were hypertensive ($P=0.001$). (P values derived by the Fisher's exact test)

The stroke group was analysed after matching for age, sex and triglyceride levels and the results are given in table 3.7.2.2. BMI, WHR, HDLC, LDLC and Lp(a) levels were all not significantly associated.

The only predisposing clinical factor for stroke thus appeared to be smoking.

Matched pairs	Males:females	Age	TG	BMI	WHR	HDLC	LDLC	Lp(a)
With stroke	10:6	53.3 (9.3)	1.9 (0.7)	27.3 (4.6)	0.95 (0.1)	0.93 (0.2)	7.4 (1.6)	25.5 (29)
Without stroke	9:7	51.6 (10.1)	1.9 (0.6)	29.1 (5.9)	0.88 (0.1)	0.96 (0.2)	7.07 (2.0)	58.6 (52)
P value	1.00	0.62	0.99	0.35	0.1	0.67	0.61	0.74

Table 3.7.2.2 Results of patients with stroke matched for age, sex and TG levels with patients without stroke in the cohort of FH subjects

Values are given in mmol/L as means with SD in brackets.
Log values of TG and Lp(a) were used for statistical comparison

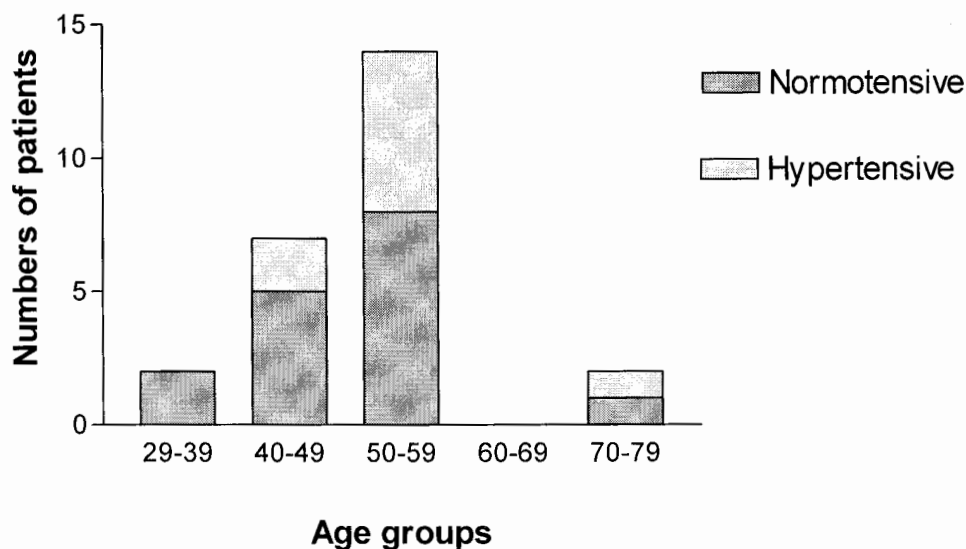


Figure 3.7.2.1 Prevalence of hypertension in patients with stroke

3.7.3 Peripheral vascular disease

Peripheral vascular disease (PVD) was uncommon, occurring in less than 4% of the FH cohort. As in stroke, the incidence of smoking in patients with PVD is high, 79%, with more males smoking (95% vs 63%). About a quarter (24%) of the cohort with PVD was diabetic.

Interestingly, one man presented with an ischaemic limb from which an embolus was removed by a catheter procedure. He had had a recent myocardial infarction and almost certainly had embolised. (Patient HAD, is a male smoker who presented with the complications at the age of 43 years. FH Afr 3 was identified as the cause of his FH).

PVD	Whole group	Males	Females
Number	38	19	19
Age of onset	50 (11)	50 (11)	54 (11)
Range	35 - 77	39 - 77	35 - 73
Smokers	30 (79%)	18 (95%)	12 (63%)
With hypertension	15 (40%)	6 (32%)	9 (47%)
With diabetes	3 (8%)	2 (10%)	1 (5%)
With DM and Hypt	6 (16%)	1 (3%)	5 (26%)

Table 3.7.3.1 Peripheral vascular heart disease in FH

Mean (SD) DM = diabetes mellitus Hypt = hypertension

3.7.4 Death from IHD in FH

More males have died during follow-up compared to females, and they died ten years earlier.

This however did not quite meet statistical significance. (P=0.09, Fisher's exact test)

DEATH	Whole group	Males	Females
Number	76	45 (71%)	31 (29%)
Mean age of death	54.9 (13)	51.4 (10)	60.5 (12)
Range	30 - 81	33 - 73	30 - 81

Table 3.7.4.1 Death from IHD in the FH cohort

Mean (SD)

In 1994 an analysis was done on FH patients presenting to the Lipid Clinic. 122 genotyped patients were analysed and the results for angina pectoris, myocardial infarction and death are given in table 3.7.4.2. These results are compared with the 1029 subjects of the current analysis for which the database was locked in April 2001.

There was no significant difference in the average age of onset of angina pectoris or myocardial infarction. This may reflect a lack of awareness of FH and the major risk for IHD in this condition, late diagnosis and initiation of treatment, or inadequate treatment of the disease. By contrast, there is a significant delay in the average age of death, from 48 years to 55 years ($P=0.012$, unpaired t tests). The apparent improvement in survival could be the result of screening programmes resulting in earlier diagnosis of milder cases or may be the result of improved drug treatment. The lower prevalence of angina in the earlier group (22% vs 36%) may have resulted from lower detection rates of FH in patients with angina, or because FH was only diagnosed after myocardial infarction.

This may well mean that if a cardiac event resulted in the diagnosis of FH being made and the subject was then adequately treated, life could have been prolonged. When comparing the incidence of events and death in the two groups, angina occurred less frequently in the earlier group ($P=0.002$), myocardial infarction much less frequently in the later group ($P=0.0008$) and death was a much rarer event in the later analysis. ($P<0.0001$) (All these values were derived by the Fisher exact test.) As some of the patients were lost to follow-up, reliable information about death was only available on 911 patients.

Reflecting back on the original publications of the age of IHD and death in FH (see chapter 1.6) the prognosis for this disease has improved considerably. The advent of statins and their availability in this country over the past fifteen years must have had an impact on the natural history of FH in this cohort, even though treatment was not optimal. It is possible that, in addition

to the statins, lifestyle management, especially aggressive lowering of cholesterol and saturated fat intake, and cessation of smoking, played a significant role.

	1994 cohort	2001 cohort	P value
Number	122	1029	
ANGINA			
Number	27 (22%)	375 (36%)	0.0017
Age (SD)	47 (13)	46 (11)	0.61
MI			
Number	46 (38%)	237 (23%)	0.0008
Age (SD)	46 (13)	45 (11)	0.43
DEATH			
Number	48 (39%)	76/911 (8%)	<0.0001
Age (SD)	48 (17)	55 (13)	0.012

Table 3.7.4.2 Comparison of angina, myocardial infarction and death in the 1994 cohort with the 2001 cohort with FH

3.8 Comparison of early onset disease with no disease after the age of 60 years.

Two sub-groups were selected out of the FH cohort of 1029 patients - those with ischaemic heart disease before the age of 35 years (56 patients) and those over the age of 60 years (39 patients) without clinically manifest ischaemic heart disease. The purpose was to identify markers of higher and lower risk in addition to that imparted by FH alone. The conventional risk factors such as age, male sex, body mass index (BMI), waist hip ratio (WHR), hypertension, diabetes and

smoking habits are given in table 3.8.1.

Patients with an early onset of IHD were predominantly males ($P=0.001$), smoked more ($P<0.0001$) but had less hypertension and/or diabetes than the older IHD free group. ($P<0.0001$) (Fisher's exact test) BMI and WHR were not significantly different. The higher prevalence of the conventional risk factors in the older IHD-free cohort likely reflects the higher prevalence of these disorders in older people and indicates that these factors are significant in those patients with earlier onset of disease, although their premorbid features may have been present. Clearly the BMI and WHR cannot be compared for the elderly disease-free cohort at the equivalent age to that of the young IHD onset group.

	IHD before 35 years	IHD free over 60 years	P value
Number	56	39	
Average age of event	30.2 (4.1)		
Age range	23 - 35	60 - 81	
Males	44	13	] 0.001
Females	12	26	
BMI	26.2 (5.1)	26.3 (4.4)	0.32
WHR	0.89 (0.19)	0.90 (0.09)	0.80
Smoking	79%	30%	<0.0001
Hypt and/or DM	15%	60%	<0.0001

Table 3.8.1 Clinical results in early onset IHD compared with no IHD after 60 years in FH

Mean (SD) DM = diabetes mellitus Hypt = hypertension

In these two groups lipid values including Lp(a) were analysed as well as LDL particle size. In the group with early onset disease lower HDL levels, higher LDL levels and LDL particles were significantly different ($P < 0.0001$, $P = 0.02$ (unpaired t tests) and $P = 0.002$ (Chi-squared test) See table 3.8.2). Lp(a) levels were not significantly different in the two groups.

Abnormalities in homocysteine metabolism have been reported as an additional risk factor for cardiovascular disease. A common mutation or polymorphism in the enzyme methylenetetrahydrofolate reductase (MTHFR) can contribute to an elevation in plasma homocysteine. (178) This mutation correlates with reduced enzyme activity and increased thermolability (hence methyl tetrahydro folate reductase). It was suggested that this polymorphism was positively related to the development of coronary arterial disease. (179) Spence has suggested however, that in contrast to this hypothesis, the T allele may be protective against IHD, independent of homocysteine levels. (180) Analysis of this polymorphism in this cohort failed to find any association with an early onset of Ischaemic heart disease, as set out in table 3.8.2. LDL receptor negative genotypes ("null" defects) compared with LDL receptor defective genotypes ("def"), were also not significantly different between the two groups.

	IHD before 35 years	IHD free over 60 years	P value
Plasma TG	1.6 (1.5)	1.80 (1.6)	0.14
Plasma TC	9.8 (1.9)	9.3 (2.0)	0.17
HDLC	0.97 (0.2)	1.28 (0.3)	<0.0001
LDLC	8.0 (2.0)	7.1 (1.9)	0.02
LDLsp A:I:B	13:22:14	18:10:10	0.002
Lp(a)	38 (2.6)	31 (2.6)	0.24
MTHFR C677T	8 (55%)	15 (45%)	0.67
Null vs Def	12:17	1:8	0.13

Table 3.8.2 Laboratory results in early onset IHD patients (before 35 years) compared with IHD free patients (over 60 years) with FH
Mean (SD) % = percentage of group with polymorphism

3.9 Carotid intima media thickness in FH

The process of atherosclerosis, which ultimately results in ischaemic heart disease, starts with morphological changes in the arterial walls. These changes are characterised by accumulation of cholesterol in macrophages in the sub-endothelial space and proliferation of smooth muscle cells, which give rise to arterial wall thickening. This thickening of the intima-media region of the carotid artery can be measured by an ultrasound technique and is considered a surrogate marker for atherosclerosis. As has been discussed in chapter 1.6, IMT progresses with age, and in FH this progression is accelerated, with the IMT of an FH subject of 40 years of age equalling that of a non-FH subject of 80 years. (88) This progression is linear, is well-documented after

the age of 10 years and the rate of progression in FH has been estimated to be twice that of normal controls (0.009 and 0.004 mm/year).

At the University of Cape Town, IMT measurements were undertaken prior to commencement of drug in a study protocol in FH adolescents, and also in patients in the FH cohort under study. The IMT was measured by high resolution B-mode ultrasound imaging which permitted safe and non-invasive assessment of arterial walls. The measurements were taken at three defined segments: the internal carotid artery, the carotid bulb and the common carotid artery. The far walls of each segment were scanned in both the right and the left carotid arteries giving a combined assessment of six segments as an estimate of the overall atherosclerotic changes in arterial walls. This was done according to the method published by de Groot. (88)

The combined results of the IMT measurements are given in figure 3.9.1. The cluster on the left is the FH adolescent group of 34 boys and girls aged between 10 and 17 years, the middle cluster is middle-aged FH patients (around 40 years), with and without IHD, and the cluster on the right comprises FH patients over 60 years of age without IHD.

There is an apparent linear progression of IMT from adolescence to older age at a rate of 0.007 mm/year which agrees well with the figure of 0.009 mm/year given by de Groot. (88) It is possible that the older group of IHD-free FH patients included in this study have a selection bias for lower IMT progression, forcing the slope of the progression down to a lower figure than reported by de Groot. (88) The IMT measurements were very varied in both these groups.

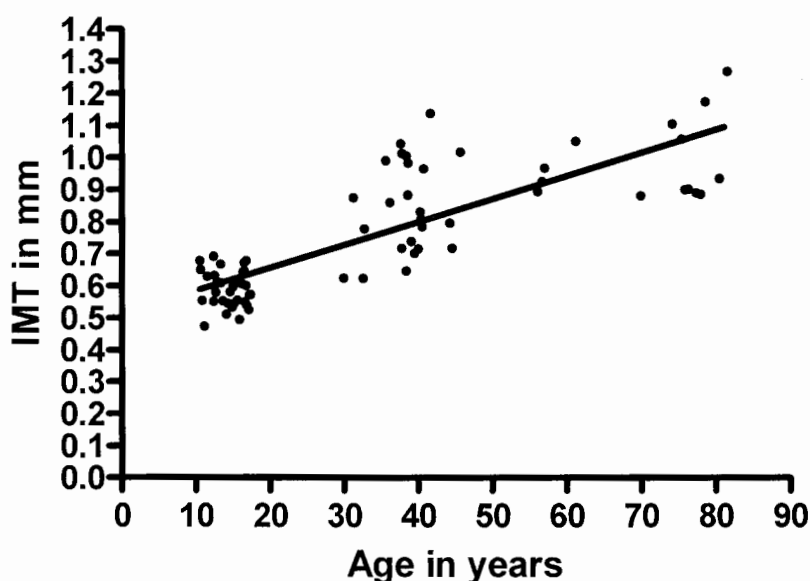


Figure 3.9.1 Combined mean IMT measurements of all segments of the carotid artery in mm in FH subjects ranging in age from 10 to 82 years
 $P < 0.0001$ $r^2 = 0.67$ Slope = $0.007 (+/- 0.0006)$

It was of interest to compare the IMT measurements of adolescent FH between Norway and South Africa, using data published by Tonstad for the Oslo group. (89) These data are displayed in table 3.9.1. The two groups were of a similar age. Tonstad also reported that 10% of the adolescents had plaque, defined as a distinct area of the vessel wall protruding into the lumen by >50% of the adjacent parts of the intima-media layer. The Cape Town study did not systematically examine the arteries for plaque but none of the IMT measurements revealed a maximum of > 1.5 mm that is also taken to represent plaque.

Comparing the IMT measurements in the carotid bulb between the Cape Town FH adolescents and the reported values for FH adolescents in Oslo, there was a 0.1mm difference for the bulb, (0.635 mm versus 0.54 mm) and 0.11 for the common carotid artery (0.597 mm versus 0.48 mm, see table 3.9.1) These differences were statistically significantly different. The Cape Town group had lower LDLC concentrations, but not significantly so ($P=0.09$). Three of the Cape

Town adolescents smoked (but only occasionally) and this was not thought to be sufficient to account for the difference. The difference may be attributed to technical factors but might also reflect differences in lifestyle, these possibly being worse in Cape Town. The nature of the dyslipidaemia was similar. The nature of the LDLR defects could not be analysed.

	Cape Town adolescents with FH	Oslo non FH controls	Oslo adolescents with FH	P value (FH vs FH)
Number	34	30	90	
Age range	10 - 17 years	10 - 19 years	10 - 19 years	
Smokers	3 (9%)	0	0	
HDLC	1.14 (0.3) mmol/L		1.2 (0.3) mmol/L	0.32
LDLC	6.0 (1.3) mmol/L		6.5 (1.5) mmol/L	0.09
Mean internal carotid	0.524 mm (0.08)			
Mean common carotid	0.597 mm (0.06)		0.48 mm (0.07)	<0.0001
Mean carotid bulb	0.635 mm (0.08)	0.50 mm	0.54 mm (0.1)	<0.0001
Mean of the means	0.591 mm (0.06)			

Table 3.9.1 Results of B mode ultrasonography in adolescents with FH in Cape Town and reported FH adolescents from Oslo and normal controls

IMT has been reported as a predictor for CVD (89) as discussed in chapter 1.6, and it was thus of interest to compare the subgroup in the FH cohort with IHD before age 35 years, with age and sex matched FH controls without IHD, and with the group over 60 years of age who were IHD-free.

Only 14 patients in the under 35 years IHD group could be contacted for ultrasonography and

in the IHD-free over the age of 60 years group only 10 were available. The average age of the young group at the time of the scan was 40.1 years (SD 7.7) and the average age of the older group at the time of scanning was 76.2 years (SD 3.4). The age difference was highly significantly different, $P < 0.0001$. (Unpaired t test) In the early IHD group there were significantly more smokers ($P = 0.0005$) and in the older IHD-free group there were more hypertensives ($P = 0.0003$). (Fisher's exact test) The BMI and WHR were not statistically significantly different (Unpaired t test). HDLC was highly significantly lower in the early IHD group and LDLC was higher, ($P = 0.003$ and $P = 0.045$, unpaired t tests) but the LDL species distribution was not different between the two groups. (Chi-squared test) The young disease group appeared to have more null defects than the old IHD-free group ($P = 0.05$, Fisher's test). As would be expected, the older group had been on drug therapy for longer, even though this was not ideal treatment. (6 versus 12 years, $P = 0.0008$, unpaired t test) The results are given in table 3.9.2.

When the means of the measurements of the internal and external carotid arteries as well as the carotid bulb were compared, only the internal carotid measurements showed a statistical difference ($P = 0.02$), with the older group having a thicker IMT measurement. There was no statistical difference in the comparison of the other segments. It would appear that the internal carotid artery (ICA) takes longer to thicken.

This comparison indicated that in this FH cohort the carotid IMT is similar in the group that developed very premature IHD and those who did not. It is possible that the older group, at a comparable age, had the same IMT measurement as the premature IHD group and progressed to a similar thickness, but remained free of complications. It is tempting to speculate that smoking introduced the complications in the younger cohort or accelerated the IMT progression, if the older disease group indeed did have thinner IMT at a comparable age. The prevalence of more patients with null LDLR defects in the young group may also be an influencing factor as has been previously discussed. The additional risk that may relate to hypertension and diabetes mellitus

in the older cohort appears not to have had an impact on the IMT or the complications

	IHD before 35 years	IHD free after 60 years	P value
Number	14	10	
Average age at scan (SD)	40.1 yrs (7.7)	76.2 (3.4) yrs	<0.0001
BMI	27.2 (5.4)	27.9 (4.0)	0.73
WHR	0.88 (0.08)	0.89 (0.08)	0.77
Smokers	12 (86%)	1 (10%)	0.0005
Hypertension	0	7 (70%)	0.0003
Diabetes mellitus	0	1	
HDLC	0.9 (0.2) mmol/L	1.4 (0.4) mmol/L	0.003
LDLC	8.4 (1.7) mmol/L	6.4 (2.4) mmol/L	0.045
LDL particle size	A+A/I : I : I/B+B 4 : 6 : 4	A+A/I : I : I/B+B 5 : 2 : 3	0.44
Mean internal carotid	0.736 mm (0.1)	0.953 mm (0.3)	0.02
Mean common carotid	0.910 mm (0.2)	0.994 mm (0.2)	0.27
Mean bulb	0.960 mm (0.2)	1.064 mm (0.2)	0.19
Mean of the means	0.864 mm (0.1)	0.972 mm (0.2)	0.12
Nul defects vs neg or unk	5 : 9	0 : 10	0.05
Years on treatment	6.4 (3.0)	12.3 (4.6)	0.0008

Table 3.9.2 Results of B mode ultrasonography of the carotid arteries of the early onset IHD group compared to the disease free over the age of 60 year group

Neg = negative Unk = unknown

The group of patients who had IHD before the age of 35 years was matched for age and sex with controls from the FH cohort who were IHD-free, to look for possible differences in IMT that might provide information of coronary risk prediction relating to the burden of atherosclerosis. Ideally

a prospective study of untreated patients should examine the IMT at presentation to avoid confounding effects of treatment. Both these groups had received treatment for about 7 years, but the treatment received did not lower LDLC by more than 50%, the level at which regression of IMT was reported in the ASAP study. (91)

Of the controls, 21% were smokers (compared to 86% in the early IHD group) which was statistically significantly different. ($P=0.002$, Fisher's exact test). There were no hypertensive or diabetic patients in either group. When comparing the lipid profiles, the early onset IHD group had lower HDLC and higher LDLC concentrations, both statistically significantly different. ($P=0.02$ and $P=0.006$, unpaired t tests). There were also more patients with null defects in the early IHD group (5 versus 0, $P=0.04$). Other commonly associated risk factors such as BMI, WHR and LDL size did not differ. These results are displayed in table 3.9.3. It would thus appear that the usual risk factors of smoking, low HDLC and high LDLC play a role in the onset of the development of early IHD. This is consistent with finding earlier MI in subjects with FH Afr 2, a null defect, associated with a higher LDLC.

Carotid IMT was no different between FH patients with early IHD and those over the age of 60 years who were IHD free. This analysis indicates that carotid artery IMT by itself may not be a good predictor of IHD as it is similar in the two groups. If the coronary burden of atherosclerotic complications is correctly reflected in the carotid arteries, it could be speculated that other factors critically promote or decrease the complications of the atherosclerotic process.

	IHD before 35 years	IHD free matched FH controls	P value
Number	14	14	
Average age at scan (SD)	40.1 yrs (7.7)	41.3 yrs (8.3)	0.7
BMI	27.2 (5.4)	25.4 (4.5)	0.35
WHR	0.88 (0.08)	0.85 (0.08)	0.33
Smokers	12 (86%)	3 (21%)	0.002
Hypertension	0	0	
Diabetes mellitus	0	0	
HDLC	0.9 (0.2) mmol/L	1.2 (0.4) mmol/L	0.02
LDLC	8.4 (1.7) mmol/L	6.8 (1.0) mmol/L	0.006
LDL particle size	A+A/I: I : I/B+B 5 : 5 : 4	A+A/I: I : I/B+B 7 : 6 : 1	0.33
Mean internal carotid	0.736 mm (0.1)	0.829 mm (0.3)	0.3
Mean common carotid	0.910 mm (0.2)	0.803 mm (0.2)	0.2
Mean bulb	0.960 mm (0.2)	0.937 mm (0.3)	0.8
Mean of the means	0.869 mm (0.1)	0.858 mm (0.2)	0.6
Null defects vs neg or unk	5 : 9	0 : 14	0.04

Table 3.9.3 Results of B mode ultrasonography of the carotid arteries of the early onset IHD group compared to disease free age and sex matched subjects with FH

() = standard deviation Neg = negative Unk = unknown

Although the experience with non-FH subjects is small, the early onset IHD group with FH was compared with age- and gender-matched non-FH controls. As expected, the LDLC was higher in the FH group (8.4 mmol/L vs 3.9 mmol/L), but the HDLC was also statistically significantly lower in the IHD group. (0.9 mmol/L vs 1.5 mmol/L, $P < 0.0001$ for both, unpaired t test). The young IHD group smoked significantly more than the controls ($P < 0.0001$, unpaired t test), but

there was no difference in the BMI or WHR. In these two groups the IMT was significantly thicker in the common carotid, the bulb and the mean of values of all the arteries in the young IHD group. ($P=0.006$, $P=0.02$ and $P=0.0009$, unpaired t test) In the non-FH controls the ICA also showed the lowest IMT. The results are displayed in table 3.9.4.

	IHD before 35 years	Non-FH matched controls	P value
Number	14	14	
Average age at scan (SD)	40.1 yrs (7.7)	42.5 yrs (6.1)	0.34
Smokers	12 (86%)	2 (14%)	<0.0001
BMI	27.2 (5.4)	27.8 (4.9)	0.57
WHR	0.88 (0.08)	0.88 (0.09)	0.76
Hypertension	0	0	
Diabetes mellitus	0	0	
HDLC	0.9 (0.2) mmol/L	1.5 (0.37) mmol/L	<0.0001
LDLC	8.4 (1.7) mmol/L	3.9 (0.77) mmol/L	<0.0001
Mean internal carotid	0.736 mm (0.1)	0.707 mm (0.13)	0.5
Mean common carotid	0.910 mm (0.2)	0.730 mm (0.1)	0.006
Mean bulb	0.960 mm (0.2)	0.806 mm (0.13)	0.02
Mean of the means	0.869 mm (0.1)	0.748 mm (0.07)	0.0009

Table 3.9.4 Results of B mode ultrasonography of the carotid arteries of the early onset IHD group compared to sex, age, IHD free matched controls with TC levels less than 6.5 mmol/L
() = standard deviation

This analysis has shown that FH is associated with thicker carotid IMT measurements when compared with matched non-FH controls, but it does not appear to be a predictor of IHD.

Smoking was more common in the FH group with early onset IHD but its effect on IMT was difficult to determine. In the early IHD group, LDLC was higher and HDLC lower when compared with the over 60 year IHD-free group as well as the young FH IHD-free group. This would appear to confirm that the well documented risk factors of high LDLC, low HDLC and smoking are more strongly associated with IHD than IMT.

Chapter four GENOTYPE - PHENOTYPE COMPARISONS

4.1 Introduction

There is considerable variation in clinical features, lipid profiles at presentation and the resultant complications in patients in this FH cohort. Although there is a variation of LDLC concentration that can be attributable to the impact of LDLR mutations ranging from no activity to considerable residual activity, many other genes and cultural/environmental factors can still play a role. While different mutations can readily be evaluated in a single cultural/socio-economic group for their impact on LDLC, physical signs and outcome, comparisons between groups found in Cape Town should be interpreted with more caution.

As a result of the founder effect in this country and identification of defined mutations it is possible to look at differences in the commonest genotypes presenting to the clinic. LDL receptor activity, as measured by fibroblast uptake of cholesterol, was related to mutations by Helen Hobbs in 1992. (181) Whilst many of the mutations in this cohort have been identified, some of the mutations have not been fully characterised, for example FH Fossum, which was reported in 1994. (172) The biochemical data may provide a guide to the severity of the LDL receptor dysfunction as was evident in a comparison of Afrikaner FH defects. (41) The functional results for LDLR defects seen in the clinic are given in table 4.1.1.

The impact of mutations on LDL receptor activity is grouped into two broad categories - the so-called defective mutations, where the activity is $> 2\%$ and the so-called null mutations where the measured LDL receptor activity is $< 2\%$. It follows that one could anticipate higher cholesterol values and consequently a worse prognosis in patients with null defects. LDL receptor activity is not easy to determine except in truly homozygous FH patients. The activity in the commonest genotypes at the clinic was confirmed in the homozygous patients attending the clinic. FH

Afrikaner 1 can be used as an example of a defective genotype and FH Afrikaner 2 as an example of a null mutation, and a comparison between these two genotypes was undertaken.

Allele designation	Exon/ intron	Codon change	Nucleotide change	Functional class	LDL receptor uptake (%)
FH Lancashire	3	E80K	GAG → AAG	2B	15 - 30
FH Afr 3	4	D154N	GAT → AAT	NR	NR
FH Lithuania	4	ΔG197	ΔGGT	2B	< 2
FH Padova	4	D200G	GAC → GGC	2B	2 - 8
FH Afr 1	4	D206E	GAC → GAG	2B	5 - 15
FH Amsterdam	6	S285L	TCA → TTA	2B	2 - 5
FH Afr 2	9	V408M	GTG → ATG	5	<2
FH Gujerat	14	P664L	CCG → CTG	2B	15 - 30
FH CT 2	In6-In8	Δ7and8	Δ 2.5 kb	3 and 5	2 - 5
FH Fr Can 3	4	E207K	GAC → AAG	2B	< 2
FH Lebanese	14	C660X	TGA → TGA	2A	< 2
FH Fossum	7	R329X	CGA → TGA	1	NR

Table 4.1.1. Molecular genetics of known LDLR genotypes in the FH cohort
 (There was only one CT 1 patient who was a homozygote)
 NR = not reported

Patient	Allele 1	Allele 2	LDL receptor % uptake
EG	D206E	D206E	14
GvdM	D206E	V408M	16
NO	D206E	D154N	16
SVD	D206E	D154N	7
YV	V408M	D154N	4
RG	D145N	D154N	3

Table 4.1.2 LDL receptor activity relative to normal in homozygous FH patients determined in the Lipid Laboratory

Differences between genotypes could also be explained by other regulatory genes which could be linked to founder effects. Mutations in such regulatory genes would include modifications in lipoprotein lipase, hepatic lipase, apo C_{ii}, apoC_{iii}, CETP, LCAT and MTP.

It is well known that the dietary intake of cholesterol and saturated fat influences lipid levels. (121; 122) The founder mutations identified in the clinic occur within specific communities who have different cultural and dietary habits. In the Afrikaner communities, which include the majority of patients with the Afrikaner mutations, there is a strong tradition of consumption of red meat. This would undoubtedly add to the cholesterol and saturated fat intake in the diet, which can change the untreated level by about 1.2 mmol/L, as has been shown in an early analysis at the Lipid Clinic. These data are presented in chapter 6.

In the Indian communities, in which FH Gujarat occurs, there is often a vegetarian or even vegan diet associated with the Hindu faith, which would improve the lipid profile relative to a fatty diet.

However, in the same communities where adherence to the Muslim faith occurs, the cultural practices are exactly the opposite. Many patients of mixed race are also Muslim, where the diet is rich in fatty and fried foods, consumed in large quantities at innumerable communal gatherings, that form an important part of their culture. Many patients come from lower socio-economic groups, where higher quantities of fatty foods and carbohydrates and eggs as a protein source would be consumed. In the rural areas the diet may be very low in animal products and thus have a beneficial effect on the cholesterol level.

4.2 Demographics

The demographics of the founder mutations as well as the commoner mutations seen at the clinic are given in table 4.2.1.

The numbers of kindreds of each genotype are given in table 3.6.1. In the FH Cape Town 2 genotype there are eight kindred in the clinic, but it is suspected that five of them may link into the same family, as their ancestors all come from the same geographical area, although they are reportedly unknown to one another.

Genotype	Mutation	Number	% of FH in clinic	Males	Females
FH Afr 1	D206E	222	22	103	119
FH Afr 2	V408M	115	11	45	70
FH Afr 3	D154N	32	3	18	14
FH Lithuania	Del G197	29	3	10	19
FH Gujarat	P664L	11	1	6	5
FH Cape Town 2	Del Ex7,8	12	1	3	9

Table 4.2.1 Demographics of the commonest FH genotypes

4.3 Physical findings

4.3.1 Anthropomorphic measurements

Anthropomorphic values are given in table 4.3.1.1.

Comparing the BMI values between the groups, subjects with FH Lithuania had significantly lower values than subjects with FH Afrikaner 1 ($P<0.01$), FH Afrikaner 2 ($P<0.01$) and FH Cape Town 2 ($P<0.001$). Subjects with FH Cape Town 2 had a significantly higher BMI than subjects with FH Gujarat ($P<0.05$). The P values were derived by ANOVA, Tukey-Kramer multiple comparison test. There was no significant difference in waist hip ratio between the genotypes. Whilst cultural differences could influence the BMI it is also possible that there is some genetic bias towards the anthropometric parameters analysed.

Genotype	Number	Body mass index	Waist hip ratio	P value
FH Afr 3	32	25.4 (5.8)	0.88 (0.1)	
FH Afr 1	222	26.1 (5.5)	0.86 (0.1)	] <0.01
FH Lith	29	21.5 (9.6)	0.84 (0.1)	
FH Afr 2	115	25.9 (5.3)	0.86 (0.1)	] <0.001
FH CT 2	12	30.5 (6.8)	0.88 (0.1)	
FH Guj	11	23.1 (3.4)	0.83 (0.1)	] <0.05

Table 4.3.1.1. Anthropomorphic measurements of the commonest FH genotypes
Figures are given as mean (SD)

4.3.2 Physical signs

The physical signs in the different genotypes are given in table 4.3.2.1. All the P values in section 4.3.2 were derived by the Fisher's exact test.

Arcus cornealis was commoner in FH Gujarat than in FH CT2 (P=0.04).

Xanthelasma in FH Gujarat (64%) was significantly more frequent than in FH Afr 1 (6%) (P=0.001). It also occurred significantly more frequently in FH Gujarat than in all the other genotypes. Xanthelasma also occurred more often in FH Afr 2 than in FH Afr 1 (P=0.03) and also occurred earlier, as is shown graphically in figure 4.3.2.1. This graph also illustrates a striking increase (6-fold) in the prevalence of xanthelasma in the 5th decade of life in FH Afr 2. Xanthelasma may partly be influenced by LDLC concentration because the higher prevalence

in FH Afr 2 than FH Afr 1 correlates with higher LDLC concentration, but the predilection for the Indians to develop arcus cornealis and xanthelasma must relate to inherent genetic differences as their LDLC concentration is at worst intermediate between FH Afr1 and FH Afr 2.

It is of interest that all the patients with the Cape Town 2 mutation had Achilles tendon xanthomata. When compared to FH Afr1, the difference is significant ($P=0.02$). Although Achilles xanthomata in FH Afr 2 appeared to be more prevalent when compared to FH Afr1 as is graphically shown in figure 4.3.2.2, this difference did not achieve statistical significance ($P=0.3$). The apparent different propensities for Achilles xanthoma formation is difficult to explain but it is interesting to note that FH Afr 2 and CT 2 are both null defects.

Genotype	Arcus	Xanthelasma	Tendon xanthoma
FH Afr 1	74 (33%)	14 (6%)	154 (69%)
FH Afr 2	39 (34%)	17 (15%)	86 (75%)
FH Afr 3	15 (47%)	5 (16%)	21 (66%)
FH Lithuania	8 (28%)	3 (10%)	15 (52%)
FH Gujerat	7 (64%)	7 (64%)	7 (65%)
FH CT 2	2 (17%)	2 (17%)	12 (100%)
Average %	145 (34%)	48 (11%)	295 (70%)

Table 4.3.2.1 Physical signs in the commonest FH genotypes
 % = the percentage of patients with each physical sign in each genotype

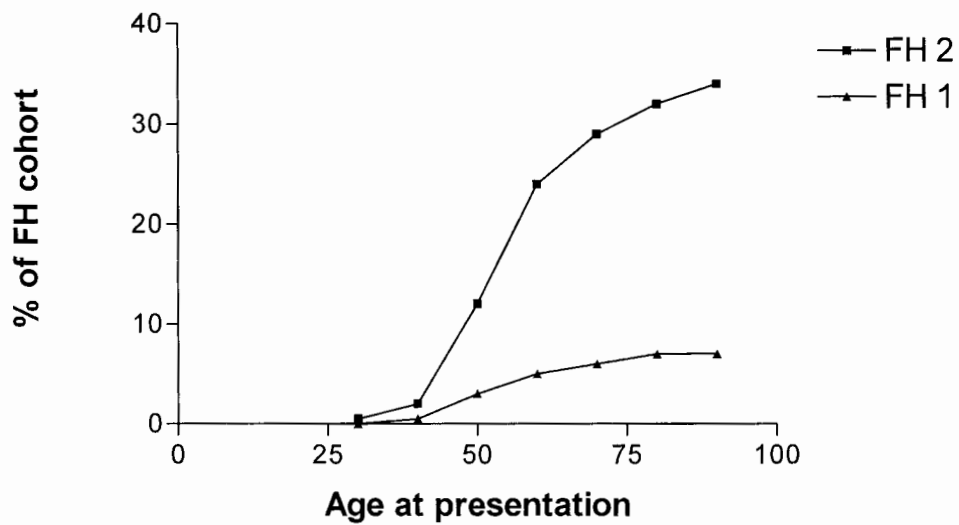


Figure 4.3.2.1 Percentage of patients with xanthelasma in FH Afrikaner 1 and FH Afrikaner 2 LDLR mutations at presentation (age in years)
P= 0.03

AGE (years)	% of FH 2	% of FH 1
12-29	0.5	0.0
30-39	2.0	0.5
40-49	12.0	3.0
50-59	24.0	5.0
60-69	29.0	6.0
70-79	32.0	7.0
80-89	34.0	7.0

Table 4.3.2.2 Percentage of patients with xanthelasma in FH Afr 1 and FH Afr 2 genotypes at age of presentation

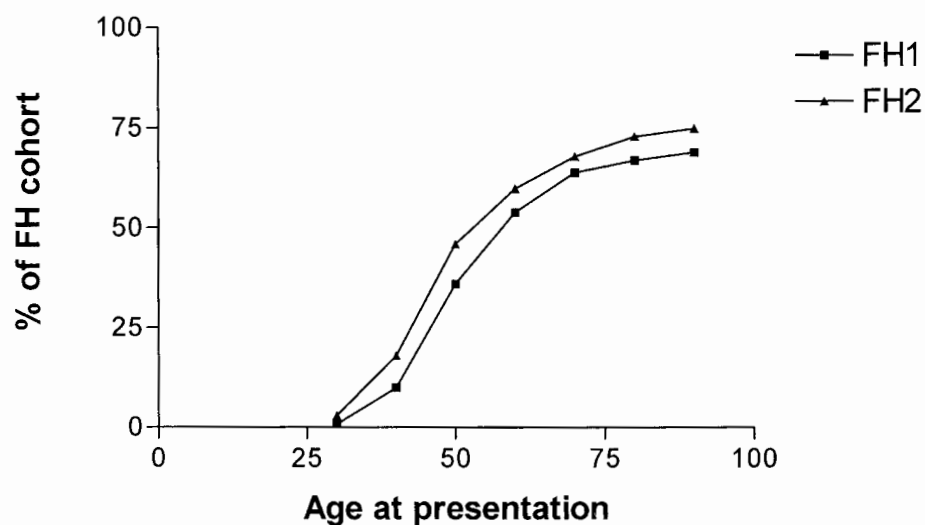


Figure 4.3.2.2 Percentage of patients with FH Afrikaner 1 and FH Afrikaner 2 LDLR mutations Achilles tendon xanthomata at presentation (age in years)
P=0.9

AGE (years)	% of FH Afr 1	% of FH Afr 2
12-29	0.9	3.0
30-39	10.0	18.0
40-49	36.0	46.0
50-59	54.0	60.0
60-69	64.0	68.0
70-79	67.0	73.0
80-89	69.0	75.0

Table 4.3.2.3 Percentages of patients with Achilles xanthomata in FH Afr 1 and FH Afr 2 in decades of age at presentation

4.4 Lipid profiles

Table 4.4.1 displays the average lipid profiles of the major genotypes in the clinic. Log values for triglyceride and Lp(a) were used, as these do not have a Gaussian distribution within the population. The ANOVA Tukey -Kramer test was used to derive the P values in this section.

FH Cape Town 2 is associated with a lower triglyceride value than FH Afr 1 ($P<0.0001$), FH Afr 2 ($P=0.001$), FH Afr 3 ($P=0.001$) and FH Lithuania ($P=0.001$) but has a higher level than FH Gujerat ($P=0.001$). The reason for this must lie in other genetic factors or lifestyle as there is no reason to suspect that the residual LDLR function has an influence since CT 2 is a null defect.

The only significant difference in total cholesterol was between FH Afrikaner 1 and 2 (10.0 ± 1.8 vs. 9.3 ± 1.6), where FH Afrikaner 2 has a higher level ($P<0.05$). (The number of CT2 is small). This was also the case for LDLC between these two genotypes - FH Afrikaner 2 had a higher level ($P<0.01$). There was no significant difference in the HDL levels nor in Lp(a). The successful demonstration of higher LDLC concentration in FH Afr 2 and not in the other null defect (CT 2) may be due to larger numbers or possibly a lower fat diet in subjects with CT 2 who generally come from a lower socio-economic group.

Genotype	No	Trig mmol/L	Tchol mmol/L	HDLC mmol/L	LDLC mmol/L	Lp(a) mg/dL
FH Afr 1	222	1.5 (1.0)	9.3 (1.6)	1.2 (0.5)	7.4 (1.6)	34 (26)
FH Afr 2	115	1.5 (0.9)	10.0 (1.8)	1.1 (0.4)	8.1 (1.7)	35 (24)
FH Afr 3	32	1.6 (1.2)	9.6 (1.7)	1.1 (0.3)	7.7 (1.5)	38 (24)
FH Lith	29	1.5 (0.8)	9.4 (1.7)	1.1 (0.3)	7.6 (1.6)	33 (28)
FH Guj	11	1.2 (0.5)	9.9 (1.9)	1.1 (0.4)	8.3 (1.7)	42 (31)
FH CT2	12	1.4 (0.7)	9.8 (2.2)	1.0 (0.4)	8.1 (2.0)	54 (29)

Table 4.4.1 Lipid profiles of the commonest FH genotypes
Mean (SD)

In order to determine whether the difference in lipid values between the genotypes was influenced by age, sex or body mass, groups were matched to correct for these factors. The matched comparison for FH Afr1 and FH Afr 2 is given in Table 4.4.2. In the analysis of the matched pairs in FH Afr1 and FH Afr 2, the difference in the total cholesterol reached greater significance ($P=0.012$) than it had in the whole group, as did the LDLC ($P<0.0001$). A novel finding was that HDLC was lower in FH Afr 2 when compared to matched subjects with FH Afr 1 ($P=0.04$). Total cholesterol was also significantly higher in CT2 than in FH Afr 1 ($P=0.03$) as was LDLC ($P=0.03$). No other values reached significant difference. All these P values were derived by the unpaired t test.

These analyses reveal that both the null defects have higher LDLC than the defective status LDLR mutations most commonly seen. Since the TG and HDLC concentrations were similar between the two groups it is unlikely that LPL activity will account for this difference. HL activity may play a role but there is no a priori reason to expect it to differ.

Genotype	Males : Females	Age (years)	BMI (kg/m ²)
FH Afr 1	62 : 67	39.7 (13))	25.5 (4.9)
FH Afr 2	43 : 67	37.7 (18)	25.9 (5.9)
P value	0.19	0.3	0.5

Table 4.4.2 Comparative values for matched pairs of FH Afr 1 and FH Afr 2
Mean (SD)

Genotype	Triglyceride mmol/L	TC mmol/L	HDLC mmol/L	LDLC mmol/L
FH Afr 1	1.5 (0.8)	9.3 (1.6)	1.25 (0.5)	7.4 (1.6)
FH Afr 2	1.5 (0.9)	9.9 (2.1)	1.13 (0.4)	8.0 (1.9)
P value	0.6	0.012	0.04	<0.0001
FH Afr 1	1.6 (1.8)	8.55 (1.0)	1.2 (0.4)	6.8 (1.2)
FH CT 2	1.4 (0.7)	9.8 (2.1)	1.0 (0.4)	8.1 (2.0)
P value	0.7	0.03	0.13	0.03

Table 4.4.3 Lipid values for matched pairs of FH Afr 1 and FH Afr 2, and FH Afr 1 and FH CT2 genotypes
Mean (SD)

4.5 Comparison of genotyped FH with non-genotyped FH

The clinical diagnosis of the FH phenotype was followed by genetic testing that led to the exclusion of familial binding defective B₁₀₀ (mutation R3500Q) and confirmed that the phenotype, based on founder genes, was due to mutations in the LDLR in approximately half of the cohort. Although the remainder may largely be due to other LDLR defects, possibly containing as yet undiscovered founder genes, other mutations rendering apo B a poor ligand for the LDLR or PCSK9 may be present. It was thus of interest to examine whether the clinical and biochemical features of the genotyped and "non-genotyped" FH were similar.

A comparison of the physical signs, plasma lipoprotein profile and ischaemic heart disease in 474 genotyped and 555 non-genotyped subjects is displayed in table 4.5.1.

	Xsma	Arcus	Ach xma	IHD	TG	TC	HDLC	LDLC	LDL particle size A+A/I ; I : I/B+B
Known genotype	53	166	338	160	1.5	9.6	1.2	7.6	209 : 118 : 66
Unknown genotype	86	205	483	217	1.9	8.8	1.2	6.8	152 : 167 : 193
P value	0.04	0.39	<0.0001	0.2	<0.0001	<0.0001	0.6	<0.0001	<0.0001

Table 4.5.1 Comparison of FH patients who had a known genotype with those with an unknown genotype

Xsma = xanthelasma Ach xma = Achilles xanthomata

The higher prevalence of tendon xanthomata is clearly due to the use of this physical sign to identify the non-genotyped cohort. The higher prevalence of xanthelasmata is interesting but the explanation is not clear. It is possible that this physical sign prompted testing for

dyslipoproteinaemia at the primary health care setting. Xanthelasma is often noted by the patient and is also more noticeable for the doctor than arcus cornealis. This could explain the similar prevalence of arcus in these two groups.

Ischaemic heart disease had occurred in 34% and 39% of the genotyped and non-genotyped cohorts respectively. The clinical diagnosis of the FH phenotype thus identifies a high risk patient for IHD, whether or not a genotypic diagnosis is confirmed. This finding justifies the clinical diagnosis and mitigates against spending limited resources on a genotypic diagnosis.

The finding of a small but significant increase in fasting plasma TG is consistent with the observation that xanthelasma was associated with higher TG concentrations in the whole cohort. (see table 3.5.3) The TC and LDLC can be viewed together and, interestingly are lower in the non-genotyped cohort. Tendon xanthomata were more prominent in subjects with the null defects (FH Afr 2 and CT 2) who had cholesterol concentrations. Therefore lower LDLC in the non-genotyped cohort suggests that this cohort may not contain many subjects with null mutations.

Compared with the genotyped cohort, the non-genotyped cohort had a more equitable distribution of LDL species, with almost a third in each of the larger, intermediate and smaller categories. The prevalence of smaller LDL species is practically doubled in the non-genotyped cohort. The small LDL species is consistent with the higher TG concentration as described in chapter 5.8. Although tendon xanthomata were associated with smaller LDL size (section 3.5.3), both increase in prevalence with age. This may be the basis for the apparent association. The influence of age was not specifically examined in this cohort owing to difficulties in matching.

4.6 ApoE genotype and Familial Hypercholesterolaemia

4.6.1 Demographics

ApoE is a well-recognised component of plasma lipoproteins including VLDL, chylomicrons and chylomicron remnants. It also occurs in some HDL particles but is absent in LDL. As indicated in chapter 1, it mediates the clearance of TG-rich lipoproteins which also contain CE that will down-regulate LDLR. As a consequence the LDLC concentration is decreased in subjects whose apoE is defective, as in the apo E2/2 genotype.

It is therefore of interest to examine the impact of apoE isoforms on LDLC concentration. To avoid the possible influence of variable LDL receptor function, the isoform was evaluated only in the commonest genotype in the clinic. ApoE genotyping was done on all the FH Afr1 patients in the FH cohort and the distribution is given in table 4.6.1. Compared to a previous analysis of the distribution of apoE isoforms in FH in Afrikaners (41), this distribution is similar although it seems to indicate an enrichment of E2/E3 in the GSH Lipid Clinic cohort, and a higher incidence of E3/E3 in the Afrikaner cohort. This could be due to the fact that the current analysis includes subjects who would not meet the criteria for being an Afrikaner. However, this did not reach statistical significance. ($P=0.24$, Chi-squared test.)

Apo E genotype	Lipid Clinic data		Study in Afrikaners	
	Number	% of FH Afr1 cohort	Number	% of cohort
E2 / E2	3	2	0	0
E2 / E3	20	9	5	4.5
E2 / E4	1	0.5	0	0
E3 / E3	153	70	87	78
E3 / E4	40	18	17	15
E4 / E4	2	0.9	3	3

Table 4.6.1 Apo E genotypes in patients with the FH Afrikaner 1 genotype

4.6.2 ApoE and triglyceride levels

In order to determine whether apoE isoforms played a role in determining the TG concentration, the FH Afr1 cohort was divided into quintiles of TG and the lowest and highest quintiles were compared for their distribution of apoE isoforms. The differences in age and average TG levels was equally striking ($P=0.0004$ and $P<0.0001$), as also the lower HDLC in the highest quintile of TG level ($P=0.011$). LDLC was not significantly different ($P=0.35$). (All these values were derived by the unpaired t test.) The difference in LDL species was statistically different ($P<0.000$, Chi-squared tests). In this analysis there was no statistically significant difference in the prevalence of IHD ($P=0.6$, Fisher's exact test).

The mild hypertriglyceridaemia in some FH subjects is thus ascribed to other genes and/or environmental factors, but is not accounted for by apoE. The hypertriglyceridaemia may represent an additional risk for CAD by virtue of overproduction of lipoproteins or under

clearance of their remnants, but this was not reflected in the LDL concentration. Similarly the CAD might also be associated with lower HDL levels that promote atherosclerosis or even the abundant preponderance of small LDL species. These results are displayed in table 4.6.2.

Apo E genotype	Highest quintile of TG level	Lowest quintile of TG level	P value
Age (SD)	45 (12)	36 (12)	0.0004
TG level (SD)	2.9 mmol/L (1.3)	0.7 mmol/L (0.16)	0.0001
Range of TG	2.0 - 5.5 mmol/L	0.3 - 0.9 mmol/L	
HDL level (SD)	1.08 mmol/L (0.5)	1.38 mmol/L (0.5)	0.011
LDL level (SD)	7.6 mmol/L (2.1)	7.2 mmol/L (1.7)	0.35
LDL species	A+A/I : I : I/B+B 6 : 17 : 13	A+A/I : I : I/B+B 31 : 6 : 2	<0.0001
E2 / E2	1	1	0.9
E2 / E3	4	5	
E2 / E4	0	0	
E3 / E3	31	24	
E3 / E4	9	9	
E4 / E4	0	1	
IHD	9 (20%)	6 (14%)	0.6

Table 4.6.2 Apo E genotyping and IHD in FH Afr 1 patients comparing the highest quintile of TG with the lowest quintile of TG

4.6.3 Lipid profiles in apoE genotypes

The lipid profiles and LDL species of the FH Afr 1 patients with apoE genotypes are given below. None of these comparisons was found to be statistically significantly different. Actual figures are given for TG and Lp(a), but for statistical comparison the logarithm of the values was used.

Genotype	Number	TG mmol/L	TC mmol/L	HDLC mmol/L	LDLC mmol/L	Lp(a) mg/dL	A+A/I:I:I/B+B
Apo E 2/2 and E 2/3 Standard deviation	23	1.3 (0.6)	9.2 (2.4)	1.3 (0.5)	7.1 (2.1)	27 (25)	12 : 3 : 5
Apo E 3/3 Standard deviation	153	1.6 (1.0)	9.4 (1.6)	1.2 (0.5)	7.5 (1.7)	33 (26)	60 : 56 : 18
Apo E 3/4 and E 4/4 Standard deviation	42	1.6 (1.1)	9.2 (1.4)	1.2 (0.3)	7.4 (1.4)	44 (27)	13 : 14 : 8

Table 4.6.3 Lipid profiles in apoE genotype cohorts in FH Afr1 patients with different apoE isoforms

This analysis indicates that apoE isoforms do not influence LDLC or LDL species significantly in the common LDLR defective form of FH in Cape Town.

4.6.4 ApoE2/2, E2/3 compared to apo E3/4, E44 isoforms in patients with IHD

In the FH Afr 1 cohort those patients with IHD who had apoE2/2 and apoE2/3 genotypes were compared with patients with IHD with apoE3/4 and apoE4/4 genotypes to determine whether

the apoE2 or apoE4 isoform had an influence on IHD and LDL species. Other traditional risk factors were also examined but the numbers were too small for analysis. The concentration of TG as well as other lipoproteins was not significantly different between the two groups. (see table 4.6.3) Table 4.6.4 depicts the findings of those patients with IHD and either apoE2 or E4 isoforms. The apo E isoforms appear to have no role in determining the IHD risk from either one or both alleles being affected. It would appear that the known prolonged post-prandial remnant particle exposure due to the apo E2 isoform was not associated with a distribution towards smaller LDL particles. (Fisher's exact test was used for IHD and sex analysis, the unpaired t test for age and the Chi-squared test for LDL species statistical analysis.)

	E2/2 and E2/3	E3/4 and E4/4	P value
IHD number	4/23 (17%)	14/42 (33%)	0.25
Males	1 (25%)	10 (71%)	] 0.25
Females	3 (75%)	4 (29%)	
Age	41 (9)	43 (11)	0.78
Smoking	1 male	9 (6m, 3f)	
Hypertension	1 female	4 (2m, 2f)	
Diabetes	0	1 male	
LDL species	A + A/I : I : I/B+B 2 : 2 : 0	A+A/I : I : I/B+B 4 : 5 : 3	0.53

Table 4.6.4 Comparison of ApoE2/2, E2/3 and apo E3/4, E4/4 isoforms in patients with IHD in the FH Afrikaner 1 cohort

4.6.5 Patients with FH and apoE2/2 genotype

Only four patients have been identified in the clinic with both confirmed FH genotyping and apoE2/2 status. ApoE2/2 genotyping has only been done on all FH Afr 1 patients and those who

had unusual presentations which merited further investigation. It is possible that some of the other mutations may coexist with apoE2/2 but there were no unusual clinical settings to investigate any other patients. The details of the patients with FH and apoE2/E2 genotypes are given in table 4.6.5.

Patient	Gene	Pres age	TG	TC	HDLC	LDLC	LDL sp	Manifest	IHD	Comment
NE (f)	FH Afr 1	35 yrs	0.7	8.2	2.0	5.9	A	Ach xma	N	BMI 30
DDK (m)	FH Afr 1	44 yrs	1.9	9.5	1.2	7.4	A/I	Ach xma Cut xma	30 yrs	BMI 26 Smoker
GVZ (m)	FH Afr 1	34 yrs	4.3	8.9	0.7	6.3	I	Ach xma Xanth Arcus Cut xma	33 yrs	BMI 28 Smoker
VF (f)	FH Lith	38 yrs	7.7	17.6	1.0	5.6	ML	Ach xma Xanth Cut xma Dig crease	N	BMI 30

Table 4.6.5 Details of patients in the clinic with FH and apo E2/2 genotype

Lipid profile in mmol/L N = none Ach xma = Achilles xanthomata
 Cut xma = cutaneous xanthomata Arcus = arcus cornealis Xanth = xanthelasma
 ML = pattern on GGE signifying remnants and VLDL2
 Dig crease = xanthomatous nodules in finger creases
 Lipid and lipoprotein concentration given in mmol/L

Patient VF was not included in the original database as she presented after the data-lock, but she has been included in this discussion as she illustrates several interesting points of relevance to FH and dysbetalipoproteinaemia and has presented a challenge to manage. Her mother originally presented to the clinic with an obvious FH phenotype in whom the genetic diagnosis of FH Lithuania was later made. On screening the family, her TC was about 6 mmol/L, whereas the other two siblings had a more obvious FH biochemical profile. The brother had a TC of 7.4

mmol/L and the sister had a TC of 8.2 mmol/L. VF was initially not thought to have FH but rather a “polygenic” dyslipoproteinaemia because of the milder profile. She presented to the clinic 10 years later. After having had children and gaining weight, she noted the development of xanthelasma. At this point the suspicion of dysbetalipoproteinaemia was confirmed by ultracentrifugation, revealing cholesterol-enriched VLDL. FH Lithuania was now confirmed genetically. Her management included strict reduction of dietary triglycerides and cholesterol as well as a trial of several statins, a fibrate and instant release niacin. She developed severe myalgia on all of the statins as well as the fibrate. Even low doses of statin could not be tolerated. Nicotinic acid was not tolerated because of persistent severe flushing. The availability of ezetimibe now presents a new therapeutic option.

Of the four cases presented, three appear to have retained the FH phenotype biochemically by having a predominant hypercholesterolaemia in which LDL is clearly defined on GGE. There is some suggestion of more severe Achilles tendon xanthomata and earlier onset of IHD. The patient VF displayed a benign phenotype in her youth before converting to a predominantly dysbetalipoproteinemia in her biochemical studies. The other subjects had not been investigated in their youth. The GGE pattern observed is typical for dysbetalipoproteinaemia. (169) It is not clear why the remnant accumulation has a stronger emphasis in this patient. While it is possible that age and insulin resistance promoted hypertriglyceridaemia, it is also possible that other genetic factors may have contributed.

4.7 Complications

Tale 4.7.1 gives the numbers, percentage of affected subjects in each genotype and the average age of occurrence of angina pectoris, myocardial infarction, stroke, peripheral vascular disease, coronary artery bypass graft (CABG) and of death in each genotype.

Genotype	Angina	MI	Stroke	PVD	CABG	Death
FH AFR1						
Number 222	71 (32%)	46 (21%)	5 (2%)	6 (3%)	33 (15%)	12 (5%)
Average age	42 (10)	41 (9)	54 (13)	51 (14)	44 (12)	50 (12)
Range (yrs)	24 - 70	24 - 60	42 - 73	39 - 77	24 - 75	38 - 76
FH AFR 2						
Number 115	35 (30%)	20 (17%)	0	0	19 (17%)	6 (5%)
Average age	44 (15)	39 (12)			43 (10)	46 (11)
Range (yrs)	21 - 74	21 - 59			26 - 60	30 - 60
FH AFR 3						
Number 32	13 (41%)	7 (22%)	0	0	6 (19%)	3 (9%)
Average age	43 (11)	45 (14)			39 (10)	52 (5)
Range (yrs)	23 - 62	23 - 63			23 - 50	48 - 58
FH LITH						
Number 29	7 (24%)	3 (10%)	1 (3%)	0	4 (14%)	3 (10%)
Average age	50 (17)	40 (6)	56		45 (8)	72 (10)
Range (yrs)	34 - 75	34 - 45			34 - 53	61 - 68
FH GUJ						
Number 11	5 (46%)	3 (27%)	0	0	4 (36%)	2 (20%)
Average age	38 (9)	35 (4)			40 (12)	52 (21)
Range (yrs)	28 - 50	31 - 38			30 - 57	37 - 67
FH CT 2						
Number 12	3 (25%)	2 (17%)	1 (8%)	1 (8%)	3 (25%)	2 (8%)
Average age	36 (5)	51 (20)	50	62	36	57
Range (yrs)	30 - 41	37 - 65			32 - 41	48 - 65
POOLED DATA						
Number	134 (32%)	81 (19%)	7 (0.2%)	7 (0.2%)	63 (16%)	28 (7%)

Table 4.7.1 Complications in the commonest FH genotypes
Average age of the event in years - mean (SD)

The table indicates a strong predominance of coronary artery disease over cerebrovascular and peripheral arterial disease. In general, coronary artery complications set in during the 5th decade, and in the FH Lithuania group, death appears delayed. This may only be a reflection of small numbers being compared and it is not statistically significantly different from FH Afr 1.

In order to try to establish whether the age of onset of the commoner complications was significantly earlier in one genotype than another, only patients presenting to the clinic between the ages of 40 and 60 were analysed. Only the Afrikaner genotypes were compared as the numbers in the other groups were considered too small. These results are given in table 4.7.2. The average age is the age of the event. The only significant result was that myocardial infarction occurred younger in FH2 than in FH1 ($P=0.03$) patients. (Unpaired t test)

The FH2 mutation being a "null" defect in the LDLR has been associated with a higher LDLC concentration and in this cohort is shown to be associated with a higher risk of myocardial infarction when compared with other Afrikaner mutations. This is novel information.

Age 40 - 60	Angina	MI	CABG	Death
FH AFR 1 Number 114	41 (36%)	27 (24%)	20 (18%)	9 (0.8%)
Average age	39 (7)	38 (6)	41 (7)	45 (6)
FH AFR 2 Number 52	15 (29%)	9 (17%)	10 (19%)	4 (8%)
Average age	36 (8)	33 (8)	41 (8)	40 (8)
FH AFR 3 Number 15	6 (40%)	3 (20%)	2 (13%)	2 (13%)
Average age	40 (5)	42 (7)	49 (3)	37 (1.4)
FH LITH Number 8	1 (13%)	1 (13%)	1 (13%)	0
Average age	34	34	34	
FH GUJ Number 3	2 (67%)	2 (27%)	1 (33%)	0
Average age	41	36	38	
FH CT2 Number 6	2 (33%)	1 (17%)	1 (17%)	1 (17%)
Average age	39 (2)	37	41	48
POOLED DATA Number	67 (34%)	43 (22%)	35 (18%)	16 (8%)

Table 4.7.2. Complications in patients presenting to the clinic between the age of 40 and 60 with the commonest FH genotypes.
 The average age is the age of the event - given as mean (SD)
 The bold typeface indicates a significant difference in the age of MI P=0.03

4.8 Geographical distribution of founder mutations.

The Cape of Good Hope Colony was established with the arrival of Jan van Riebeeck in 1652. Initially the occupation was centred around the Castle, for protection and also for the availability of fresh produce and communal goods. With the passage of time, land was granted to free burgers and land occupation spread, with farms and small communities establishing themselves further afield. This migration was gradual and often determined by geographical factors such as availability of water and physical obstacles such as rivers and mountains. Initially, land settlement was mainly up the west coast and along the south western regions of the Cape, with no real migration north.

In the 1830's mass emigration of colonists, mainly of Dutch origin, took place. Angered by restrictive legislation and the perceived lack of understanding of the problems of the farmers by the British Colonial Office, inadequate compensation for the freeing of slaves and general dislike of taxation and centralised administration, the colonists moved north in a mass migration which became known as the Great Trek. The trekkers were of the same religious persuasion and cultural background, stayed in the same communities and married within the group, which gave rise to expansion of the emerging Afrikaner gene pool. Owing to the small numbers of Settlers and their isolation in the early stages of migration, it is possible that founder effects could occur in the West and South coast and that the founder mutations could redistribute in the North as a result of the Great Trek.

From the map it can be seen that a large mountain range runs roughly in the North-South direction separating the West coast from the drier inland region. Similarly, an East-West range separates the drier inland (the Karoo) from the South coast. Migration north first required crossing a mountain range approximately 50 kilometres from Cape Town. The Eastern Cape was settled in 1820 by British Settlers and thus did not attract much migration.

The prevalence and distribution of Afrikaner mutations throughout South Africa has been previously described. (182) The relative distribution of the three Afrikaner mutations was found to be similar in the two regions of the Transvaal and the Cape Province. However in the Transvaal Province the three gene mutations explained 98% of the FH and in the Cape they accounted for only 74% of the FH seen in Afrikaners. In this FH cohort, 76% of the FH seen in Afrikaners was accounted for by FH Afr 1, FH Afr 2 or FH Afr 3 mutations.

When new patients are assessed at the clinic and a family tree is obtained, information about origins of the family is sought. Figure 4.8.1. represents the geographical origins of each index case of a kindred with an Afrikaner mutation in the clinic. A dot represents each kindred about whom such information was reasonably accurately known, and is colour-coded for each of the Afrikaner genes. It clearly shows that FH Afrikaner 3 spread almost exclusively along the southern districts. FH1 and FH2 were predominantly taken north by the trekkers.

Table 4.8.1 gives the demographics of the kindreds of Afrikaners with one of the three Afrikaner mutations and the number who could give the area of origin of their forbears.

	FH Afr 1	FH Afr 2	FH Afr 3
Number of patients	222	115	32
Number of kindred	176	66	25
Afrikaner kindred	103	57	17
Origins known	69 (67%)	43 (75%)	12 (71%)

Table 4.8.1 Numbers of patients, numbers of kindred and numbers of Afrikaner kindreds with the three Afrikaner genes, and the numbers and percentages of the known areas of origin of each kindred

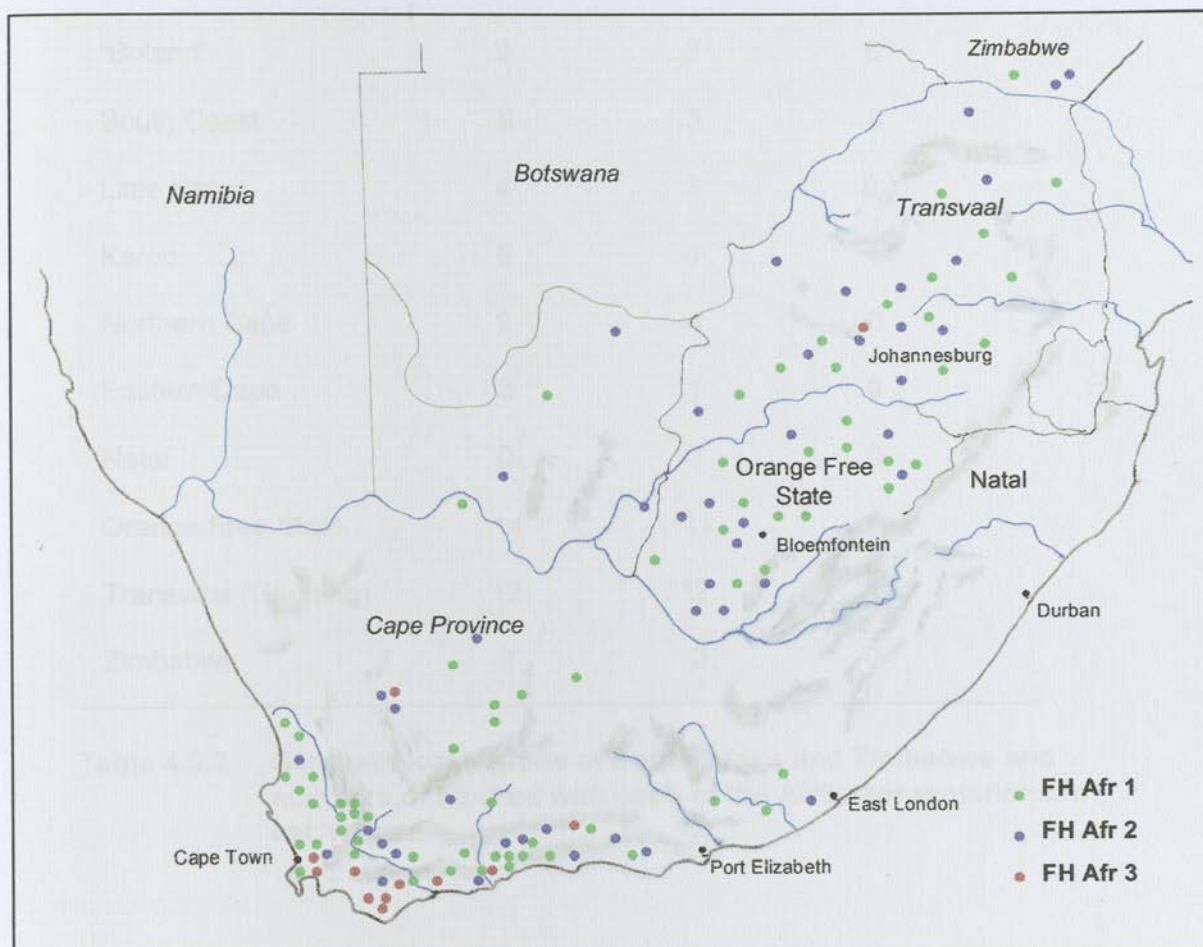


Figure 4.8.1 The geographical areas of origin of the Afrikaner kindreds of the three Afrikaner genotypes. The names of the old provinces of South Africa are used for historical accuracy

REGION	FH Afr 1	FH Afr 2	FH Afr 3
Greater Cape Town	3	1	1
West Coast	6	1	0
"Boland"	9	3	0
South Coast	8	3	8
Little Karoo	4	3	0
Karoo	6	4	1
Northern Cape	2	4	0
Eastern Cape	3	1	0
Natal	0	0	0
Orange Free State	14	11	0
Transvaal (Gauteng)	12	12	1
Zimbabwe	1	2	0

Table 4.8.2 Geographical regions of South Africa and Zimbabwe and numbers of kindred with each of the Afrikaner mutations in each area

The clinical phenotype is the most practical means of making the diagnosis of a high risk condition in medical practise. The geographical distribution of the mutations is of historical and genetic interest. Should a confirmatory diagnostic test be required, this information would be a useful guide to more appropriate testing.

Chapter five LIPOPROTEINS

5.1 Introduction

An elevated LDL cholesterol has been shown to be a major risk factor for coronary heart disease in humans in epidemiological studies and in clinical trials. In contrast other mammals are generally resistant to atherosclerosis even on diets associated with hyperlipidaemia, as they lack LDL. All modern clinical guidelines (NCEP, European, SA) emphasise the central role of LDL in atherosclerosis. FH can be regarded as an extreme model for evaluation of the effect of LDL on atherosclerosis. The large cohort of FH subjects at the Lipid Clinic makes it possible for the impact of lifestyle and other disease parameters to be evaluated, and to assess whether they play a role in the atherosclerosis observed in this cohort.

Plasma low density lipoproteins (LDL) consist of a mixture of particles which vary in size and density and in composition of protein and lipids. Traditionally, LDL has been defined according to ultracentrifugational separation in a density range of 1.020 to 1.063 g/mL, with recognised subcategories within this range. Since lipids impart buoyancy, smaller particles with less lipid will have a greater density. Separation of LDL according to its diameter is also possible by non denaturing gel electrophoresis which is inexpensive and less demanding on laboratory facilities.

Krauss and Burke first described the identification of subclasses of LDL by electrophoresis after ultracentrifugation in 1982. (183) Evaluation of LDL subclasses has further shown that the small dense LDL particle size is associated with an increased risk of myocardial infarction independent of sex, age or weight. (184) In this study by Austin there was a three-fold increased risk of myocardial infarction associated with small dense LDL particle size. There has been no systematic study to date to evaluate the influences of the factors associated with the modulation of LDL particle size in familial hypercholesterolaemia, nor whether the LDL particle size has an

influence on atherosclerosis and its complications in this disorder.

5.2. Factors Influencing LDL species

LDL particle size is directly influenced by the concentration of plasma triglyceride, hepatic lipase activity, cholesterol ester transfer protein activity, and lipoprotein lipase activity. Small dense LDL is associated with higher plasma triglyceride levels, VLDL, IDL and a reduction in HDL levels. LDL particle size is also affected by other factors or conditions such as the metabolic syndrome relating to insulin resistance, which has recently become a recognised entity for clinical management. (185) Associated conditions include age, obesity, physical inactivity, and hypertension. Low oestrogen levels (186) and raised plasminogen activator inhibitor-1 (PAI-1) levels (187) are also associated with small dense LDL particles. There may also be a direct genetic influence on LDL particle size, as Austin has demonstrated that LDL subclass patterns are influenced by a common allele at a single gene locus. (188)

Triglyceride

The circulating plasma triglyceride (TG) appears to be the single most important factor influencing LDL particle size. Increasing plasma triglyceride levels result in smaller LDL particles. This was clearly shown in an analysis of the Framingham study cohort. (189)

Griffin and co-workers found that the "threshold value" for modulation of LDL species was a fasting TG value >1.5 mmol/L. (17) Above this triglyceride level there is a significant change to small dense LDL. They postulate that an increased production of large, triglyceride rich VLDL1 gives rise to the small dense LDL through exchange and delipidation.

Hepatic lipase

Hepatic lipase (HL) is thought to be synthesised in the liver and is bound to the luminal surface of sinusoids where remodelling of VLDL and IDL to LDL may take place. Low HL activity has been associated with large LDL species, and conversely, higher HL levels were associated with small dense LDL particles. (191) HL is also responsible for conversion of large buoyant HDL2 particles to the smaller denser HDL3 particles.

Intensive lipid lowering therapy causes a marked reduction in HL, resulting in larger more buoyant LDL and HDL species, suggesting a mechanism for improved cardiovascular disease in response to lipid lowering. (192) HL has also been found to be significantly raised in diabetic patients which may also be a contributing factor in determining the small dense LDL found in these patients. (193)

CETP

The transfer of neutral lipids, such as cholesterol esters (CE) and triglyceride, between plasma lipoproteins, is mediated by cholesterol ester transfer protein (CETP). This reaction results in a net transfer of CE from HDL to VLDL and LDL particles. It also transfers TG from VLDL to LDL and HDL. In patients with high triglyceride levels, CETP in addition enhances the formation of small dense LDL particles by preferential CE transfer from HDL to LDL whereafter the HL can hydrolyse the TG. (194) There is also an indirect mechanism involving CE transfer from HDL to large VLDL, the precursors of small dense LDL particles (195) An extreme model for the role of CETP is hereditary CETP deficiency, which results in very high levels of HDL. These are mainly large, less dense HDL particles, enriched in CE and apoE.

5.3 LDL particle size analysis

Figure 5.3.1 is a schematic illustration of the Gradient Gel Electrophoretogram (GGE) that is used to define problems of lipoproteins containing apoB. When patients present to the clinic, routine GGE estimation is done as part of the biochemical investigation. An in-house notation was developed to describe the pattern. The LDL particle sizes are graded from large to small and labelled A, A/I, I, I/B and B, with B being the small dense particles. Ae and Bp are rarely encountered extremes of LDL size. The first four columns demonstrate Type I, III, IV and V dyslipidaemia. The next two columns illustrate the pattern seen with Lipoprotein X and lipoprotein (a) pattern. The first five columns are not relevant to this thesis.

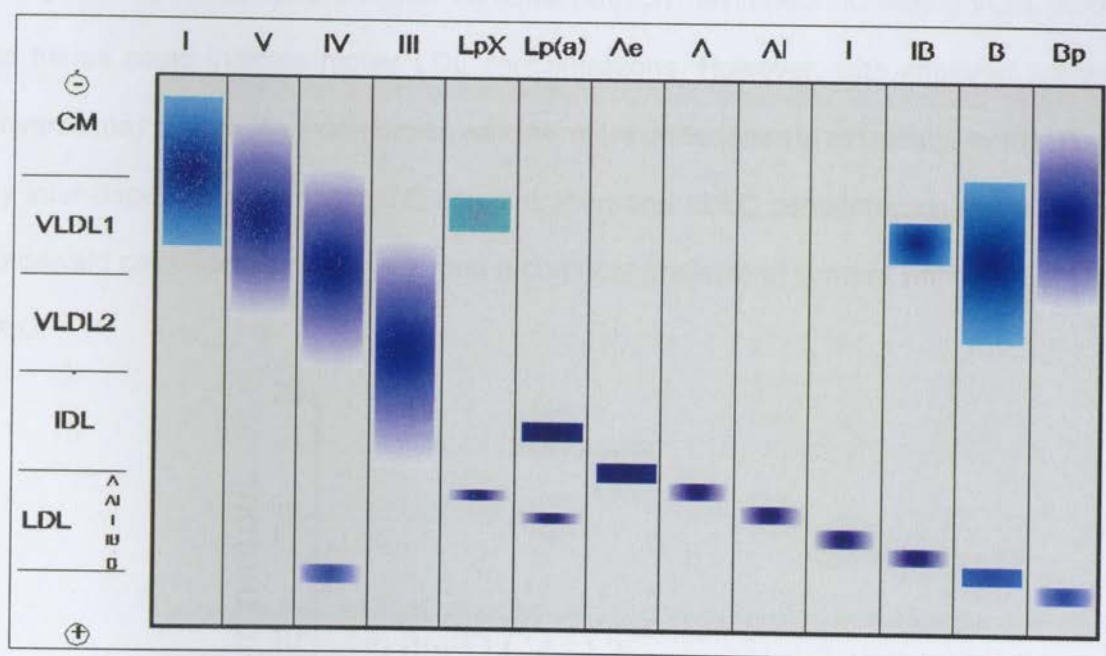


Figure 5.3.1 Schematic illustration of a gradient gel electrophoretogram

The calibration by ultracentrifugally prepared lipoproteins is presented on the left.

CM	Chylomicrons
VLDL1	Very low density lipoprotein 1
VLDL 2	Very low density lipoprotein 2
IDL	Intermediate density lipoproteins
LDL	Low density lipoproteins
LpX	Lipoprotein X
Lp(a)	Lipoprotein (a)

I	Type 1
III	Type 3
IV	Type 4
V	Type 5
Ae	A early
Bp	B post

5.4 Triglyceride, body mass, age and LDL cholesterol.

5.4.1 Correlation of triglyceride and LDL cholesterol concentrations.

From the conventional scheme of lipoprotein metabolism it is generally accepted that a LDL receptor defect will result only in an increase in LDL concentration. VLDL concentration is thus viewed as an independent variable in the FH lipoprotein profile. Triglyceride concentration reflects VLDL concentration in the fasted state and is known to be influenced by age, obesity and insulin sensitivity as well as oestrogen levels. Since LDL is the final product of VLDL, the fasting VLDL (triglyceride) concentration may give an indication of the LDL concentration. In the setting of complete lipolysis, a higher TG concentration may reflect increasing VLDL production and hence could indicate higher LDL concentrations. However, with impaired lipolysis the converse may be true. An examination was therefore undertaken to establish whether there was any inter-dependency between TG concentration and LDLC concentration knowing that the Friedewald calculation already assumed a constant cholesterol content within VLDL to derive LDLC.

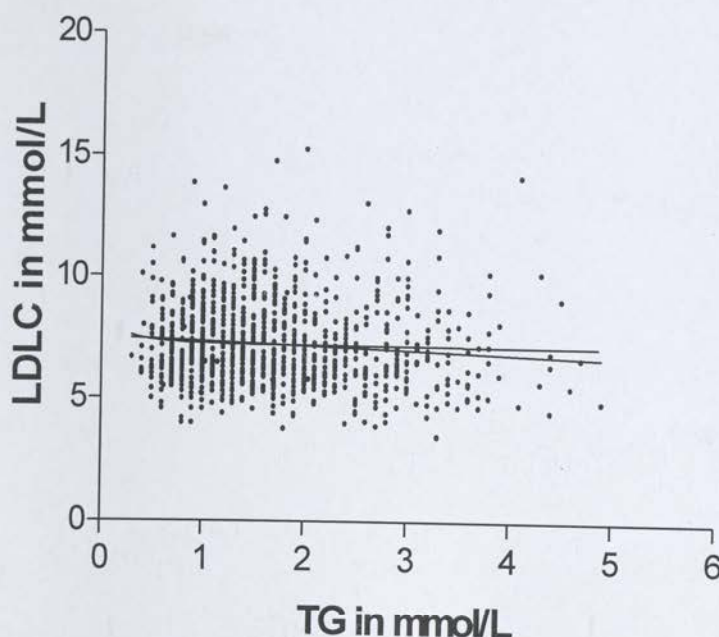


Figure 5.4.1 Correlation of TG and LDL concentrations in the FH cohort
 Linear regression analysis.
 Slope = -0.16 $r^2 = 0.006$ $P < 0.02$

Figure 5.4.1 indicates the wide scatter of LDLC against TG concentration and the linear regression revealed that only a small percentage (0.6) of the variance in LDLC is determined by the TG and the negative slope indicates an inverse relationship. Although this result by linear regression is statistically significant ($P < 0.02$), the slight contribution of the TG concentration revealed by the variance, is not clinically important as a quantitative phenomenon. The marked qualitative effect of TG concentration on LDLC is demonstrated by the analysis of LDL particle size. (See section 5.8) It may be appreciated that the individual LDLC concentration reflects a larger LDL particle number if the size is smaller. Since the concentration of LDL particles cannot be quantified exactly, it is not possible to correlate the TG concentration with LDL particle concentration. The general conclusion is therefore that TG concentration does not powerfully influence LDLC concentration but the impact on particle size is such that it may not be altering the particle.

5.4.2 Correlation of triglyceride and body mass index.

Plasma triglyceride levels are known to increase with increasing body mass and this correlation was examined in the FH cohort. The correlation was highly significant ($P < 0.0001$ by linear regression), but the TG concentration only explains 6% of the variance. The wide scatter indicates that other factors play a role in determining the TG concentrations.

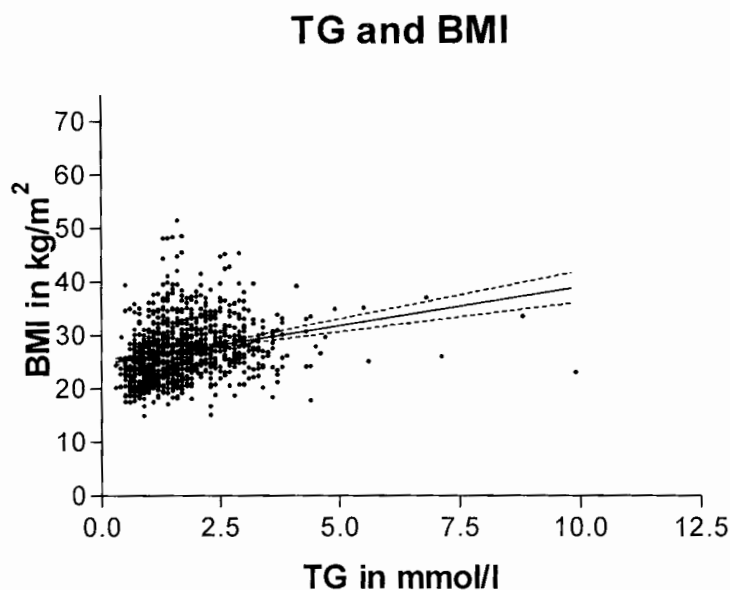


Figure 5.4.2 Body mass index (kg/m^2) and TG concentrations in the FH cohort

Linear regression analysis.

Slope 1.5 r^2 0.06 $P < 0.0001$

5.4.3 Correlation of age with body mass index.

In the FH cohort the correlation of increasing age with body mass index was investigated. As would be expected there was a positive association ($P < 0.0001$, Linear regression) The influence of age and body mass index on LDL particle size is discussed in section 5.5 and 5.6. Age explains only 5% of the variance in BMI but confirms the general trend in almost all population studies.

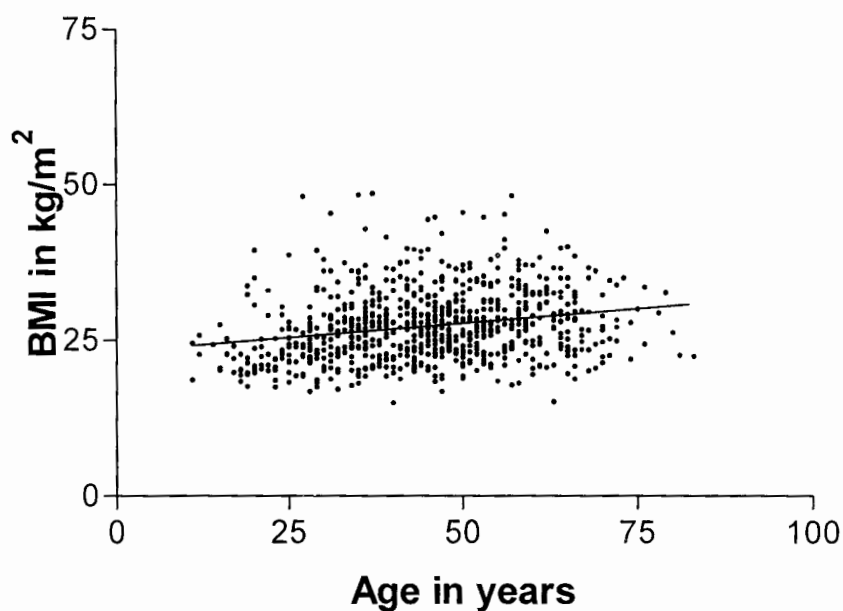


Figure 5.4.3 Correlation of age in years with body mass index (kg/m^2) in the FH cohort

Linear regression analysis.

Slope 0.09 $r^2 = 0.05$ $P < 0.0001$

5.5 Age and LDL particle size in the FH cohort

5.5.1 Age and LDL particle size in the FH cohort

The influence of age on LDL particle size in the whole cohort is clearly illustrated in figure 5.5.1, with an increase in small dense LDL occurring with increasing age. The two largest and smallest species were combined to more simply detect changes in distribution. The presence of smaller LDL species more than doubles. The change in distribution is highly significant ($P < 0.0001$). All P values in this section were derived by the Chi-squared test unless stated otherwise. The cohort was divided into quintiles consisting of 172 subjects in each.

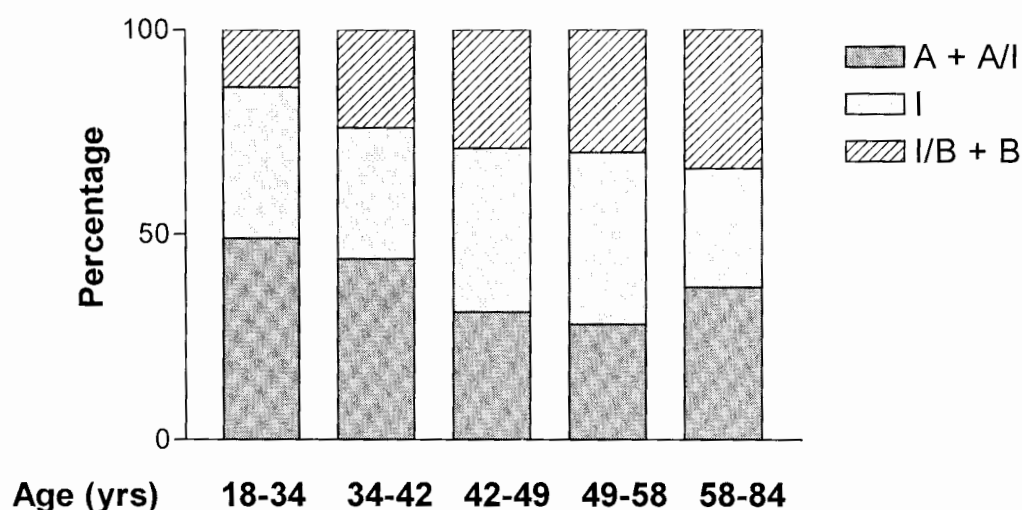


Figure 5.5.1 Age and LDL particle size in whole cohort with FH

Age	A + A/I	I	I + I/B
12 - 31	84 (49%)	64 (37%)	24 (14%)
32 - 41	75 (44%)	56 (32%)	41 (24%)
42 - 48	54 (31%)	69 (40%)	49 (29%)
49 - 57	49 (28%)	72 (42%)	51 (30%)
58 - 84	64 (37%)	49 (29%)	59 (34%)

Table 5.5.1 Number and percentage of particle sizes in each quintile of age

Although BMI and TG concentration could influence LDL size distribution other influences may may also play a role. As hormonal factors relating to gender and age may also influence LDL particle size an examination was undertaken in males and females.

5.5.2 Age and LDL particle size in males with FH

For the analysis of LDL particles in males and females, the age group quintiles of the cohort were retained for better comparison between the sexes. As indicated in chapter 3.2, there were fewer males in the cohort and they presented earlier. In males with FH the change to small dense LDL occurs fairly early, around the fourth decade, where-after the prevalence remains fairly constant. The change in distribution was however not quite statistically significant ($P=0.068$), although it can be regarded as a trend.

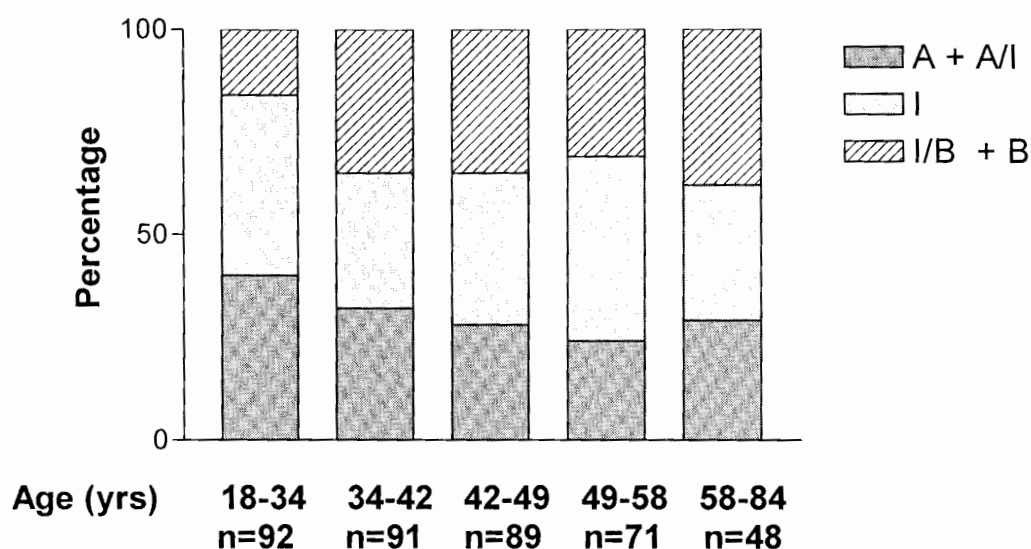


Figure 5.5.2 Age and LDL particle size in males with FH

Age	A + A/I	I	I/B + B
18 - 34	37 (40%)	40 (44%)	15 (16%)
34 - 42	29 (32%)	30 (33%)	32 (35%)
42 - 49	25 (28%)	33 (37%)	31 (35%)
49 - 58	17 (24%)	32 (45%)	22 (33%)
58 - 84	14 (29%)	16 (33%)	18 (38%)

Table 5.5.2 Number and percentages of particle size in each age group of males with FH

5.5.3 Age and LDL particle size in females with FH

In this analysis the same age groups as in the quintile analysis of the whole cohort were retained. As previously noted, there were more females in the FH cohort and they presented later. In females it would appear that the age for change to small dense LDL particles is much later, around the fifth decade. This also coincides with expected menopausal changes including weight gain and diminishing oestrogen levels. The change in distribution was statistically significant ($P < 0.0001$).

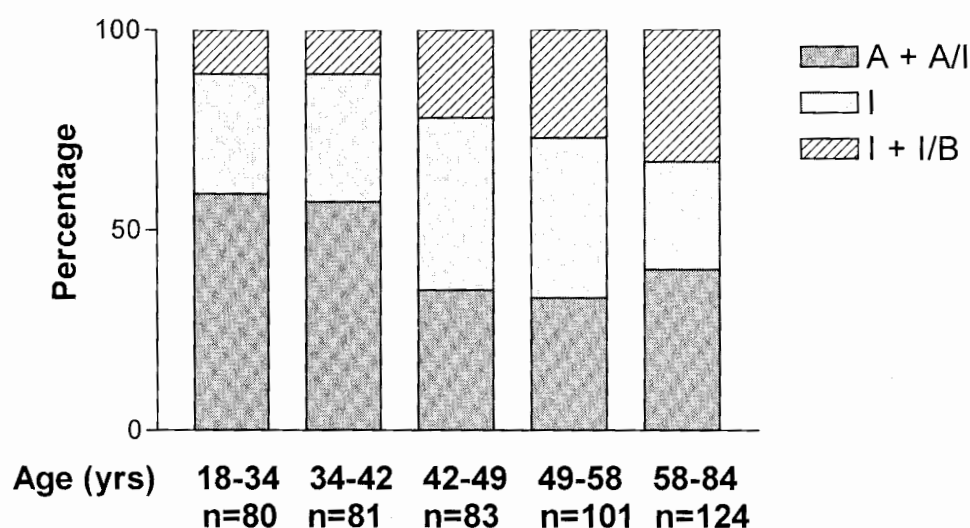


Figure 5.5.3 Age and LDL particle size in females with FH

Age	A + A/I	I	I + I/B
18-34	47 (59%)	24 (30%)	9 (11%)
34-42	46 (57%)	26 (32%)	9 (11%)
42-49	29 (35%)	36 (43%)	18 (22%)
49-58	33 (33%)	40 (40%)	29 (29%)
58-84	50 (40%)	33 (27%)	41 (33%)

Table 5.5.3 Number and percentage of particle size in each age group of females with FH

5.6 Body mass index (BMI) and LDL particle size in FH

5.6.1 BMI and LDL particle size in the FH cohort

As body mass index increases in the whole cohort the distribution of LDL particles changes from large to smaller, denser species. The number in each quintile is 170. The distribution is statistically different across the quintiles ($P<0.0001$, Chi-squared analysis was used for all P values in this section) with an approximately 2.5 times higher prevalence of smaller LDL species if the BMI is greater than 30 kg/m² compared to less than 23 kg/m².

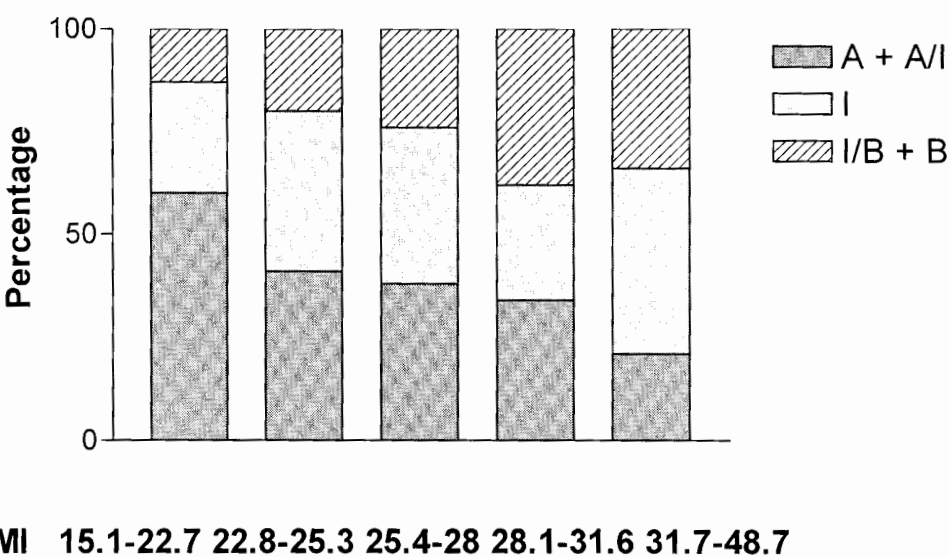


Figure 5.6.1 BMI (kg/m²) and LDL particle size in the whole cohort of FH

BMI	A + A/I	I	I/B + B
15.1-22.7	102 (60%)	46 (27%)	22 (13%)
22.8-25.3	69 (41%)	66 (39%)	35 (20%)
25.4-28.0	64 (38%)	65 (38%)	41 (24%)
28.1-31.6	58 (34%)	47 (28%)	65 (38%)
31.7-48.7	36 (21%)	76 (45%)	59 (34%)

Table 5.6.1 Number and percentage of particle size in each quintile of BMI in the FH cohort

5.6.2 BMI and LDL particle size in males with FH

The same BMI groups as in the quintiles for the FH cohort were retained for the analysis of particle size in males and females to illustrate the difference between the sexes. The numbers for each group are given below. The analysis of the LDL species distribution of BMI in males reveals that LDL species redistributes from large to small dense particles progressively with an increase in the BMI. The number of subjects in each group is indicated. The prevalence of smaller denser LDL changes approximately 2.5 fold from BMI less than 23 kg/m² to BMI greater than 32 kg/m² (P=0.0001). A BMI of >28 kg/m² seems to confer a cut point for significant change in LDL species.

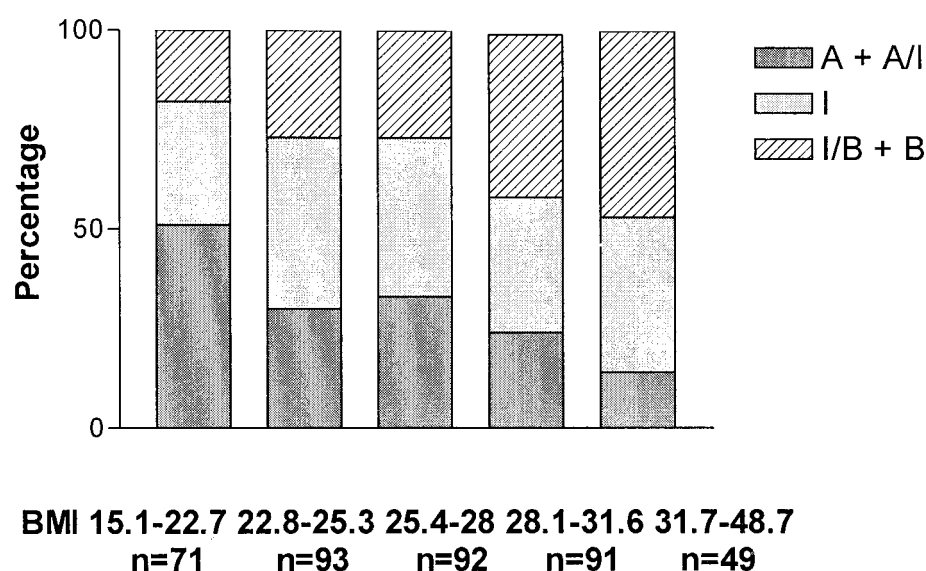


Figure 5.6.2 BMI (kg/m²) and LDL particle size in males with FH

BMI	A + A/I	I	I + I/B
15.1-22.7	36 (51%)	22 (31%)	13 (18%)
22.8-25.3	28 (30%)	40 (43%)	25 (27%)
25.4-28	30 (33%)	37 (40%)	25 (27%)
28.1-31.6	22 (24%)	31 (34%)	37 (41%)
31.7-48.7	7 (14%)	19 (39%)	23 (47%)

Table 5.6.2 Percentages of particle size in quintiles of BMI in males with FH

5.6.3 BMI and LDL particle size in females with FH

An analysis of the distribution of LDL species in groups of BMI in females also shows a gradual increase in the prevalence of small particles with increasing BMI. ($P < 0.0001$) The number of subjects in each group is given below. The large percentage of intermediate species in the last quintile is not explained. If not due to random sampling variation, it could indicate some intrinsic resistance in females to modulation of LDL species to a very small size or a propensity to store fat in the peripheral adipose tissue rather than viscerally so that it does not promote smaller species. There is an approximately 3-fold increase in the prevalence of smaller LDL species from a BMI of less than 23 to greater than 32 kg/m^2 . The sensitivity to LDL size modulation by BMI thus appears similar between males and females, with a BMI of $>28 \text{ kg/m}^2$ being a similar point for decreasing LDL size.

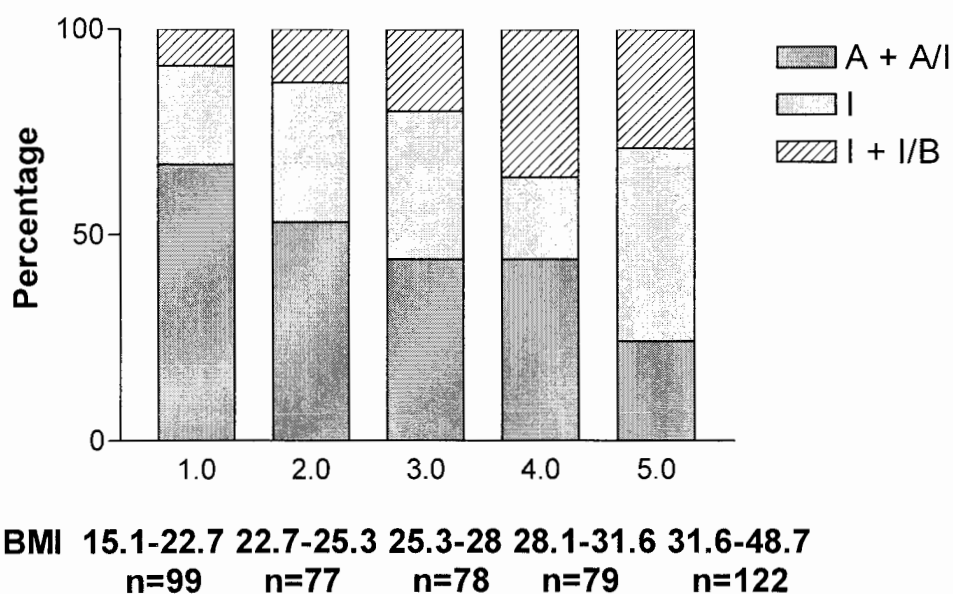


Figure 5.6.3 BMI and LDL particle size in females with FH

BMI	A + I/B	I	I + I/B
15.1-22.7	66 (67%)	24 (24%)	9 (9%)
22.8-25.3	41 (53%)	26 (34%)	10 (13%)
25.4-28	34 (44%)	28 (36%)	16 (21%)
28.1-31.6	35 (44%)	16 (20%)	28 (35%)
31.7-48.7	29 (24%)	57 (47%)	36 (29%)

Table 5.6.3 Percentage of particle size in each group of BMI in females with FH

5.7 Waist hip ratio (WHR) and LDL particle size in FH

5.7.1 WHR and particle size in the whole FH cohort

Whilst BMI is indicative of obesity, its central location may be of greater influence on LDL size. The definition of the metabolic syndrome by including a waist circumference reflects this. As the patients attending the Lipid Clinic come from many genetic and socio-economic backgrounds that could impact on the lean body size, a waist/hip ratio was utilised rather than waist circumference.

In the whole cohort there is a progressive change to small dense LDL particles with increasing waist hip ratio ($P < 0.0001$). (The Chi-squared test is used for analysis in this section.) The prevalence of smaller species increases approximately 6-fold. Each quintile comprised 147 individuals. The change in LDL species with WHR is more dramatic than with BMI, indicating that central obesity is more important in modulating LDL species, presumably as part of the metabolic syndrome. The cut point for transition to smaller species appears to be at 0.91, although there appears to be a continuum of influence. Clearly other factors also influence the LDL size: small LDL species were also observed in the lowest quintile

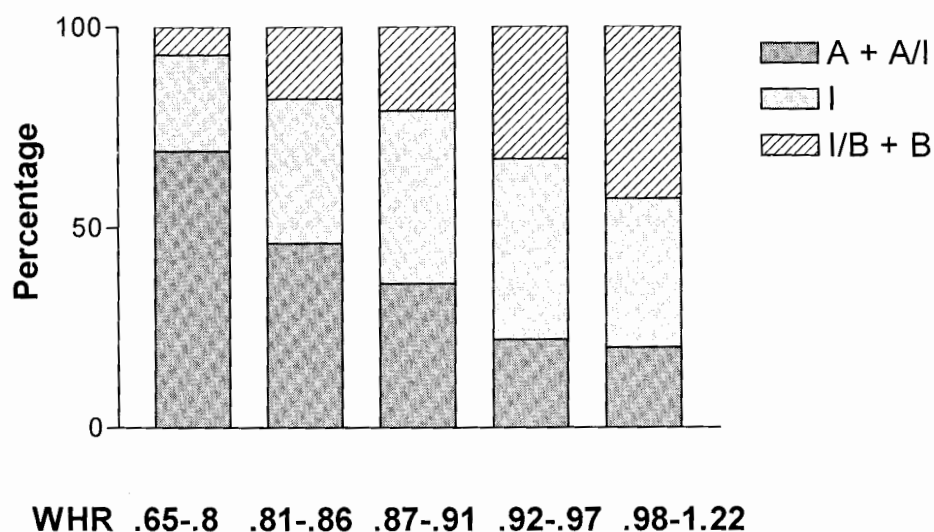


Figure 5.7.1 WHR and LDL particle size in the whole cohort of FH

WHR	A + A/I	I	I/B + B
.65-.8	102 (69%)	35 (24%)	10 (7%)
.81-.86	67 (46%)	53 (36%)	27 (18%)
.87-.91	54 (36%)	63 (43%)	31 (21%)
.92-.97	33 (22%)	66 (45%)	48 (33%)
.98-1.22	29 (20%)	54 (36%)	64 (44%)

Table 5.7.1 Number and percentage of LDL particle sizes in each quintiles of WHR

5.7.2. WHR and LDL particle size in males with FH

In males the progression to small dense LDL particle size is progressive with increasing waist hip ratio measurement ($P < 0.0001$). The WHR measurements were grouped as for the quintiles of the whole group for comparison of the sexes and the number in each group is given in the table. The apparently small number of patients with small dense LDL particles in the second quintile is not explained. It is suggested that this is random variation. It would appear that a WHR of about 0.91 represents the point at which LDL particle size is likely to be modulated by the increase in central fat mass. In male subjects with a WHR of >1.00 , the majority will still not display smaller LDL species although the prevalence is raised about 3-fold.

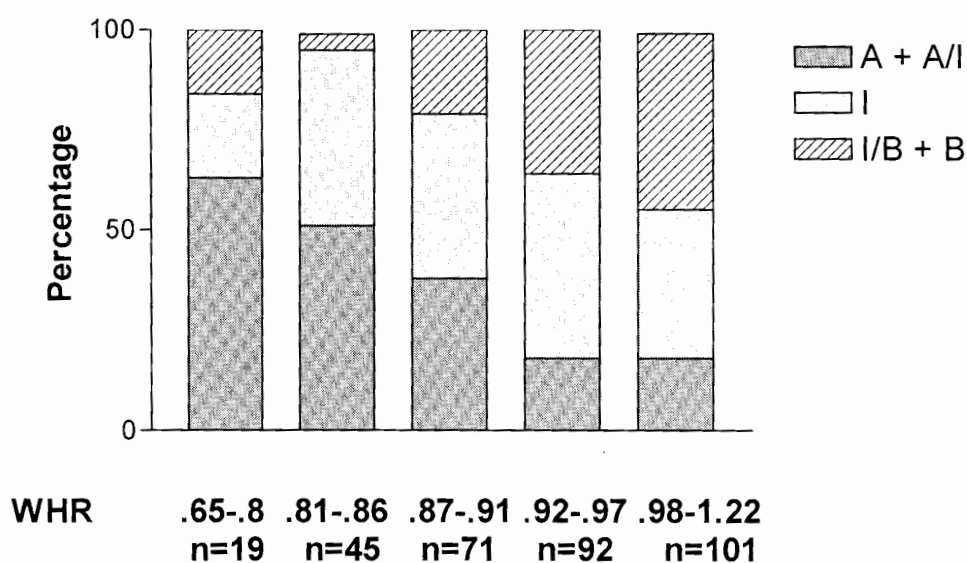


Figure 5.7.2 Waist hip ratio and LDL particle size in males with FH

WHR	A + A/I	I	I/B + B
.65-.8	12 (63%)	4 (21%)	3 (16%)
.81-.86	23 (51%)	20 (45%)	2 (4%)
.87-.91	27 (38%)	29 (41%)	15 (21%)
.92-.97	17 (18%)	42 (46%)	33 (36%)
.98-1.22	18 (18%)	37 (37%)	45 (45%)

Table 5.7.2 Number and percentages of LDL particle size in groups of WHR in males with FH

5.7.3 WHR and LDL particle size in females with FH

The groups of WHR were retained as for the quintiles of the whole cohort. The number in each group is given in the figure. In females there is a steady increase in the prevalence of small dense species with increasing waist hip ratios ($P<0.0001$). There is an 8-fold increase in the prevalence of smaller LDL species in women whose WHR is >1.0 . Females, like males, have better LDL size prediction from WHR than from BMI. Similarly, 0.91 seems to be a reasonable cut-point above which particles are likely to be modulated to a smaller size, though only about 40% will have small LDL.

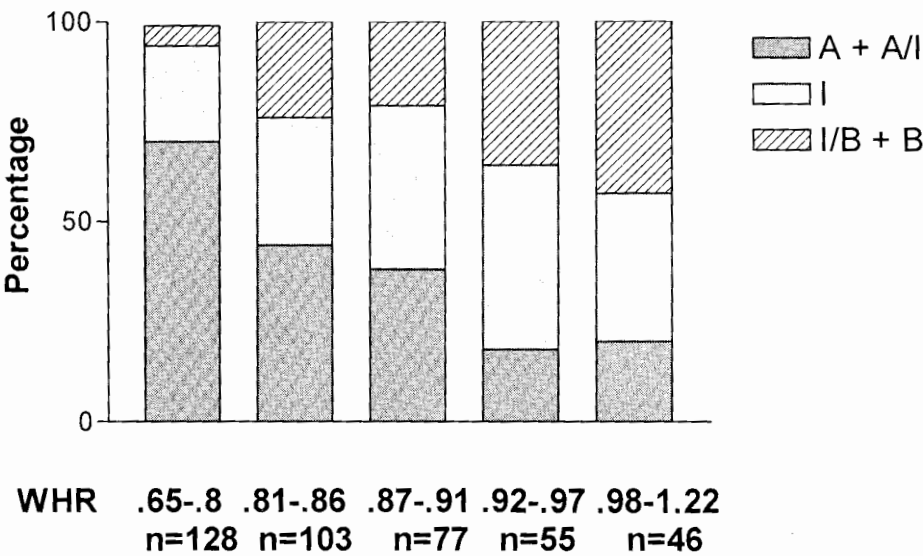


Figure 5.7.3 Waist hip ratio and LDL particle size in females with FH

WHR	A + A/I	I	I/B + B
.65-.8	90 (70%)	31 (24%)	7 (5%)
.82-.86	41 (44%)	33 (32%)	25 (24%)
.87-.91	27 (38%)	34 (41%)	16 (21%)
.92-.97	16 (18%)	24 (46%)	15 (36%)
.98-1.22	9 (20%)	17 (37%)	20 (43%)

Table 5.7.3 Number and percentage of LDL particle size in groups of WHR in females with FH

5.8 Influence of plasma triglyceride level on LDL species

5.8.1 Triglyceride levels and LDL particle size in the whole FH cohort

The influence of triglyceride on LDL particle size in FH is discussed in section 5.1. The distribution of particle size changes from large to small with increasing TG level across the quintiles of concentration. The number of patients in each quintile is 178 and the distribution is statistically significantly different ($P=0.0001$, Chi-squared) across the quintiles. It is clear that LDL species in FH are varied and are influenced by the concentration of TG. In the highest quintile 35% will have the smallest LDL species and another 20% will be smaller than intermediate. As HL is promoted by androgens that could influence LDL particle size, males and females were analysed separately. There are a few individuals who have small LDL species even with very low TG concentration indicating that other parameters can influence LDL size.

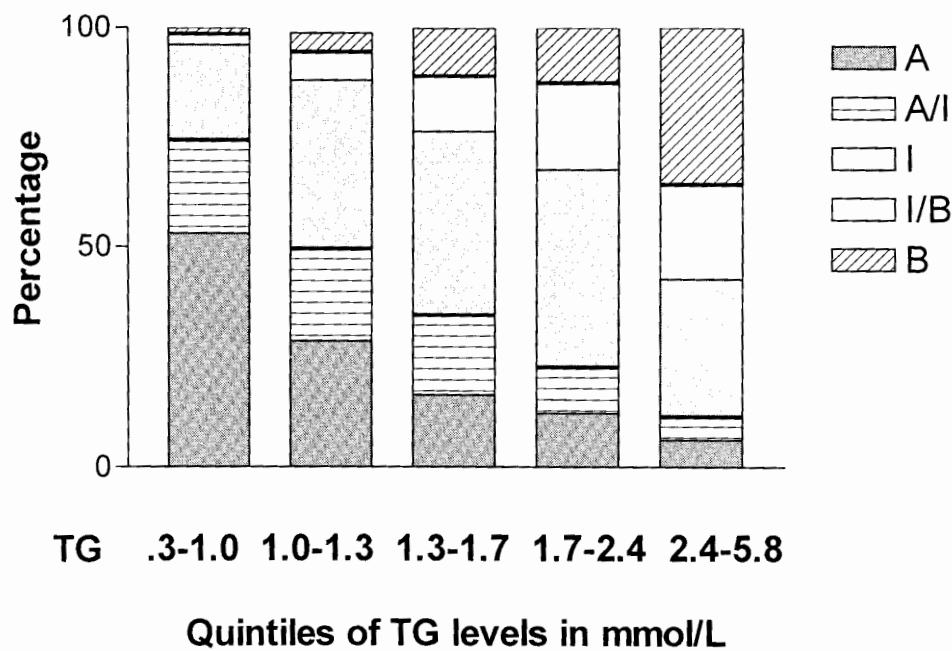


Figure 5.8.1 Triglyceride and LDL particle size in the cohort of FH

TG level	A	A/I	I	I/B	B
.3-1.0	94 (53%)	38 (22%)	38 (22%)	5 (2%)	2 (1%)
1.0-1.3	51 (29%)	38 (21%)	68 (38%)	12 (7%)	9 (5%)
1.3-1.7	29 (16%)	33 (18%)	74 (42%)	23 (13%)	19 (11%)
1.8-2.4	22 (12%)	19 (11%)	80 (45%)	35 (20%)	22 (12%)
2.4-5.8	11 (6%)	10 (6%)	55 (31%)	39 (22%)	63 (35%)

Table 5.8.1 Number and percentage of LDL particle size in each quintile of TG

5.8.2 Triglyceride levels and LDL particle size in males with FH

For the analysis of TG and LDL particle size in males and females, quintiles of males and females in the FH cohort were used. In males the same change occurred in LDL particle size from large to small dense particles with increasing levels of triglyceride as were observed in the whole cohort. The statistical analysis of the LDL species in the quintiles, each of which comprises 82 subjects, indicated a significant difference ($P=0.0001$, Chi-squared test). Taking the LDL species B and I/B together as smaller species particles, it is striking to note that the prevalence changes from 6% to 67% across the quintiles of TG concentration.

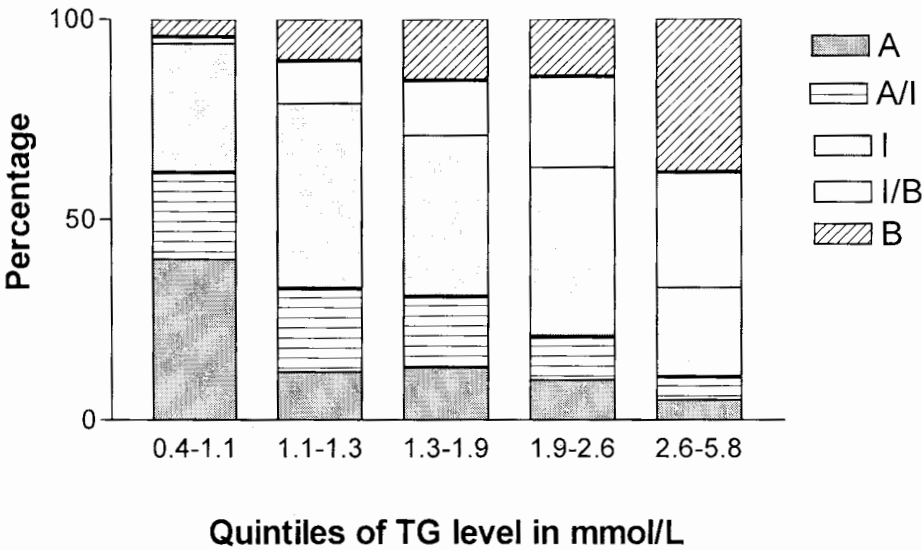


Figure 5.8.2 Triglyceride and LDL particle size in males with FH

TG level	A	A/I	I	I/B	B
0.4-1.1	33 (40%)	18 (22%)	26 (32%)	2 (2%)	3 (4%)
1.1-1.3	10 (12%)	17 (21%)	38 (46%)	9 (11%)	8 (10%)
1.3-1.9	11 (13%)	15 (18%)	33 (40%)	11 (14%)	12 (15%)
1.9-2.6	8 (10%)	9 (11%)	34 (42%)	20 (23%)	11 (14%)
2.6-5.8	4 (5%)	5 (6%)	18 (22%)	24 (29%)	31 (38%)

Table 5.8.2 Number and percentage of particle size in each quintiles of TG in males with FH

5.8.3 Triglyceride levels and LDL particle size in females with FH

In females with FH a similar change in LDL particle size is seen with increasing TG levels. The number of patients in each quintile of TG level is 96 and the difference between the quintiles was statistically significant ($P < 0.0001$, Chi-squared test). Grouping the LDL species B and I/B together as the smaller species, the prevalence again, as was observed in males, changes from 3% to 54% across the quintiles of TG concentration.

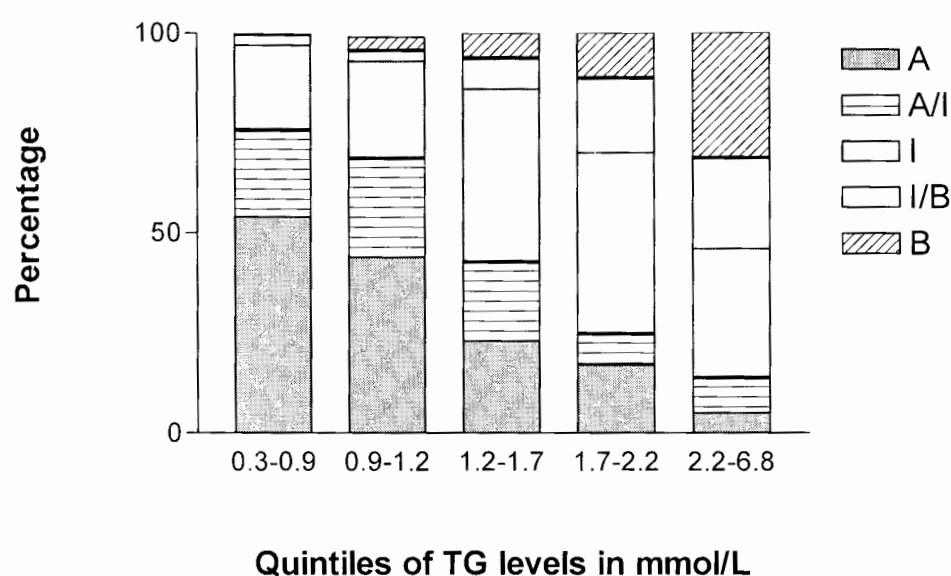


Figure 5.8.3 Triglyceride level and LDL particle size in females with FH

TG level	A	A/I	I	I/B	B
0.3-0.9	52 (54%)	21 (22%)	20 (21%)	3 (3%)	0
0.9-1.2	42 (44%)	24 (25%)	24 (25%)	3 (3%)	3 (3%)
1.2-1.7	22 (23%)	19 (20%)	41 (43%)	8 (8%)	6 (6%)
1.7-2.2	16 (17%)	8 (8%)	44 (45%)	18 (19%)	11 (11%)
2.2-6.8	5 (5%)	9 (9%)	31 (32%)	23 (23%)	30 (31%)

Table 5.8.3 Number and percentage of particle size in each quintile of TG level in females with FH

The prevalence of the smallest LDL species and the largest LDL species is altered in a reciprocal fashion from the lowest to the highest quintile of TG concentrations, the prevalence differing 8-fold. It is important to note that in the whole cohort small LDL species could be found in 2 individuals who had a TG less than 1.1mmol/L. Large LDL species could still be found in the highest quintile of TG concentration (2.6 - 5.8 mmol/L) in 11 subjects. These subjects had a TG concentration between 2.5 and 4.0 mmol/L.

The TG concentrations that indicate the occurrence of LDL B and LDL I/B are given in table 5.8.4. This shows that above a concentration of TG of >3.8 mmol/L small LDL species will almost invariably be found.

No of patients	TG concentration	I/B species	B species	Prevalence I/B and B species
541	0.6 - 1.7 mmol/L	38	33	13%
290	1.7 - 2.6 mmol/L	48	36	35%
110	2.7 - 3.7 mmol/L	17	34	43%
22	3.8 - 5.6 mmol/L	5	13	92%

Table 5.8.4 TG concentration and prevalence of small LDL species in the FH cohort

5.9 Diabetes in the FH cohort.

5.9.1 LDL particle size in diabetics in the whole FH cohort.

In the total cohort of FH there were 85 (8%) patients who had diabetes mellitus. All of these were type 2 diabetics. In the diabetic FH patients there was a particularly high prevalence of small dense LDL particles. The redistribution to smaller LDL species was statistically significant when compared with the whole cohort. ($P<0.0001$, Chi-squared test)

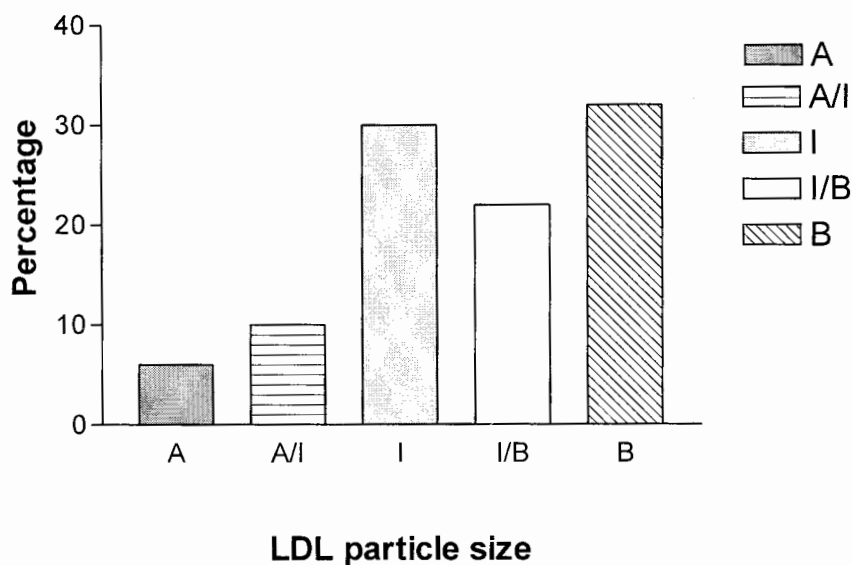


Figure 5.9.1 Percentage of LDL particle size groups in diabetic patients with the FH

LDL particles size	A	A/I	I	I/B	B
Number of patients	5	8	25	18	27
Percentage	6%	10%	30%	22%	32%

Table 5.9.1 Numbers of diabetic FH patients with each LDL species

5.9.2 Diabetic and non-diabetic FH patients matched for sex and age

Diabetes is considered an independent risk factor for IHD and some of the atherogenesis may be due to the alterations in lipoproteins. To establish the role of diabetes alone on LDL particle size, the group of FH diabetics was initially matched for sex and age with non-diabetic FH patients. The results are given in table 5.9.2.1. The actual TG levels are given, but logarithmic transformation was used in the statistical analysis. LDLC levels were calculated by the Friedewald equation. The higher TG values which could influence the composition of VLDL could affect the calculated value of LDLC.

As expected for type 2 diabetes, the affected subjects had statistically significantly higher triglyceride concentrations ($P<0.0001$) and lower HDLC concentrations ($P=0.003$), but they also had lower LDLC concentrations, ($P=0.03$). The diabetic patients also had greater waist/ hip ratios ($P<0.0001$) and body mass indices ($P<0.0001$). (All these values were derived by the unpaired t test).

WHR, BMI and triglyceride levels have been shown to influence LDL particle size, thus the group was matched for these factors to exclude their influence on particle size.

	Diabetics	Non diabetics	P value
Number	85	85	
Males : Females	32 : 53	32 : 53	
Age (SD)	53.2 (9.6)	49.9 (9.2)	0.2
Triglyceride	2.5 mmol/L (1.3)	1.8 mmol/L (0.9)	<0.0001
Total cholesterol	8.7 mmol/L (1.4)	9.1 mmol/L (1.6)	0.11
HDLC	1.1 mmol/L (0.3)	1.3 mmol/L (0.4)	0.003
LDLC	6.6 mmol/L (1.5)	7.1 mmol/L (1.6)	0.03
LDL species	A : A/I : I : I/B : B 5 : 8 : 25 : 18 : 27	A : I/A : I : I/B : B 24 : 9 : 25 : 4 : 13	<0.0001
Waist hip ratio (SD)	0.97 (wst/hip) (0.1)	0.87 (wst/hip) (0.1)	<0.0001
Body mass index (SD)	30.7(kg/m ²) (4.7)	27.6 (kg/m ²) (5.2)	<0.0001

**Table 5.9.2 Diabetic and non-diabetic patients in the FH cohort
matched for age and sex**

5.9.3 Diabetic and non-diabetic FH patients matched for sex, age and TG level.

When diabetic FH patients were matched for age, sex and triglyceride level with non-diabetic FH patients, there was still a preponderance of small dense LDL particles ($P=0.04$, Ch-squared analysis). This suggests that the diabetic state alters the LDL distribution by additional mechanism(s) to hypertriglyceridaemia but WHR could still play a role.

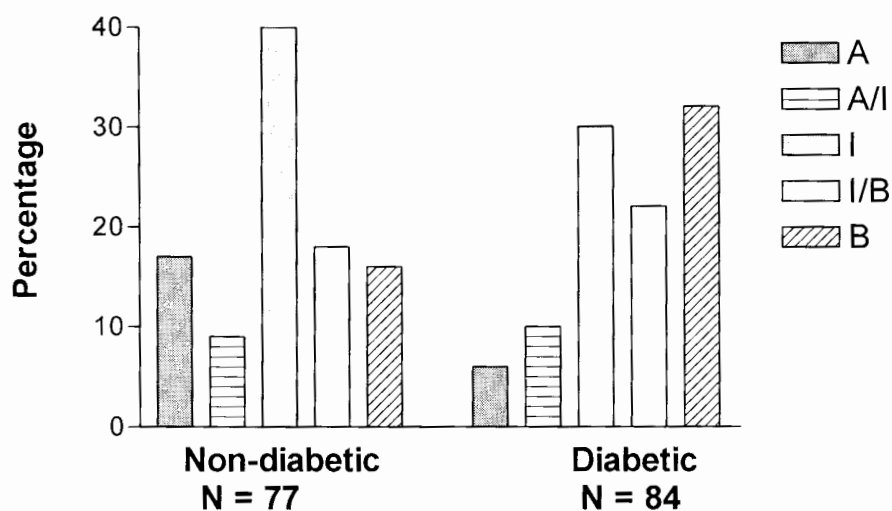


Figure 5.9.3 LDL particle size in diabetic FH patients matched for sex, age and TG level with non diabetic FH patients

	A	A/I	I	I/B	B
Non-diabetic	13 (17%)	7 (9%)	31 (40%)	14 (18%)	12 (16%)
Diabetic	5 (6%)	8 (10%)	25 (30%)	18 (22%)	27 (32%)

Table 5.9.3 Number and percentage of particle size in diabetic and sex, age and TG level matched non diabetic patients in the FH cohort

5.9.4 Diabetic and non-diabetic patients with FH matched for sex, age and WHR

Since WHR appeared a more powerful factor influencing the LDL species than BMI, further matching of the diabetic and non-diabetic subjects was undertaken. When matched for age, sex and waist hip ratio, diabetic FH patients have an even greater preponderance of small LDL species when compared to matched non-diabetic FH patients ($P=0.05$, Chi-squared test). The changes that may be conferred by an increased WHR are thus not likely to account for the higher prevalence of smaller LDL species in diabetes mellitus.

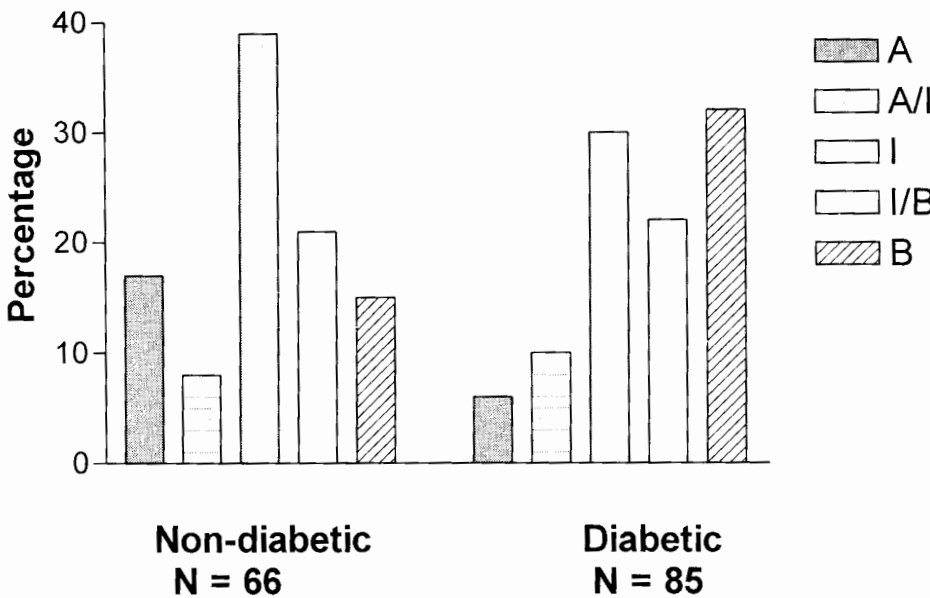


Figure 5.9.4 LDL particle size in diabetic FH patients matched for sex, age and WHR with non diabetic FH patients

	A	A/I	I	I/B	B
Non-diabetic	11 (17%)	5 (8%)	26 (39%)	14 (21%)	10 (15%)
Diabetic	5 (6%)	8 (10%)	25 (30%)	18 (22%)	27 (32%)

Table 5.9.4 Number and percentage of particle size in diabetic and sex, age and WHR non diabetic matched patients in the FH cohort

5.9.5 Diabetic and non-diabetic patients matched for sex, age and body mass index.

The two groups were also matched for BMI to establish the role of BMI in modulating LDL particle size. The diabetic group still had a preponderance of small dense LDL particles ($P=0.03$, Chi-squared test), thus the changes that may be conferred by an increased BMI are also not likely to account for the higher prevalence of smaller LDL species in diabetes mellitus.

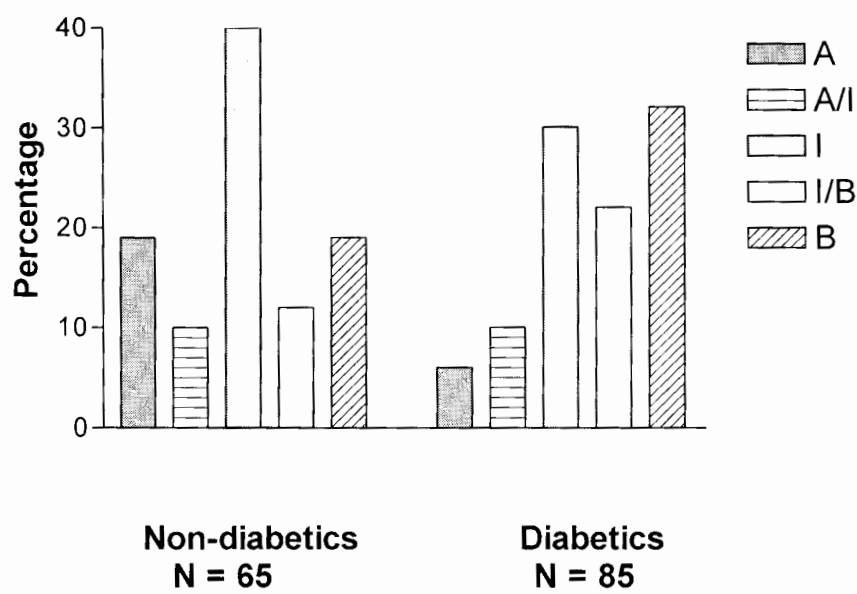


Figure 5.9.5 LDL particle size in diabetic FH patients matched for sex, age and BMI with non diabetic FH patients

	A	A/I	I	I/B	B
Non-diabetics	12 (19%)	6 (10%)	27 (42%)	8 (12%)	12 (19%)
Diabetics	5 (6%)	8 (10%)	25 (30%)	18 (22%)	27 (32%)

Table 5.9.5.1 Number and percentage of particle size in diabetic and sex, age and BMI matched non diabetic patients in the FH cohort

Small dense LDL particle size has been associated with a greater risk of myocardial infarction independent of sex, age or mass, as has been discussed in section 5.1. In order to determine the impact of the preponderance of smaller LDL particles in FH subjects with diabetes, the matched diabetic and non-diabetic cohorts (matched for age and sex as described in table 5.9.5.2) were compared to evaluate cardiovascular complications.

In this matched cohort the diabetics had significantly more hypertension ($P=0.003$, Fisher's exact test) which might be related to the greater mass noted in the diabetic subjects or changes brought about in insulin resistance. In the comparison of the cardiovascular complications, diabetics did not have angina or myocardial infarction any more frequently, nor was there an increased death rate ($P=0.65$, $P=1.0$ and $P=0.63$, Fisher's exact test). They, however, had myocardial infarction significantly later than matched non-diabetics ($P=0.003$). This was not the case for angina pectoris or death. ($P=0.2$ and $P=0.3$, unpaired t test)

It would thus appear in this cohort that despite imparting more small LDL particles, diabetes did not appear to confer greater cardiovascular risk above that of FH alone. The unexpected finding of a later average age of MI, despite the greater prevalence of hypertension in this comparison cannot be explained readily, especially when LDL_B seems to be an additional risk factor. It is possible that the lower LDL concentration is protective. Alternatively the duration of diabetes mellitus may be too short to impact on the risk. A lower LDL concentration is also somewhat unexpected but may reflect some metabolic effects of the diabetes, either to reduce LDL production or to increase LDL clearance. The latter may theoretically relate to increased glycation as a result of hyperglycaemia. Decreased production of LDL might also be related to increased levels of insulin as it is permissive for VLDL catabolism. The influx of fatty acids may also result in a greater production of VLDL1 rather than cholesterol-rich VLDL2.

Whilst the exact duration of diabetes is difficult to account for, it is generally accepted that type 2 diabetes is preceded by a prolonged period of insulin resistance that is viewed as an atherogenic state, either on its own or in association with the metabolic syndrome. It is also possible that the risk factors might have introduced a selection bias in that only relatively atherosclerotic resistant survivors ultimately attended the clinic. The controls were randomly selected for gender and age matching so that it is unlikely that there is a selection bias for younger subjects, especially since the average age of MI is similar to that in this control group and in the rest of the cohort. The distribution of the ethnic groups was representative of the whole clinic, avoiding another possible bias. If anything the diabetic male FH subjects smoked more than their controls. (see table 5.9.6.1)

	Diabetics	Non diabetics	P value
Number	85	85	
Males : females	32 : 53	32 : 53	
Age (SD)	53.2 (9.6)	49.9 (9.2)	0.2
Triglyceride	2.5 mmol/L (1.3)	1.8 mmol/L (0.9)	<0.0001
Total cholesterol	8.7 mmol/L (1.4)	9.1 mmol/L (1.6)	0.11
HDLC	1.1 mmol/L (0.3)	1.3 mmol/L (0.4)	0.003
LDLC	6.6 mmol/L (1.5)	7.1 mmol/L (1.6)	0.03
LDL species	A : A/I : I : I/B : B 5 : 8 : 25 : 18 : 27	A : I/A : I : I/B : B 24 : 9 : 25 : 4 : 13	<0.0001
Waist hip ratio (SD)	0.97 (wst/hip) (0.1)	0.87 (wst/hip) (0.1)	<0.0001
Body mass index (SD)	30.7(kg/m ²) (4.7)	27.6 (kg/m ²) (5.2)	<0.0001
Smoking - number	56 (66%)	50 (59%)	0.43
Hypertension - number	50 (59%)	30 (35%)	0.003
Angina - number	42 (49%)	38 (45%)	0.65
- average age	49.2 (4.8)	45.5 (8.9)	0.2
MI - number	25 (29%)	24 (28%)	1.00
- average age	51.4 (4.6)	44.9 (9.4)	0.003
Death - number	11 (13%)	8 (9%)	0.63
- average age	58.4 (7.2)	54.5 (8.9)	0.3

Table 5.9.5.2 Diabetic and non-diabetic subjects in the FH cohort matched for age and sex

5.9.6 Comparison of male and female diabetics.

Although the diabetic cohort comprised only 8% of the subjects with FH, they were of sufficient interest to study in greater detail. The male and female comparisons are given in table 5.9.6.

There were more female FH patients with diabetes than males, but not significantly so. The males however presented significantly earlier, 48.8 years compared to 55.8 years of age in the females ($P=0.0009$, unpaired t test). In the comparison of TG, TC and LDLC there was no difference, and HDLC showed only a trend towards lower levels in the males ($P=0.06$, unpaired t test). Both these HDLC levels were below the cut-off point for the metabolic syndrome. (See section 5.11) The other interesting feature relating to the metabolic syndrome was the fact that 85% of the females had a waist measurement greater than 89 cm, which is the cut off for females for the metabolic syndrome. This, together with a trend towards a greater body mass index in the females ($P=0.07$, unpaired t test), could be modifying factors for the LDL species, as the females had a higher prevalence of small species than the males. This however did not reach statistical significance ($P=0.7$, Chi-squared test).

The males tended to have more angina ($P=0.08$) and the females more hypertension ($P=0.07$), but these differences also did not reach statistical significance. In the male cohort, both angina ($P=0.02$) and myocardial infarction ($P=0.04$) occurred significantly earlier (Fisher's exact test).

In the male diabetic cohort, 84% smoked, which was significantly different from the 55% of female diabetics ($P=0.009$). This was also a higher percentage of male smokers than in the whole FH cohort. (84% compared to 63%, $P=0.02$, Fisher's exact test) The definitive comparison between diabetics and matched non-diabetics is given in the previous table. (5.9.5.2)

	Males	Females	P value
Number	32	53	0.07
Average age (SD)	48.8 (9.3)	55.8 (8.9)	0.0009
Triglyceride (SD)	2.3 mmol/L (0.7)	2.6 mmol/L (1.5)	0.47
Total cholesterol (SD)	8.8 mmol/L (1.6)	8.7 mmol/L (1.4)	0.76
HDL cholesterol (SD)	1.02 mmol/L (0.3)	1.14 mmol/L (0.3)	0.06
LDL cholesterol (SD)	6.8 mmol/L (1.5)	6.5 mmol/L (1.5)	0.34
LDL species	A : A/I : I : I/B : B 2 : 1 : 10 : 7 : 9	A : I/A : I : I/B : B 3 : 7 : 16 : 11 : 13	0.68
Waist (SD)	98.8 cm (8.9)	99.3 cm (12)	0.85
	37%>102 cm 44%<102 cm	15%<89 cm 85%>88 cm	0.0009
Waist hip ratio (SD)	0.99 (wst/hip) (0.05)	0.96 (wst/hip) (0.09)	0.12
Body mass index (SD)	29.3 (kg/m ²) (4.2)	31.3 (kg/m ²) (5.1)	0.07
Smoking - number	27 (84%)	29 (55%)	0.009
Hypertension - number	15 (47%)	35 (66%)	0.11
Angina - number	20 (63%)	22 (42%)	0.08
average age	45.8 (6.9)	52.5 (9.7)	0.01
MI - number	12 (38%)	13 (25%)	0.22
average age	48.2 (6.8)	54.7 (7.7)	0.04
Death - number	7 (22%)	4 (8%)	0.09
average age	53.3 (9.6)	63.5 (9.1)	0.12

Table 5.9.6 Comparison of male and female FH patients with diabetes mellitus

5.10 The metabolic syndrome in the FH cohort

In the general population, the metabolic syndrome is known to be a major contributor to the risk of ischaemic heart disease. According to the NCEP III guidelines the metabolic syndrome consists of three or more of the following - waist circumference in males greater than 102 cm and in females greater than 88 cm, TG concentration greater than 1.7 mmol/L, HDLC concentration less than 1.04 mmol/L for males and 1.3 mmol/L for females, diastolic blood pressure greater than 85 mmHg and systolic greater than 130 mmHg or treated hypertension, and fasting blood glucose greater than 6.1 mmol/L and less than 7 mmol/L. (184)

This study was not designed to look at the metabolic syndrome and therefore blood glucose levels were not captured in the original database. Having the other data for diagnosing the metabolic syndrome, its influence on the risk factors and the complications of FH was undertaken. In this analysis the emphasis is therefore given to hypertension and central obesity. In the male FH cohort only 10 patients (2%) met the criteria that were used to identify the metabolic syndrome - raised blood pressure, TG > 1.7 mmol/L and waist circumference >102 cm. These subjects were matched for age with male FH subjects with a TG level <1.7 mmol/L. The same study was then done for females where 28 (5%) had the metabolic syndrome.

The results of the matched comparisons are given in tables 5.10.1 and 5.10.2. The lipid values were analysed by the unpaired t test, the LDL species analysis was done by contingency tables including Fisher's exact test. In affected males, compared to the rest of the cohort, TG values, hypertension and waist circumference were statistically highly significantly different, as would be expected, as these were selection criteria. There were significantly more small LDL species in the metabolic syndrome group ($P=0.045$), but no other comparisons reached statistical significance. Diabetes and smoking were no different between the two groups.

It appears that the metabolic syndrome does not impact on the coronary artery complications of FH, but this may be as a result of small numbers and incomplete information or relatively short duration of the condition. Of interest is the observation that the HDLC concentration was not significantly higher in the matched cohort without the metabolic syndrome. This may be due to the lowering of HDLC by FH as previously observed. (196;197)

MALES	With metabolic syndrome	Without metabolic syndrome	P value
Number	10	10	
Hypertension number	10	1	0.0001
Waist measurement (SD)	111.5 cm (7.4)	89.9 cm (4.2)	<0.0001
Diabetes number	0	0	
Smoking number	9	6	0.3
Triglyceride (SD)	2.7 mmol/L (0.8)	1.2 mmol/L (0.3)	<0.0001
Cholesterol (SD)	8.4 mmol/L (1.2)	9.0 mmol/L (1.4)	0.35
HDLC (SD)	0.9 mmol/L (0.2)	1.1 mmol/L (0.5)	0.31
LDLC (SD)	6.3 mmol/L (1.3)	7.3 mmol/L (1.6)	0.13
LDL species	A : A/I : I : I/B : B 0 : 0 : 5 : 1 : 4	A : A/I : I : I/B : B 0 : 1 : 7 : 0 : 0	0.045
Angina - number	6	6	1.35
Average age (SD)	45.5 (5.3)	46.2 (5.9)	0.83
MI - number	6	4	0.66
Average age (SD)	39.3 (4.2)	41 (9.5)	0.7
Death - number	1	3	
Average age (SD)	53	55 (6.1)	

Table 5.10.1 Males with FH and the metabolic syndrome matched for age with males in the FH cohort with TG < 1.7 mmol/L.

In the female FH cohort, 28 patients (5%) were identified with the metabolic syndrome, using the same criteria as for males but a waist circumference of >89 cm. This group was also matched for age with FH females who had a TG concentration <1.7 mmol/L. The same statistical analyses were undertaken. Once again the selection criteria of TG level, hypertension and waist circumference were significantly different as would be expected. However, in the matched group the average waist measurement was also greater than 88 cm. This may indicate that in FH, a different waist measurement may apply for discrimination of the metabolic syndrome or that the absolute measurement of 89 cm is not appropriate for the Cape Town subjects.

In the female cohort HDLC was also significantly different, with the metabolic syndrome group having lower levels ($P=0.002$). In the female comparison the difference in LDL species reached greater significance ($P=0.0002$), with smaller species in the metabolic syndrome cohort. In this group there was also no increase in cardiac events or death.

FEMALES	With metabolic syndrome	Without metabolic syndrome	P value
Number	28	28	
Hypertension number	28	6	<0.0001
Waist measurement (SD)	100 cm (9)	90 cm (14)	0.003
Diabetes number	0	0	
Smoking number	14	12	0.8
Triglyceride (SD)	2.6 mmol/L (0.7)	1.2 mmol/L (0.4)	<0.0001
Cholesterol (SD)	9.0 mmol/L (1.4)	9.5 mmol/L (2.0)	0.3
HDLC (SD)	1.1 mmol/L (0.3)	1.5 mmol/L (0.6)	0.002
LDLC (SD)	6.7 mmol/L (1.3)	7.5 mmol/L (1.9)	0.07
LDL species	A : A/I : I : I/B : B 1 : 2 : 9 : 10 : 6	A : A/I : I : I/B : B 9 : 7 : 7 : 2 : 1	0.016
Angina - number	17	14	0.6
Average age (SD)	52.3 (10)	51.6 (10)	0.85
MI - number	8	5	0.53
Average age (SD)	50.8 (5)	49.6 (4)	0.66
Death - number	1	2	
Average age (SD)	50	61 (0)	not determinable

Table 5.10.2 Females with FH and the metabolic syndrome matched for age with females in the FH cohort with TG < 1.7 mmol/L.

The relationship between waist measurement and small LDL species was also examined. In this comparison only waist circumference was identified as an influencing factor on LDL species. These data are given in table 5.10.3. The Fisher's exact test was used for the analyses. The impact of a larger waist measurement was greater in males than it was in females. Twenty four percent of males whose waists exceeded the cut point for the metabolic syndrome waist circumference by NCEP III criteria had the smallest LDL particles, significantly more than in females ($P=0.05$). However, when I/B and B particles were grouped together, the difference between the sexes was lost.

All ages	Males	Females	P value
Number	66	189	
Waist	> 102 cm	> 88 cm	
LDL I/B+B sp	29 (43%)	60 (32%)	0.1
LDL B species	16 (24%)	25 (13%)	0.05

Table 5.10.3 Waist circumference and LDL particle size in males and females in the FH cohort

In females, oestrogen levels are known to impact on the lipid profile and the risk of IHD is markedly increased after the menopause. As the age of the menopause had not specifically been documented, an arbitrary age of 50 years was chosen and LDL species distribution was examined in females before and after 50 years of age. These results are given in table 5.10.4. The impact of menopause, judged by this criterion, revealed significantly more smaller LDL species in a cohort where the waist circumference was already a contributing factor in both groups, according to NCEP III criteria ($P=0.03$, Fisher's exact test).

Females	Under 50 yrs	Over 50 yrs	P value
Number	81	106	
Waist	> 88 cm	> 88 cm	
LDL I/B + B sp	19 (24%)	41 (39%)	0.03

Table 5.10.4 Waist circumference and LDL species in women with FH before and after the age of 50 years

5.11 LDL particle size in the common genotypes

Patients in the common genotypes found in the clinic were matched for age and sex to determine whether any given genotype was associated with a different distribution of LDL particles. As a result of the small numbers when matching was performed, the LDL species were pooled for larger (A + A/I) and smaller (I/B + B) species. FH Lithuania had significantly fewer patients with small LDL particles when compared to FH Afrikaner 1 ($P=0.06$) as a trend, but not statistically significantly so. Although the numbers are small, it can be suggested that null mutations (FH Afr 2 and FH CT 2) do not alter the LDL size distribution when compared with defective mutations (FH Afr 1, FH Afr 3 and FH Gujerat).

Genotype	A + A/I	I	I/B + B		p value
FH Afr 1	51	37	13	]	0.74
FH Afr 2	51	36	9		
FH Afr 1	16	11	5	]	0.98
FH Afr 3	15	10	4		
FH Afr 1	6	5	1	]	0.21
FH CT2	5	2	4		
FH Afr 1	1	5	5	]	0.47
FH Gujerat	3	5	3		
FH Afr 1	9	13	7	]	0.06
FH Lithuania	14	6	2		

Table 5.11.1 LDL particle size in FH Afrikaner 1 patients matched for age and sex with the commoner FH genotypes
(Numbers are numbers of patients with each particle size)

In a previous comparison, FH Lith had a significantly lower BMI than FH 1 (see ch 4.3.1). When these two groups were now matched for age, sex and BMI, the difference in LDL particle size was no longer significant ($P=0.17$). The P values were derived by the Chi-squared test for independence. The difference in BMI was thus attributable to the influence of BMI.

Genotype	A + A/I	I	I/B + B	]	p value
FH Afr 1	10	14	3		
FH Lithuania	14	6	2		0.17

Table 5.11.2 LDL particle size in FH Afrikaner 1 patients matched for age, sex and BMI with FH Lithuania

FH, like other dyslipidaemias, requires comprehensive management of all risk factors, but clearly the severe LDL hypercholesterolaemia is the prime target for modulation. Such comprehensive management may make high demands on resources but the high risk and anticipated benefit justify such an approach. Medical evaluation includes lifestyle evaluation and counselling but additional input, especially from a dietician, would enhance management. Initially patients should be seen at short intervals for titration of drug therapy. Once stabilised, six monthly or annual visits for ongoing safety and efficacy surveillance should suffice. Lipid Clinics can play an important role in the management of FH.

6.1 Lifestyle modification.

6.1.1 Dietary modification.

At the first visit to the Groote Schuur Hospital (GSH) Lipid Clinic a dietary history was taken to reflect the contents of the diet. Appropriate counselling was then given by a dedicated dietician whenever possible, or else this was given by the doctor who admitted the patient to the clinic. As far as possible dietary modification was encouraged in line with the AHA step III guidelines. Follow-up was generally done after 3 months of the new lifestyle before drugs were prescribed.

The analysis done of the first 122 genotyped FH patients attending the clinic (see chapter 3.7.4) included assessing the impact of dietary advice. The average LDLC was reduced by 1.2 mmol/L. These results are given in table 6.1.1. This study confirmed the utility of introducing a low fat diet along the lines of the AHA step III diet. In the FH cohort the reduction was not quite as good as those reported by Connor, as shown in chapter 1, table 1.10.1.1. The lower response may be due to an intrinsic difference in response to diet in FH but is more likely due to an improved diet

advised to the patient before attending the Lipid Clinic, or to an incomplete adherence to dietary advice given.

	TG	TC	HDLC	LDLC
At presentation (SD)	1.5 mmol/L (0.8)	10.2mmol/L (1.8)	1.15mmol/L (0.3)	8.3 mmol/L (1.8)
After diet change (SD)	1.5 mmol/L (1.3)	8.8 mmol/L (1.8)	1.16mmol/L (0.3)	7.1 mmol/L (1.5)
Change		-1.5 mmol/L		-1.2 mmol/L
% change		- 15%		- 14.5 %
P value		< 0.002		< 0.002

Table 6.1.1 Lipid profiles at presentation and after dietary modification for three months in 122 patients with FH

6.1.2 Cessation of smoking

At the Lipid Clinic a smoking history was always taken and the risks of smoking were clearly explained to the patient. Patients were strongly urged to stop and their smoking status was monitored at subsequent visits. Accurate assessments of smoking habits are notoriously difficult. On questioning, many individuals appeared to smoke less. The best results in cessation of smoking were achieved after a cardiac event in a patient or a near relative, with few returning to the habit. One drug trial involving 101 FH subjects of whom 22 % were smoking, reviewed smoking status at frequent intervals for one year. On average the patients smoked 8 cigarettes per day, ranging from 15 - 4. Only 2 (9% of smokers) stopped smoking during this time. Ten (45% of smokers) were smoking as much as at entry to the study and 10 (45%) reduced the number of cigarettes by more than 50%, from 7/day to 3/day (range 4 to occasional). In this

group there were patients who seemed to have no insight into the risk of smoking despite clear counselling and there were those who appeared to be true addicts, unable to stop the habit despite a concerted effort.

6.1.3 Regular exercise and maintenance of ideal body weight.

The promotion of regular exercise is an integral part of the management of patients with FH. At the Lipid Clinic patients were counselled on the benefits of regular exercise and encouraged to make exercise a part of their daily living routine. Although high intensity exercise is required to substantially increase fitness and VO₂ max, regular moderate exercise sustained over a period of time is encouraged in the light of the findings discussed above. Patients were encouraged to perform 30 minutes of moderate-intensity exercise on most days of the week. (198) These recommendations were not quantitatively monitored at the Lipid Clinic.

6.2 Lipid modifying drug therapy.

6.2.1 HMG -CoA reductase inhibitors or statins.

At the UCT clinical research facility many drug studies in patients with FH using different statins have been conducted, with some patients being exposed to a number of different statins. The results of some of the completed studies are given below.

A dose titration study with Parke-Davis enrolled 22 patients with FH. They were started on a dose of 10 mg daily of atorvastatin and the dose was doubled every 4 weeks. (Parke-Davis study 981 - 198) In this group of FH patients none reached the LDLC goal of 3 mmol/L using

40 mg of atorvastatin, and only 32% reached this target on 80 mg daily. Each doubling of the dose in this study resulted in approximately a further reduction of between 5% and 8% in the LDLC level. The results are given in table 6.2.1.1

Daily dose	LDLC mmol/L	% change from baseline	% reduction over initial dose
0	7.9 (1.1)	0	
10 mg	5.2 (0.9)	35.4 (7.9)	
20 mg	4.4 (0.8)	43.7 (8.8)	8%
40 mg	4.1 (0.7)	48.6 (6.9)	5%
80 mg	3.5 (0.8)	55.6 (8.8)	7%

Table 6.2.1.1 Response in LDL cholesterol to incremental doses of Atorvastatin in patients with FH
(Standard deviation)

Using rosuvastatin in the FH cohort, patients were enrolled on a 24-week randomised double-blind multi-centre multinational trial to evaluate the efficacy and safety of rosuvastatin and atorvastatin in the treatment of subjects with FH. (AstraZeneca study IL4522/0030.) The results are given for the patients taking rosuvastatin in table 6.2.1.2. A 20 mg daily dose of rosuvastatin was as effective as 40 mg atorvastatin and doubling of the dose gave the anticipated further reduction.

Daily dose	LDLC mmol/L	% change from baseline	% reduction over initial dose
0	8.5 (1.5)	0	
20 mg	4.3 (1.0)	49.6 (7.9)	
40 mg	3.6 (0.6)	58.1 (7.4)	7%
80 mg	3.2 (0.7)	61.8 (7.3)	5%

Table 6.2.1.2 Response in LDL cholesterol to incremental doses of Rosuvastatin in patients with FH
(Standard deviation)

When rosuvastatin was used in incremental doses in the different Afrikaner FH genotypes, no significantly different responses were noted between the groups, despite the apparent differences in the percentage reduction. These data were obtained from pooled data from AstraZeneca studies - IL4522/0034, 0091 and 0099 which were open label, multi centre, multinational, phase IIIb studies to evaluate the efficacy and safety of rosuvastatin.

Genotype	Number	Daily dose	% reduction from baseline	P value
FH Afr 1	26	40 mg	57.8 (8.3)	0.9 (FH Afr 1 vs FH Afr 3)
FH Afr 2	11	40 mg	53.5 (9.3)	0.2 (FH Afr 1 vs FH Afr 2)
FH Afr 3	4	40 mg	58.4 (5.0)	0.3 (FH Afr 2 vs FH Afr 3)

Table 6.2.1.3 Response to the same dose of Rosuvastatin in the Afrikaner FH genotypes
(Standard deviation)

In these studies only 31% of subjects reached the LDL target of 3 mmol/L. Initially study 0034 investigated a 80 mg dose of rosuvastatin but this was reduced to 40 mg daily for the remainder of the study. Higher doses of statins may achieve target LDLC concentrations but it appears that toxic manifestations may limit the use of doses that lower LDLC by more than 60%. One patient on the study, CFM, developed heavy proteinuria, elevated serum creatinine and numerous granular casts on 80 mg rosuvastatin, and on detailed investigation (including renal biopsy) this was shown to be tubular toxicity, presumed to be related to the study drug. On discontinuing rosuvastatin, renal function returned to normal and has remained so on a lower dose of both simvastatin and atorvastatin. (199)

LDLR negative (null) mutations have higher LDLC concentrations compared with defective mutations indicating that the mutated LDLR may play a role in the cholesterol balance. (See chapter 4.4) This may also influence the response to the statin. There have been varied reports about differences in response to statin therapy in differing LDLR defects. Jeenah reported that FH Afrikaner 2 appeared to have a greater percentage reduction in response to 40 mg Simvastatin when compared to FH Afrikaner 1 but achieved the same LDLC concentration. (200) As shown above, there were no significantly different responses between the genotypes in the studies conducted of relatively small numbers of subjects with the three Afrikaner mutations. It is possible that the small numbers might not reveal the differences that were shown between genotypes in the Canadian study reported by Couture. (201)

6.2.2 Bile acid sequestrants or resins.

The experience at the Lipid Clinic has been that bile acid sequestrants have been difficult to take because of the unusual and inconvenient way of administering the drug and the frequency of side effects. These include nausea, a feeling of "bloating", dyspepsia, abdominal cramps

and, in particular, constipation have been major factors contributing to non-compliance. The cost of these drugs has also increased enormously, and their use is now reserved for pregnant women with very high LDL levels, patients intolerant of statins or as additional therapy to statins in an attempt to reach target levels.

The new compound, Colesevelam, which is taken in tablet form, is tolerated more easily by patients, without many of the side effects of the older bile acid sequestrants, but is not yet available in this country.

6.2.3 Nicotinic acid or Niacin.

In the setting of limited resources nicotinic acid is a useful lipid modifying agent and has been tried in a number of patients attending the Lipid Clinic. Few patients have managed to persist with the large doses of nicotinic acid required for therapeutic effect, due to the frequently encountered side effects and the problem of taking large numbers of tablets at frequent intervals.

A notable exception was patient SA, a female with the FH Afrikaner 1 mutation at the LDLR, who experienced her first MI at the age of 42 years followed by coronary artery bypass grafting (CABG), only to have a second MI five years later also followed by CABG. With the combination of 40 mg Simvastatin daily and 2 grams of Nicotinic acid three times a day she reduced her LDLC from 7.8 mmol/L to 2.8 mmol/L. At the age of 65 years she is still playing badminton regularly.

Another patient RA, with FH Afrikaner 3 and an LDLC of 8.2 mmol/L reduced the LDLC to 2.9 mmol/L with a combination of Simvastatin 20mg and Niacin 2 g three times a day.

6.2.4 Ezetimibe

Ezetimibe has only recently been registered in South Africa and has not been used in the clinic to treat patients with FH. It would appear to offer a greater possibility of getting patients with FH to target levels, when added to statin therapy.

6.2.5 Fibric acid derivatives or fibrates.

These drugs are not the first line of treatment for FH and have not been used extensively in FH at the Lipid Clinic. However, they are used occasionally in FH patients with unusually low HDLC levels, where raising the HDLC would be expected to improve the risk profile or where hypertriglyceridaemia co-exists. The experience is limited and is not reviewed here.

6.3 Plant sterol-enriched margarines

A randomised double-blind cross-over study in which patients with FH consumed 20g of regular margarine or phytosterol enriched margarine for a period of six weeks on each margarine, followed by a washout period, thus giving two baseline levels for comparison. (Sitosterol-enriched margarine study, Unilever 2000) 42 patients completed the study and their data was analysed. This study formed the basis of an MSc (Med) Nutrition Dietetics thesis submitted by Ms Zarina Hamdulay.

Compared to regular margarine, LDLC was reduced by 6.5% ($P=0.005$) and TC was reduced by 6.4% ($P=0.002$). These reductions, whilst statistically significant, are considerably lower than the published lowering effect of the sterol margarines, which were not tested in FH patients.

Even though this appears to be a modest reduction in patients with FH, its effect is equivalent to the doubling of a statin dose and would be cost-effective in the treatment of this disease.

6.4 Ileal bypass surgery

With the advent of statins and the effective lipid lowering they achieve, this form of therapy is obsolete. However, two interesting patients attending the Lipid Clinic merit mentioning.

Patient PVZ with FH Afrikaner 1 had a total cholesterol of 14 mmol/L in 1978. In 1980 he underwent partial ileal bypass surgery and was seen at the GSH Lipid Clinic in 1993. At this visit he was asymptomatic, had no stigmata of FH and a lipid profile of TG 6.5 mmol/L, TC 8.9 and HDLC 0.8 mmol/L. He was not on lipid lowering therapy at the time. He has not returned to the clinic as he lives too far away.

Patient AVB also had the FH Afrikaner 1 gene. She presented in 1991 with a TC of 11.4 mmol/L, TG 1.5 mmol/L, HDLC 0.7 mmol/L and LDLC 10.0 mmol/L. She had xanthelasmata and Achilles tendon xanthomata. In 1998 she was admitted to hospital with intestinal obstruction due to a bowel infarction that appeared to follow blunt trauma. After surgery for the resection of the necrotic bowel she was left with 60cm of small bowel and half of her colon. She returned to the Lipid Clinic in 2005. On no lipid lowering therapy her profile was now TG 1.8 mmol/L, TC 6.1 mmol/L, HDLC 0.6 mmol/L and LDLC 4.6 mmol/L - a more than 50% reduction in LDLC by the resection of her intestines.

6.5 Plasmapheresis

This form of therapy is now infrequently used in heFH as statins are effective and are the therapy

of choice. At the Lipid Clinic the use of this procedure has been limited to the treatment of hoFH, where many patients have been treated since 1976 with considerable success and undoubted prolongation of life.

6.6 Portocaval shunt

This form of therapy has only been undertaken in patients with hoFH at the Lipid Clinic, prior to the advent of statins and where these patients did not have access to plasmapheresis.

6.7 Future treatment strategies

6.7.1 New drug development

The most rational strategies to limit risk in FH are likely those that modify the primary lipoprotein defect (that is LDLC) or atherosclerotic process. New drugs have been tested or are under development.

The **microsomal triacylglycerol transfer protein (MTP) inhibitors** have been shown to inhibit LP production in the Watanabe rabbit, a model for hoFH. (202) Unfortunately this drug causes fatty liver and development has ceased.

The **acylCoA:cholesterol acyltransferase (ACAT) inhibitors** may prevent foam cell formation but this could enhance macrophage apoptosis or even cause necrosis that could incite inflammation and result in a higher risk of complications.

The **cholesterol ester transfer protein (CETP) inhibitors** which raise HDL may not provide

adequate increase in reverse cholesterol transport or might not be adequately antioxidative for the LDL load seen in FH.

6.7.2 Liver transplantation and gene therapy

Neither of these forms of therapy has been used at the Lipid Clinic. The advent of statins, especially in combination therapy, made the control of heFH so successful that neither of these options will be likely to be used for this condition. However, the new approach of inhibiting the production of LP containing apoB₁₀₀ through the use of anti-sense mRNA holds much promise for the future.

Chapter seven LESSONS LEARNT AND RECOMMENDATIONS

This thesis set out to analyse the experience with FH as a clinical diagnostic entity in Cape Town. Though retrospective, this study describes a large cohort of FH patients referred from outlying clinics, hospitals and private practitioners.

The high prevalence of FH in South Africa is generally appreciated locally and internationally but apart from delineating the chemical effects and genetic causes of LDLR mutations in South Africa, little further information is available. The clinical experience, the impact of the genetic defects on clinical features, the utility of genetic diagnosis, the means for detecting higher and lower risk in affected individuals and the lipoprotein sizes and their response to the conventional modulators have been investigated in this thesis. A limited number of genotypes previously described account for almost half of the FH seen and the clinical experience indicates that FH is present in all local populations with additional founder effects and is also found in recent immigrants who do not come from populations with founder effects.

7.1 Clinical findings

7.1.1 Demographics

FH is not the predominant dyslipidaemia referred to the GSH Lipid Clinic. The estimated number of subjects with FH in the area within an hour's drive of Cape Town was almost 10,000. Considering the numbers of referred patients and knowing that the majority of patients requiring treatment in this group would require diagnosis and treatment at a tertiary hospital, suggests that FH is under-diagnosed. This problem is also encountered in other countries. (203)

Of the patients presenting with hyperlipidaemia only 23% had the FH phenotype. Dyslipidaemic

White patients accounted for 40% of the clinic referrals and 55% of these had FH. White males and females presented earlier than those of other racial groups. These observations suggest a greater awareness of FH in Whites by the population and/or medical practitioners. The Coloured group formed the largest component of the clinic accounting for 56% of patients presenting with hyperlipidaemia, but comprising only 43% of the FH cohort. In the Asian group, who only formed 2% of those who presented to the clinic, the diagnosis of FH was made in 20% of patients. The relatively frequent occurrence of two mutations accounting for FH in this community suggests the presence of a founder effect as a cause of dyslipidaemia. In the Black population the diagnosis of FH is probably seldom considered as this large community accounts for only 1% of patients presenting to the clinic and they formed 1% of the FH cohort. It would appear that FH could be highly under-diagnosed in the Coloured and Black communities where private healthcare is limited.

In the whole FH cohort, males tended to present earlier, with 70% presenting before the age of 50 years. Women tended to present later with the greatest percentage between 40 and 60 years. This time of presenting may coincide with menopausal changes and awareness of cardiovascular risk.

Greater awareness of this condition needs to be created in the public domain and amongst the medical fraternity, especially as regards the Black population. Promotion of FH as a clinical diagnosis would encourage medical practitioners to recognise this high risk condition. Routine cholesterol measurement and in particular family screening where an index case has been identified would lead to identification and earlier treatment of many more individuals with FH. FH is a sufficiently common condition that could be managed at primary health care level after diagnosis and introduction of appropriate treatment at a Lipid Clinic.

7.1.2 Clinical signs

7.1.2.1 Xanthelasma

Xanthelasma, known as a relatively poor indicator for dyslipidaemia, was not examined in the non-FH cohort and its value as a diagnostic criterion for the diagnosis of FH could consequently not be determined.

Xanthelasmata occurred in 14% of the FH cohort, with the sign being found more frequently in women (72% vs.28%). In males the sign was present by the age of 30 years and showed no increase in prevalence after this age, whereas in women there was a steady increase in prevalence throughout life. Xanthelasmata were associated with higher TG, TC and LDLC levels. They were present in 64% of FH Gujarat patients which was higher than in any other genotype. This may reflect a higher background prevalence or other predisposing causes such as greater vascular permeability in this group. In FH Afr 2 xanthelasmata were found more frequently than in FH Afr 1 and appeared at an earlier age. In this genotype there was a marked increase after the age of 50 years. This may be due to the higher LDLC concentration found in FH Afr 2. In both males and females the presence of xanthelasmata was not more common in smokers nor was it associated with increases of angina pectoris or myocardial infarction.

As discussed in chapter 1 this sign is not pathognomonic for FH as up to 50% of people with xanthelasma will be broadly normolipidaemic, but it should nevertheless alert the clinician to the possibility of FH.

7.1.2. Arcus cornealis

Arcus cornealis was found in 32% of FH subjects, present in about 25% by the age of 40 years

and 45% by the age of 50 years. Arcus cornealis occurred more commonly in males and was seen at an earlier age in this gender. Arcus cornealis increased with age in both sexes and was present in more than 80% of patients over the age of 70 years. Arcus cornealis was also more common in smokers and was more commonly found in FH Gujarati subjects than in other genotypes. FH subjects with arcus cornealis had higher TC and LDLC concentrations than those without this sign and their LDL particle size distribution was shifted towards a larger species. The pathogenesis of arcus cornealis is thus more related to LDLC concentration and larger size may lead to larger aggregates that scatter light to produce this sign.

Arcus cornealis is a potentially useful physical sign but has poor sensitivity and low specificity above the age of 50 years. Nevertheless, arcus cornealis in a young person suggests the presence of significant hyperlipidaemia and requires further investigation.

7.1.2.3 Tendon xanthomata

Although this sign may appear to be somewhat subjective and dependent on experience, it remains a valuable physical sign in the diagnosis of FH. This study included subjects in whom genotyping confirmed FH when xanthomata were absent at presentation but FH was suspected by experienced clinicians. This approach might detect most of the incomplete FH phenotype in Afrikaners and Asians but would fail to identify the LDLR mutations in other population groups seen at the clinic. In such groups a milder phenotype may elude diagnosis and this may bias the experience to more severe phenotypes.

Achilles tendons are for practical purposes the best physical sign for diagnosis. Tendon xanthomata were present in 80% of the FH cohort. Only 1% of the patients had tendon xanthomata only in the extensor tendons of the fingers without the presence of Achilles tendon

xanthomata. Although not specifically indicated in the notes, it was the impression of the clinicians that less than 0.1% of the entire cohort had been referred with the documentation of a tendon xanthoma. The utility of this clinical sign should be emphasized at under- and postgraduate teaching level. It is well worth additional studies with ultrasound description of Achilles tendon dimensions for enhancing the detection of the FH phenotype.

In both sexes tendon xanthomata were well established by the age of 40 years, 85% in males in whom the prevalence remained the same in all subsequent decades, and 76% in females, in whom the prevalence continued to increase in succeeding decades to 91% by the age of 70 years. An interesting observation was the rapid rise in the prevalence of tendon xanthomata between the second and third decades, being present in only 12% before the age of 25 and increasing to more than 75% by the age of 40 years. It could be speculated that young men may be predisposed to Achilles xanthoma by greater physical activity, yet a marathon runner had no Achilles xanthomata whilst a disabled individual had gross xanthomata, suggesting that physical activity is not a prerequisite for the formation of xanthomata.

Patients with tendon xanthomata had significantly higher concentrations of TG, TC and LDLC as well as Lp(a), and the HDLC concentration was significantly lower. Although FH patients with tendon xanthomata were found to have a distribution towards smaller LDL particles compared to FH patients without xanthomata on initial analysis, further analysis that matched xanthomatous FH subjects for age with non-xanthomatous FH subjects indicated that LDL species was not significantly associated with xanthomata. Every patient with FH CT2 had an Achilles tendon xanthoma and the finding was also more prevalent in FH Afr 2 than in FH Afr 1. It is suspected that null mutations render a more complete FH phenotype. When correlating the physical sign with clinical findings, it was found less frequently in smokers and patients with Achilles tendon xanthoma had a lower incidence of angina pectoris and myocardial infarction. Tendon xanthoma could lead to earlier diagnosis of FH a consequent reduction of the risk of cardiovascular complications.

7.2 Genotypic findings

Genotyping is not a prerequisite for deciding on the class or dose of medication but offers the possibility of a more accurate diagnosis in subjects with borderline cholesterol values and may be of use in decisions about termination of pregnancy in couples in which both parents have FH. The policy in the clinic is to systematically examine FH candidates for locally prevalent mutations and according to the information in the pedigree. In the Cape Town setting a clinical phenotype would suggest a LDLR defect rather than FDB which accounts for only 1% of patients seen at the clinic.

7.2.1 Results of genotyping

In the GSH Lipid Clinic 46% of the patients with the FH phenotype were genotypically identified. The founder effects in South Africa accounted for the identification of 94% of defects in the Indian population, 61% in the Whites, of whom the majority were Afrikaners, and only 23% in those patients of mixed ancestry. Within Afrikaners at the clinic, 76 % of the FH phenotype could be accounted for by the D206E, V408M and D154N mutations. This confirms previous experience and emphasises the situation of Cape Town as the original portal of entry for the Afrikaner population.

Although the introductory paragraph suggests little benefit to genotyping, other considerations may apply. The Health Scheme Act 131 of 1998 made hyperlipidaemia a treatable condition. The proof of FH genotyping may have the benefit that the Medical Insurance companies will support treatment. The experience with such patients who are members of medical schemes is that a risk calculation is wrongly applied and treatment may not be provided when a clear genetic diagnosis is not supplied. Such genetic information may, however, be a disadvantage when

applying for life insurance.

7.2.2 Genotype comparisons

Specific comparisons of genotypes for the impact of residual LDLR function are of interest although FH in general imparts such marked metabolic changes that the consequences are generally severe. The best cohorts in which defective versus negative status could be examined were FH Afr 1 (defective) and FH Afr 2 (null).

Subjects with FH Afr 2 had a higher TC than FH Afr 1. Eliminating the effect of gender, age and mass, matched pairs in these two genotypes were compared. This revealed that FH Afr 2 had higher TC and LDLC concentrations and significantly lower HDLC levels. FH CT2, another null defect, also had higher TC and LDLC concentrations than FH Afr 1. As TG concentrations are influenced by apoE, and LDLC is lower in normal subjects with the apoE2/2 isoform, quintiles of TG concentrations in FH Afr 1 were examined to determine whether apoE isoforms had a similar impact in FH. However, ApoE isoforms were found to have no influence on the lipoprotein concentrations in FH Afr 1. The small number of subjects with apoE2/2 and FH suggests that the early lipid profile may be ameliorated whereas later physical signs and complications may be aggravated.

When examining differences in clinical complications between genotypes, myocardial infarction occurred at a significantly younger age in FH Afr 2 than in FH Afr 1 in the 40 - 60 year old group. This group was analysed separately as it represents the time frame in which most typical referrals occurred. The increased risk may be as a result of the worse lipid profile in this group and is similar to the experience reported with other null defects. This may support risk stratification by genotyping but the LDLC may already provide insight into the higher risk.

The geographical distribution of the three Afrikaner genes was demonstrated to support the contention that there was a common portal of entry and the original distribution may have been determined by geographical features. The Great Trek later led to a recombination but different distribution between the north and the south. Not only may the region of origin of the patient allow cost-effective determination of the genotype for the region but also careful family history in the rest of the country may allow a better strategy for determining the LDLR defect.

Although the Afrikaners at the clinic have three founder mutations, these mutations have been found in British immigrants as well as in subjects with mixed ancestry. Within the Afrikaner cohort at the clinic, other mutations including FH Lithuania have been documented. FH CT 2 is currently restricted to the mixed ancestry group. Changes in South Africa may result in a greater variety of mutations and complicate strategies for genotyping.

7.3 Lipoproteins

LDL particle size, studied previously in non-FH subjects, is modulated by genetic and environmental factors in such a way that obesity, insulin resistance and metabolic syndrome are correlated with smaller species that are regarded as atherogenic. No systematic evaluation has been reported for FH in which it is generally assumed that large LDL will be present

Factors determining LDL particle size were examined in detail. As published for non-FH subjects, LDL particles become smaller with increasing age. In this study, the change occurred around 38 years in men and around 46 years in women. Large LDL particles decrease in prevalence approximately 3-fold with increasing BMI, with the effect becoming especially noticeable at 28 kg/m^2 in males and females. The particles also become smaller with increasing WHR, with the change occurring around 0.91 in both sexes. In men the increase in the

prevalence of small dense LDL in WHR quintiles was 3-fold and in women there was a striking 8-fold increase. This strikingly different responsivity to WHR may reflect the higher concentration of small LDL in men, possibly by virtue of higher HL activity. With increasing TG concentration there was a marked increase in the prevalence of small dense LDL particles. In women the increase was from 6% in the lowest quintile to 67% in the highest quintile and in males the change was from 3% to 54% across quintiles. Once the TG level had reached 3.8 mmol/L small LDL particles were found in 92% of subjects.

In diabetic patients with FH the prevalence of small dense LDL particles was 2.5-fold higher than in matched non-diabetic FH patients. Diabetic FH subjects also showed the additional changes in the lipoprotein particles that are reported for non-FH subjects. When matched with non-diabetic FH patients, the diabetic cohort had higher BMI values and greater waist/hip ratios. They also had higher TG values and lower HDLC concentrations. These observations are typical of diabetes. Interestingly they displayed lower LDLC concentrations and their prevalence of small LDL particles was higher. When the cardiovascular complications were examined, patients in the diabetic cohort did not experience angina pectoris, myocardial infarction or death any earlier than in the matched non-diabetic FH cohort. In fact, they had their myocardial infarction significantly later than the matched cohort. It is possible that glycated LDL enhances LDL clearance, protecting the subject from additional risk in FH. Alternatively, type 2 diabetes may not have had adequate time to enhance IHD risk in these high risk subjects.

When FH patients with the metabolic syndrome were matched with FH controls, the only significant differences in males were higher TG concentrations and a higher prevalence of small LDL species in the metabolic syndrome group. In females HDLC was also lower in the metabolic syndrome group and the higher prevalence of small LDL also reached statistical significance. When a waist measurement greater than 88 cm in women was compared in FH subjects before and after the age of 50 years (roughly the time of menopause) there was a significant change

to small LDL species in the older age group. In neither males nor females was there an increase in angina pectoris or myocardial infarction with the metabolic syndrome. The apparent lack of additional risk from the metabolic syndrome may also be due to the high back-ground risk of FH and a relatively short time for the metabolic syndrome to have an impact.

The apparent lack of additional cardiovascular risk imposed by the concurrence of either diabetes mellitus and/or the metabolic syndrome in patients with FH was surprising. This finding might merely be the result of such a powerful effect of the FH that the additional risk of diabetes mellitus/metabolic syndrome is obscured. Other possible explanations are discussed in chapter 5.9 and 5.10.

The value of determining LDL size in FH may be that the electrophoretic technique can exclude dysbetalipoproteinaemia and might support the diagnosis of insulin resistance and diabetes. Therapeutic modulation of LDL size in FH has not been evaluated but may be an additional strategy to combat the high risk of coronary artery disease. Apart from reduction of mass and improving the WHR, strategies to modulate the LDL size could also include drugs that reduce TG concentration such as fibrates and niacin.

7.4 Cardiovascular complications

In the FH cohort 43% had ischaemic heart disease, of whom 57% were male subjects. Both angina pectoris and myocardial infarction were more common in males and occurred earlier. (43 years vs. 49 years). These figures for men correlate exactly with those published by Slack in 1969 and are 4 years younger than she found for women, in an era when no effective lipid lowering therapy was available. Sixty eight percent of those developing IHD smoked and a higher percentage of males smoked. Hypertension was present in 24% with a higher percentage

in females. Diabetes was uncommon, occurring in 5% of both males and females.

Stroke was surprisingly uncommon, occurring in only 1.6% of FH subjects, which is in accordance with published data. (see ch 1.6) The prevalence of smoking in those with stroke was 94%, 55% were hypertensive and only 6% were diabetic. TIA and PVD were also uncommon. PVD was found in less than 4% of FH patients, but occurred equally in men and women. In this group there was also a very high prevalence of smoking, especially in women where the prevalence was 95%, and 25% of those with PVD were diabetic. It is clear that smoking increases cardiovascular risk, and especially in FH.

The mean age of death was about 55 years, with men dying about ten years earlier than women. In the experience of the clinic this showed a significant improvement over a period of 7 years during which statin therapy had been administered, even though not at internationally accepted dosages.

7.4.1 Cardiovascular disease in the young versus the older FH subjects

The high risk for cardiovascular disease in FH appears to be affected by lifestyle and/or genetic factors but the impression gained from comparing those who developed IHD before the age of 35 years and those who were beyond the age of 60 years with no manifest IHD was that only smoking and low HDLC and possibly small LDL species were associated with increased risk. The thicker IMT did not predict risk in the younger age cohort, further suggesting that not atheroma per se, but lifestyle, other diseases, genetic factors, haemostatic or tissue properties may affect outcome. It is possible, however, that carotid IMT may not be a good surrogate marker for atheroma and complications of coronary artery disease in FH. Electron beam calcium tomography is not available in South Africa to discern whether coronary atherosclerosis markers

are better predictors of risk.

7.4.2 Carotid IMT in FH

Although the IMT studies involved relatively small numbers of FH subjects, the findings are likely reliable as the protocol for analysis was strictly adhered to by sonographers who had undergone training for reproducibility as part of international studies. The IMT in FH was seen to progress with age at a similar rate as previously reported. Whilst middle-aged FH subjects with and without heart disease had no significantly different IMT readings, they clearly had thicker IMT than non-FH controls. The carotid IMT may be a good surrogate marker for comparing controls to FH or other high risk groups or for studying atheroma, but appears not to be a good marker for coronary atherosclerosis complications in FH.

With further studies it may become clear whether a rate of progression of IMT in a child or adolescent could assist with the initiation or intensity of treatment.

7.5 Management

Statins, though successful in treating IHD in secondary and primary prevention in non-FH subjects, probably make a greater contribution to risk reduction in FH subjects. Whilst the LDLC reduction in FH subjects may be similar to that in non-FH subjects, the high absolute concentration of LDL may make it difficult to achieve ideal concentrations with monotherapy.

In patients attending the Lipid Clinic, FH was shown to respond to dietary counselling with an average LDLC reduction of 1.5 mmol/L. This stresses both the need for patient education and

the need for a dietician in the management of FH. In the near-ideal situation of a drug study, with frequent dietary and drug compliance monitoring, only 32% of a FH study cohort using 80 mg atorvastatin reached the SA national guideline LDLC target of 3 mmol/L. In another study using 40 mg rosuvastatin, similar results (31%) were found. There was no difference in response to statin therapy between the three Afrikaner genotypes.

In patients with FH, combination therapy would be required in at least two thirds of subjects to reach the targets set for ideal treatment. These targets have become even lower after publication of recent studies. (see chapter 6.3.1)

The least that could be done in FH to achieve regression appears to be a reduction in LDLC of approximately 50% or to achieve a LDLC concentration of around 4 mmol/L, according to the ASAP study. (90) However, preceding exposure to high LDLC may place FH subjects at higher risk than peers without FH. This may be offset by less aggressive treatment commencing at a younger age. Future studies are required to evaluate the ideal time for commencement of treatment and the extent to which this treatment should be taken.

7.6 Recommendations

7.6.1 Public awareness

Familial hypercholesterolaemia is markedly under-diagnosed. It occurs in 1/500 in the general population, and as high as 1/72 in the Afrikaners. While screening and diagnosis appear more urgent in these founder populations, the expected prevalence of 1/500 in other population groups also justifies public awareness and detection of this treatable disease which causes significant morbidity and mortality. Heightened public awareness of the disease, especially amongst Blacks, who are in transition to higher risk lifestyles and longer life expectancy should be encouraged.

Public awareness can be raised by public media, radio and television, particularly if real-life cases are involved. The impact that a public figure with the disease can make is immense. The Heart Foundation could play a major role in these strategies. Active screening campaigns in public venues for cardio-vascular risk aimed at moderate dyslipidaemia with hypertension and diabetes will obviously unmask severe hyperlipidaemia. Such identification of significant hypercholesterolaemia is only meaningful if the subject will be correctly evaluated and treated by the medical profession. The identification of subjects with FH and appropriate education, counselling and treatment should reduce the risk of cardiovascular disease in these high risk population groups and the occurrence of homozygous FH can be avoided or anticipated.

A dedicated organisation involving the public (affected persons), medical and healthcare workers could render valuable services. Apart from an involvement in screening, setting up a registry could prove useful. This may assist in the avoidance of the tragic problem of homozygous FH or could make certain that this is diagnosed early and treated appropriately. Support groups, especially for families with hoFH, would provide education, social contact and practical assistance. Legislation protecting the rights of patients against discrimination resulting from the diagnosis of a genetic disorder lessen the ethical concerns about a registry of FH. Such a body may also provide and fund research to improve detection and treatment for those affected, especially hoFH, where the treatment is costly.

7.6.2 Awareness in health professionals

In the medical teaching curriculum at UCT the biochemistry and genetics of FH are adequately covered, but there is limited, if any, exposure to the management of patients in the outpatient

clinic setting. Limited patient access to drugs also detracts from undergraduate and post graduate experience. Continuing medical education and congresses take time but are nevertheless worthwhile avenues to improve the awareness of FH. Although the emphasis should be on clinical diagnosis and treatment, medical practitioners should be aware of the recent advances in genotyping and risk prediction. Utilisation of specialised investigations needs to be appropriate as LDLR mutational analysis will only be cost-effective after thorough clinical assessment including insight into the ancestry that may be the cause of the mutation.

7.6.3 Healthcare

7.6.3.1 Public healthcare

In South Africa the public healthcare system is failing to appreciate the prevalence and severity of FH. Screening should be available at all levels of healthcare and adequate drug therapy should be provided, given the evidence from major studies to support the treatment of hyperlipidaemia (see chapter 1.10) and the fact that the generic statins are now affordable. This is a condition that can be diagnosed and treated at primary health care level, with only complicated problems needing the assistance of a specialist Lipid Clinic.

7.6.3.2 Private healthcare

In the private healthcare sector, Medical Aid Schemes should provide the appropriate treatment demanded by the seriousness of this disease. The medical Schemes Act 131 of 1998 declared dyslipidaemia, including FH, a chronic condition requiring treatment. In the private healthcare sector Medical Aid Schemes have mostly incorporated treatment for general hyperlipidaemia but their processing of patient data has often lead to denial of treatment especially when the doctor

had not made it clear that the patient has a genetic disorder.

The analysis of this FH cohort has shown that the prevalence of IHD is the same in non-genotyped individuals as that in individuals with a genetically proven defect. Treatment should thus be provided on the basis of a clinical diagnosis of FH, without the need for the additional expense of a genetic diagnosis.

7.6.4 The role of a lipid clinic

A Lipid Clinic should be a tertiary referral centre based in a teaching hospital. It should provide expertise and information, investigating and managing complicated patients and researching clinical problems. It should also train and teach at undergraduate and postgraduate level. It should investigate new forms of diagnosis and therapy and research the pathogenesis of atherosclerosis and dyslipoproteinaemia. It should also work in close association with primary and secondary healthcare services to provide appropriate levels of care.

The clinic at the teaching hospital and its link to the University has enabled this review and thus has set up additional studies that may permit the identification of more mutations responsible for FH, genes that influence the course of the disease and test new agents for control of dyslipidaemia and atherosclerosis.

7.7 Future challenges and opportunities

It is clear that the greater proportion of the FH phenotype has not been genetically identified. Other LDLR mutations, other FDB and PCSK9 mutations must account for the clinical phenotype

of the “unknown” genotypes seen at the clinic. It is even possible that newer mechanisms of LDL hypercholesterolaemia may be identified.

The risk factors for IHD - mainly smoking, age, male sex, raised LDLC, low HDLC, increased BMI, WHR and small LDL particle size all contribute to the burden of premature ischaemic heart disease and should be addressed individually and collectively. The complications of FH are still unpredictable for their timing and IMT has not been shown to be a very useful adjunct to predicting cardiovascular disease.

Policies regarding cost-effective treatment should be implemented at regional and national level, with lipid clinics assisting with complex diagnostic and clinical management problems. The treatment of FH has made enormous advances in the past decade. Statins have probably reached the limit of their power with toxic effects shown at very high doses. They remain the treatment of choice with good potential of achieving significant LDLC reduction but a two thirds of FH subjects will not reach the target of 3 mmol/L. Additional lowering may be achieved with combination therapy and the development of new classes of drugs such as torcetrapib and genetic therapies hold hope for the future.

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APPENDIX

Detailed University of Cape Town Lipid Laboratory Methods.

1.	Technique for gradient gel electrophoresis.	1
2.	Parzer's rapid method for extraction of genomic DNA from blood.	3
3.	Heteroduplex assay for LDLR mutations in the 3' portion of exon 4.	5
4.	PCR assay for LDLR mutation R329X (FH Fossum).	10
5.	Exon amplification for heteroduplex analysis R57C and R60C.	12
6.	LDLR Exon 7,8 deletion diagnosis by PCR - FH Cape Town 2	14
7.	Detection of FH Afrikaner 2 and Familial Defective apoB ₁₀₀ - Arms assay.	16
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9.	PCR assay: Exon 14 - FH Gujarat	19
10.	Apolipoprotein E genotyping	20

1. TECHNIQUE FOR GRADIENT GEL ELECTROPHORESIS

This technique allows separation of lipoprotein particles by size. Polyacrylamide mini-gels (6 cm long) are freshly cast. A 30% stock solution of acrylamide with 2.7% bisacrylamide is used to prepare an 8% solution of acrylamide in a buffer containing 13.6 g% Tris and 30% (v/v) glycerol at pH 8.8. A 2% acrylamide solution was prepared in Tris 13.6 g%. Polymerisation was activated in 4 ml of each solution by adding 30 μ l of ammonium persulphate (100 mg/ml) and 1.5 μ l of tetramethylethylenediamine (TEMED).

The activated solutions are placed in the two chambers of a gradient maker with a small magnet in each chamber to permit mixing as the gel was poured from the chamber containing the 8% acrylamide solution. The stacking gel contains 3% acrylamide stock in 1.68 g% Tris at a pH of 6.8. Once the separation gel has set, the stacking gel solution is polymerised by adding 100 μ l of ammonium persulphate and 4.0 μ l of TEMED. Using this solution and a comb 15 lanes are prepared for electrophoresis

. Fasted EDTA- plasma samples are pre-stained for lipids with Sudan Black using a similar method to Gambert *et al*. Briefly, 100 μ l of plasma is incubated with 50 μ l of lipid stain (1 % w/v Sudan Black in ethylene glycol) for an hour at 4°C and then spun for 20 min at 10000G. Equal volumes of supernatant and saturated sucrose are mixed and 12 μ l of the resultant solution is loaded per well.

Gels were run for 17 hours at 120 V at 4° C in a Tris Glycine (pH 8.3) buffer utilising the BioRad Minigel apparatus in a refrigerator. The gels are subsequently analysed without any further processing

The gels are visually inspected in a blinded fashion and reports use a simple terminology that avoids inferring lipoprotein separation by density. The bands corresponding to LDL species usually appear sharp and well-defined and migrate to the distal fifth of the gel (anodal end). Migration of lipoproteins on successive gels are controlled relative to two control lanes, in which previously selected plasma with a known large (A) and small (B) LDL species are run.

Since the gel was developed to look at "LDL", the zone between the origin and the LDL is referred to as "mid" and the staining in this region is referred to as M, which later modified to M-early or M-late depending on whether the particles were larger or smaller. Subsequently it became clear that chylomicron-like particles tended to remain at the origin of the separation gel, and hence were termed O. At the "LDL" range, the largest particle is designated "A", the intermediate band "I" and the smallest (densest) LDL band "B". Subsequently it became clear that even further size distinctions can be made, now abbreviated to A-early, A/I, I/B and B-post. Sometimes a band between A and M-late

can be made out and was then referred to as pre-A.

(Illustration from chapter 5.3)

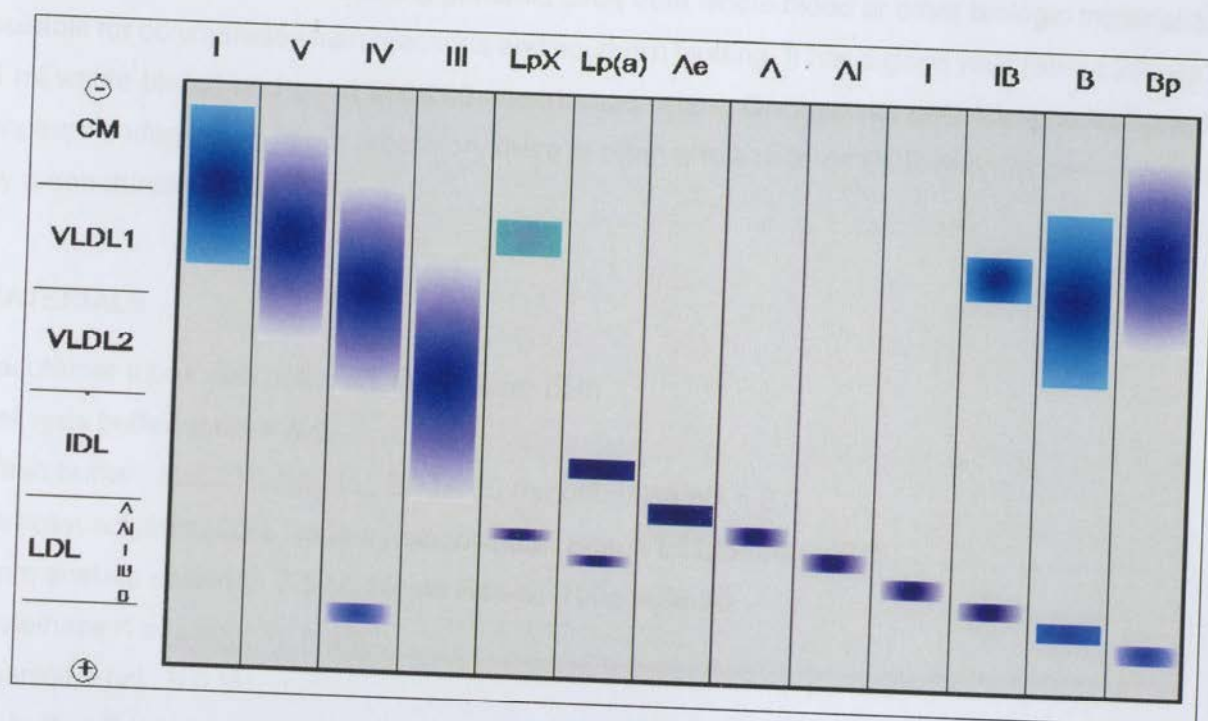


Figure 5.3.1 Schematic illustration of a gradient gel electrophoretogram

The calibration by ultracentrifugally prepared lipoproteins is presented on the left.

CM	Chylomicrons	1	Type 1
VLDL1	Very low density lipoprotein 1	2	Type 2
VLDL 2	Very low density lipoprotein 2	3	Type 3
IDL	Intermediate density lipoproteins	4	Type 4
LDL	Low density lipoproteins		
LpX	Lipoprotein X	Ae	A early
Lp(a)	Lipoprotein (a)	Bp	B post

REFERENCE

Blom DJ, Byrnes P, Jones S, Marais AD. Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia. *Journal of Lipid Research* 2003; 44(1):212-217.

2. PARZER'S RAPID METHOD FOR EXTRACTION OF GENOMIC DNA FROM BLOOD

GENERAL

This rapid method is for extracting genomic DNA from whole blood or other biologic material and is suitable for polymerase chain reactions and southern blotting. It has a good yield (about 250 μ g from 5 ml whole blood) and good 260/280 absorbance ratios. On agarose gel electrophoresis the DNA appears undegraded. In our laboratory there is often a failure to do PCR and this can be corrected by a denaturation step.

MATERIALS

Vacutainer tubes with anticoagulant. Water bath.

Cell lysis buffer at pH = 8.0.

Wash buffer: NaCl 10 mmol/L, EDTA 10 mmol/L, also pH = 8.0.

Sarkosyl solution: 20% Na laurylsarcosinate. Sigma L5125. 50g = \$10.

Amm acetate solution: 7.5 M. Sigma A1542. 100g = \$8.50.

Proteinase K solution: 10 mg/ml.

Guanidine hcl: 6.0 M.

TE buffer: Tris HCL 10 mmol/L and EDTA 1 mmol/L at pH = 8.0.

Ice cold ethanol.

PROCEDURE

The directions are for 5 ml frozen blood. Thaw rapidly at 37°C and mix with 50 ml cell lysis buffer. Incubate for 15 min. on ice before centrifuging at 6800G for 10 min. at 4°C. Decant the supernatent.

Thoroughly resuspend the pellet in wash buffer. Spin at 6300G for 10 min. at 4°C. Siphon off the supernatent.

To the almost white pellet add the following in the order stipulated -

Sarkosyl soln 350 μ l

Amm acetate soln 250 μ l

Guanidine soln 3.5 ml

125 μ l proteinase K.

Incubate at 60°C until clear (10-15 mins).

Cool to 0°C.

Precipitate the DNA by adding 10 ml ice cold ethanol. The DNA is spooled off with a hooked glass rod. Gently wipe off the excess alcohol on the rod.

Dissolve the DNA in TE buffer for 1 hr at 37°C. The material is usually incompletely soluble but can be clarified by a brief centrifugation (15 secs) to precipitate the insoluble material. Store at - 20°C.

DENATURATION

To 10 µl of the DNA in TE buffer, add 10 µl of 0.1M NaOH. Boil in waterbath or heating block for 10 min. Snap cool on ice. Add 2.5 µl of 1M Tris at pH 8.0.

REFERENCE

Parzer S, Mannholter S. Biochem J 1991;273:229-231. A rapid method for the isolation of genomic DNA from citrated whole blood.

3. HETERODUPLEX ASSAY FOR LDLR MUTATIONS IN THE 3' PORTION OF EXON 4

INTRODUCTION

This method was introduced to screen for several mutations in the 3' end of exon 4 in the LDL receptor gene since 20 Jul 99. The mutations are: FH Afr3 (D154N), FH Lithuania (delG197), FH Padova (D200G) G), GSH4 (D203A), FH Afr1 (D206E), French Canadian 3 (E207K). All of these are added as controls. Multiple loadings of the gel after intervals permit several runs of PCR to be analysed on the same gel. This method is best analysed over a UV light with a Polaroid picture than by exposure in the gel documentation apparatus.

PROCEDURE

Conditions for PCR

Primer G102: 5'- CGA GGC CTC CTG CCC GGT GCT CAC C-3' (25mer)
 Primer G103: 5'- GGG ACC CAG GGA CAG GTG ATA GGA C-3' (25mer)

	Stock concentration	Volume	Final concentration
Water		32.3 µl	
Buffer	10x	5.0 µl	1x
dNTP	2 mmol/L	2.5 µl	100 µmol/L
OligoG102	10 pmol/µl	3.0 µl	30 pmol/assay
OligoG103	10 pmol/µl	3.0 µl	30 pmol/assay
MgCl ₂	25 mmol/L	2.0 µl	1mmol/L
Taq polymerase	5 U/µl	0.2 µl	1U/ assay
DNA		2.0 µl	

Programme: 10: 95°C for 35 sec, 68°C for 1 min, 72°C for 1 min for 30 cycles.
 Extension 72°C for 10 min.

10% Acrylamide gel with 1% crosslinks

9 g urea in 41.4 ml of water

15 ml acrylamide (40% stock solution: 80 g acrylamide & 0.8 g bisacrylamide in 200ml water)

3.6 ml 10X TBE (final concentration 0.6X)

0.4ml 10% ammonium persulphate

40 µl TEMED

Running buffer 1X TBE: Tris, borate, EDTA.

Formamide loading buffer.

Assemble the large gel plates. Pour the gel made as above, consuming practically all of the 60 ml but leaving a space for inserting the 15 well comb. Allow to polymerise for 40 min. Wash out the urea from the wells with syringe and running buffer, very carefully so as not to dislodge the wells.

Load 9 µl of product with 3 µl of formamide loading buffer into the well. Run at 350V for 20 min. Wash wells again before loading the second lot of samples and controls. Run at 350V for another 20 min and repeat for the 3rd load of samples and controls. Run at 190-200V overnight for 16-17h until the xylene cyanol dye (turquoise) nears the lower end of the gel. Stain with ethidium bromide and examine under UV light. Photograph with polaroid camera.

The hetero duplex formation and small deletions allow detection of the mutations outline above. Note that the distance between the heteroduplex bands can aid in the identification of the different mutations but specific restriction patterns must still be used for proper identification of the mutations.

Confirmation of FH Afrikaner 1

Cut 20 µl of product with 1 µl dil Dde1 (diluted 1+ 1,5 µl H₂O) at 37°C overnight. Spin. Add 3 µl sucrose loading buffer. Load on 12% acrylamide gel. Stain with ethidium bromide.

Dde1 cuts at C↓TNAG. Normals have 1 cutting site giving 2 fragments 197 and 136, HeFH 197, 136, 72, and 64, and HoFH 197 and 64 and 72.

Confirmation of FH Afrikaner 3

Cut 10 µl of product with 3 units Mbo11 (0.5 µl stock + 3.5 µl H₂O) @ 37°C overnight. Spin. Add 2µl sucrose loading buffer and load on 6% acrylamide gel. Stain with ethidium bromide.

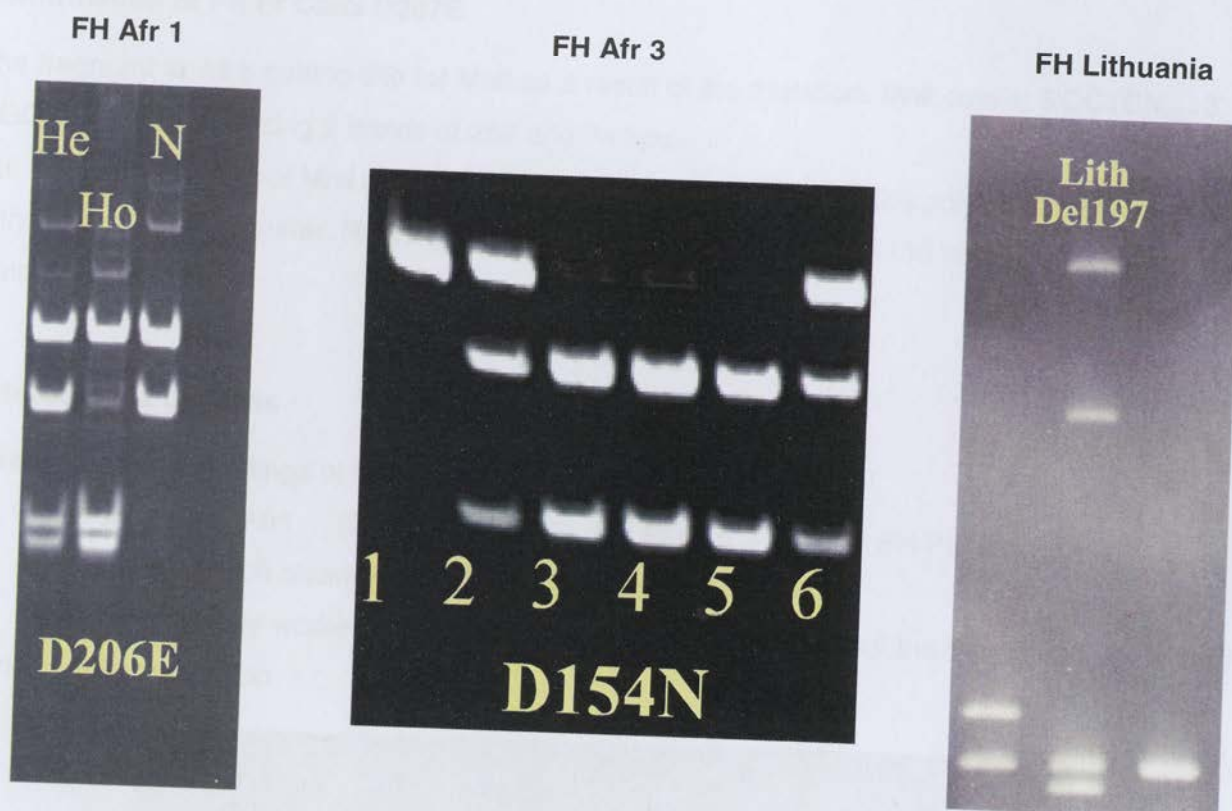
The mutation loses the only cutting site. HoFH does not cut and HeFH has bands 333, 217 and 116 and normal cuts into 2 bands of 217 and 116 bp.

Confirmation of FH LITHUANIA

The Lithuanian deletion is clearly seen on the long gel as a deletion and hetero duplexes of both bands are formed.

Figures below:

Left panel shows PCR result with DdeI for mutation D206E (AfrFH1) with fragments for heterozygote, homozygote and normal subjects (24Oct01). Middle panel demonstrates cut fragments with MboII : lane 1 uncut, lane 2 & 6 heterozygotes, and normal in lanes 3 to 5. The panel on the right shows the full pattern on hetero duplex for the Lithuanian mutation.



Confirmation of FH Padova D200G

The mutation creates a cutting site with MspI at 5'C↓CGG3'. In the normal product there will be no cutting whereas the mutated fragment will have 3 bands, 1 normal and 2 restriction products of 333, 243 and 90 bp respectively.

Cut 20 µl of the PCR product with 1 µl of MspI (10U of Boehringer supply) at 37°C overnight. Load into a well of a 12% polyacrylamide gel together with sucrose loading buffer. Running buffer is 1X TBE. Voltage at 100-110 for about 3h. Remove and stain with ethidium.

Confirmation of FH GSH4 D203A

The fragment gains an extra cutting site for *HaeIII*, which cut 5' GG↓CC3'. Normal bands are 93, 80, 61, 55 and 44 nucleotides. The heterozygote will display the normal sizes as well as an additional band but the mutation allows the 93 band to be cut into 79 and 14 nucleotides.

Cut 20 μ l of product with 3 units of *HaeIII* at 37°C overnight. Load into a well of a 12% polyacrylamide gel together with sucrose loading buffer. Running buffer is 1X TBE. Voltage at 100-110 for about 3h. Remove and stain with ethidium.

Confirmation of FH Fr Can3 D207E

The fragment loses a cutting site for *MnII* as a result of the mutation. *MnII* cuts at 5'CCTCN₍₇₎↓3' and 3'GGAGN₍₆₎↑5' producing 2 bands of 259 and 74 bps.

Cut 10 μ l with 1 μ l (2U) of *MnII* at 37°C overnight. Load into a well of a 12% polyacrylamide gel together with sucrose loading buffer. Running buffer is 1X TBE. Voltage at 100-110 for about 3h. Remove and stain with ethidium.

Heteroduplex patterns

Three successive loadings of PCR product for hetero duplex analysis.

- (1) Controls for FH Afr1 (2) FH Afr3 (3) FH Fr Can 3 (4) FH Padova
- (5) FH Lithuania which clearly shows 4 bands (6) FH GSH4.
- (7) is an incompletely worked up sample displaying equal intensity of the bands and suspected of being an 18 bp insertion.



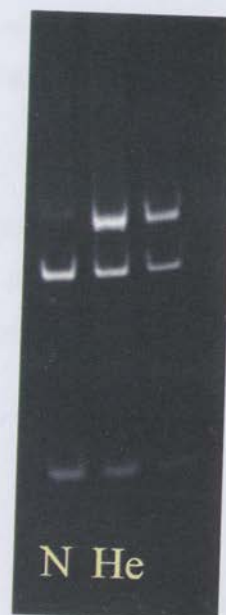
FH Padova



FH GSH 4



FH Fr Can 3



REFERENCE

Rubinsztein DC, van der Westhuyzen DR, Coetzee GA. S Afr Med J 1994;84:339-344. Monogenic primary hypercholesterolaemia in South Africa.

4. PCR ASSAY FOR LDLR MUTATION R329X IN EXON 7 (FH Fossum).

INTRODUCTION

The R329X mutation in the LDLR was previously described and subsequently identified in Afrikaner families by Kotze at the University of Stellenbosch and later independently at the University of Cape Town (through Academisch Medisch Centrum, Amsterdam). The mutation is in exon 7. It may have taken origin in Scandinavia. (Solberg et al). The assay was devised from an exon 7 amplification and the known cutting sites for BsrI.

The assay involves producing a fragment of 234bp with 1 normal cutting site with the restriction enzyme BsrI: 2 bands 204 and 30bp. BsrI cuts at 5'-ACT GGN↓...-3' or 3' TGA C↓CN...-5'. The mutation creates a second cutting site, yielding fragments of 130, 74 and 30bp. The heterozygote will thus display 4 bands (204, 130, 74, 30bp).

Primers G134: 5'- GGC GAA GGG ATG GGT AGG GG-3' (20mer)

Primers G135: 5'- GTT GCC ATG TCA GGA AGC GC-3' (20mer)

PROCEDURE

PCR

	Stock concentration	Volume	Final concentration
Water		35.3 µl	
Buffer	10x	5.0 µl	1x
dNTP	2 mmol/L	2.5 µl	100 µmol/L
Oligo G134	20 pmol/µl	1 µl	20 pmol/assay
Oligo G135	20 pmol/µl	1 µl	20 pmol/assay
MgCl ₂	25 mmol/L	3 µl	1.5 mmol/L
Taq polymerase	5 U/µl	0.2 µl	1U in assay
DNA		2 µl	

Program 1: 94°C for 1 min, 60°C for 1 min, 74°C for 1 min, 35 cycles.
74°C for 10 min extra.

Restriction conditions: BsrI is incubated at 65°C overnight, using 1 µL (5 units) per 20 µL of PCR product.

GEL

The small 12% polyacrylamide gel is prepared in TBE. Of the PCR product, load the restriction preparation in the well with sucrose loading buffer. Run the gel at 100-105 mV over approximately 4h until the bromophenol blue is near the edge. Stain with ethidium and view under UV illumination. Document the image on the Geldoc.

INTERPRETATION

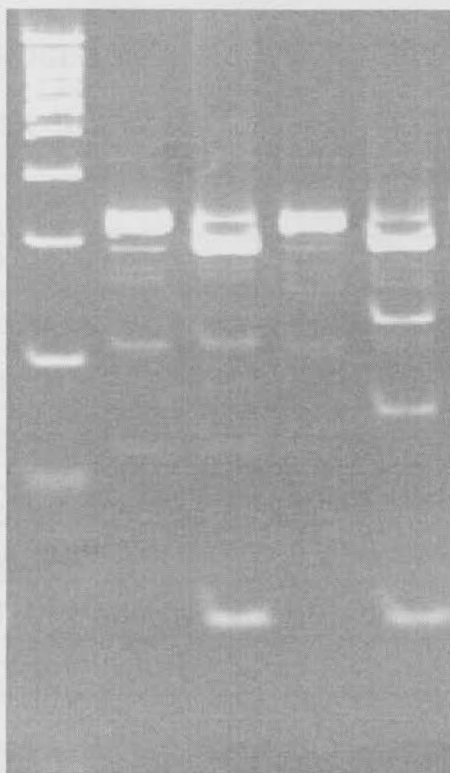


Figure 1. PAGE of BsrI restriction enzyme digest of PCR fragment amplified for mutation R329X in exon 7 of the LDLR as above.

Lane 1 MW markers

Lane 2 Uncut normal (234 bp)

Lane 3 Cut normal (234, 204, 30 bp)

Lane 4 Uncut abnormal

Lane 5 Cut abnormal (234, 204, 130, 74, 30 bp)

REFERENCES

1. Primers (FH 118 and FH 138) Leitersdorf et al. JCI. 1990; 85:1014
2. Solberg K, Rodningen OK, Tonstad S, Ose L, Leren TP. Familial hypercholesterolaemia caused by a nonsense mutation in codon 329 of the LDL receptor gene. Scand J Clin Lab Invest 1994;54:605-609.

5. EXON 3 AMPLIFICATION FOR HETERO DUPLEX ANALYSIS (R57C, R60C)

INTRODUCTION

The mutations R57C and R60C were discovered in patients with familial hypercholesterolaemia at the lipid clinic of Groote Schuur Hospital and were named FH GSH1 and GSH2 respectively. Other mutations may also be discovered in this region of the LDLR gene as it is part of the binding domain. Originally the work-up was done in collaboration with Dr M J Kotze at Human Genetics based in Obstetrics and Gynaecology at the University of Stellenbosch.

PROCEDURE

PCR Conditions

	Stock concentration	Volume	Final concentration
Water		17.2 μ l	
Buffer	10X	2.5 μ l	1X
dNTP	2 mmol/L	1.25 μ l	100 μ mol/L
OligoG55 (1/10)	12 pmol/ μ l	1 μ l	12 pmol/assay
OligoG54 (1/10)	13.5 pmol/ μ l	1 μ l	13.5 pmol/assay
MgCl ₂	25 mmol/L	1 μ l	1 mmol/L
Taq Polymerase	5U/ μ l	0.1 μ l	0.2 U/assay
DNA		1 to 2 μ l (denatured)	

Program 5: 95°C for 5min 1 cycle, (93°C for 1min, 58°C for 1min, 72°C for 2min) for 35 cycles, 72°C for 10min for 1 cycle.

Oligo G55: 5'- CTG TCT CTT CTG TAG TGT CT- 3' (20mer)

Oligo G54: 5'- ACT TAC GAC AGC CTT GCT CG-3' (20mer)

Gel Conditions (10% Acrylamide gel with 1% crosslinks)

9g urea in 41.4 ml of water

15 ml acrylamide (40% stock solution: 80g acrylamide & 0.8g bisacrylamide in 200 ml water)

3.6 ml 10X TBE (final concentration 0.6X)

0.4 ml 10% ammonium persulphate

40 μ l TEMED

Running buffer 1X TBE: Tris, borate, EDTA.

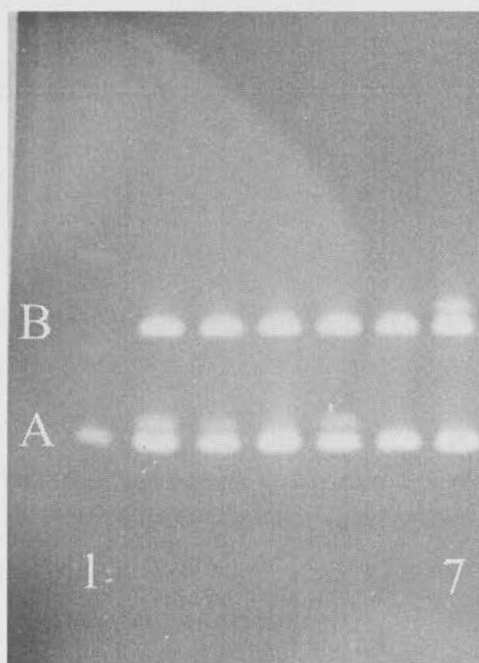
Formamide loading buffer

Assemble the large gel plates. Pour the gel made as above, consuming practically all of the 60 ml but leaving a space for inserting the 15 well comb. Allow to polymerise for 40 min. Wash out the urea from the wells with syringe and running buffer, very carefully so as not to dislodge the wells.

Load 10 μ l of product with 3 μ l of formamide loading buffer into the well. Run at 350V for 20min. Wash wells again before loading the second lot of samples and controls. Run at 350V for another 20 min and repeat for the 3rd load of samples and controls. Run at 140-150V overnight for 16-17h until the xylene cyanol dye (turquoise) nears the lower end of the gel by approx 2.5 cm. Stain with ethidium bromide and examine under UV light. Photograph with polaroid camera.

Confirm R60C mutation by restriction enzyme digest, using *Acil*. The R57C mutation was described by sequencing at Tygerberg Hospital.

Figure 1. Heteroduplex assay for R57C and R60C mutations in exon 3 of the LDL receptor gene. Row A was loaded first followed by row B as described in the text. Result A1 reflects the normal pattern whilst A3 was the R60C heterozygote control and B7 was the R57C control.



6. LDLR EXON 7,8 DELETION DIAGNOSIS BY PCR - FH Cape Town 2

GENERAL

Exon 7 and 8 deletions have been described as the cause of familial hypercholesterolaemia (FH) from the Netherlands and South Africa. In the former case there is a 4 kb deletion and in the latter there is a 2.5 kb deletion. The South African deletion appears to be particularly prevalent in the mixed ancestry (coloured) group in which we have 2 unrelated families with homozygous FH children and several unrelated heterozygotes have been found.

This method supercedes the Southern blot method originally described for the lesion. The PCR amplification is of a 6.5 kb fragment while the 2.5kb deletion renders a 4kb fragment. The amplification is from 5' of exon 6 to the middle of exon 9.

MATERIALS

Extracted DNA 1µl.

Primer B2 (G127): 5' - CTCTggCTCTCACAgTgACACTCTgCgAgg-3' (exon 6)

Primer A3 (G126): 5' - gATTCTATTgCTggCCACCTCCgTgTCCAg-3' (exon 9)

Use Expand Long Template PCR system - Boeringer.

Mastermix 1 Use 1.75 µl of each of the 10 mmol dATP, dCTP, dGTP, dTTP solutions. Combine with 50 pmol/assay A3 (0.3µl) and 50 pmol/assay B2. Bring to 24 µl by adding 16.3 µl of water.

Mastermix 2 Buffer 1 as 10X concentrate, 5µl and enzyme mix 0.75 µl and water 19.25 µl to make 25 µl.

PROCEDURE

Prepare mastermix 1 and 2 on ice. Pipette 24 µl master mix 1 into an eppendorf tube. Add 1 µl DNA. Add 25 µl master mix 2 - mix well. Overlay with 2 drops oil. Start therm cycling immediately.

Program 21:

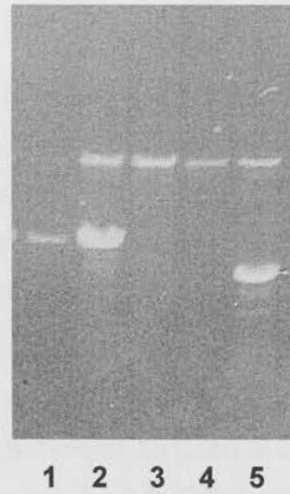
1 cycle	-	94°C for 2 mins	
10 cycles	-	94°C for 10 secs	(denaturing)
		57°C for 30 secs	(annealing)
		68°C for 8 mins	(elongation)
20 cycles	-	94°C for 10 secs	
		57°C for 30 secs	
		68°C for 8 min + 20 secs each cycle	(extended elongation)
1 cycle	-	68°C for 7 mins	(prolonged elongation)

Run 10 µl product on a 0.7% LE agarose gel with ethidium bromide in gel and buffer.

Figure 6.1

Reading the lanes from left to right -

1. Homozygous for 2.5kb deletion
2. Heterzygous for 2.5kb deletion
3. Negative for 2.5kb deletion
4. Negative for 2.5kb deletion
5. Estimated at 3 kb deletion



REFERENCE

MJ Kotze. 1997. Personal communication.

7. DETECTION OF FH AFRIKANER-2 AND FAMILIAL DEFECTIVE APO B-100 ARMS ASSAY (Amplification Refractory Mutation System)

GENERAL

This assay determines whether the DNA contains the exon 9 mutation V408M (Afrikaner FH2) in the LDL receptor, and/or whether there is binding defective B₁₀₀ from the mutation R3500W or R3500Q, and possibly R3531C. The Afrikaner 2 mutation constitutes about 20% of FH in Afrikaners and confers a biochemically worse disease, while familial binding defective B₁₀₀ is responsible for about 1% of the xanthomatous hypercholesterolaemia phenotype at the UCT Lipid Clinic.

The ARMS PCR method is used to detect the FH Afrikaner-2 mutation while the same tube also contains oligonucleotides that amplify the apo B gene which serves as an internal control for the PCR. Additionally, a normal allele can be amplified as indicated below for 9.6 (G87) instead of the mutant primer 9.7 (G88) to differentiate a homozygote from a heterozygote.

The five oligos used in this assay are

AB-1 (G84)	24mer :	5'- GGAGCAGTTGACCACAAGCTTAGC-3'
AB-2 (G85)	24mer :	5'- CAGGGTGGCTTTGCTTGTATGTTC-3'
9.5 (G86)	25mer :	5'- GCTCACCTGCAGATCATTCTCTGGG-3'
9.7 (G88)	25mer :	5'- AGCCTCATCCCCAACCTGAGGACCA-3'
9.6 (G87)	25mer:	5'- AGCCTCATCCCCAACCTGAGGACCG-3'

PROCEDURE

	Stock concentration	Volume	Final concentration
dd water		38.0 µl	
Buffer with MgCl ₂	10x	5.0 µl	1.5 mmol/L
dNTPs	2 mmol/L	5.0 µl	200 µmol/L
AB - 1	50 pmol/µl	0.1 µl	5 pmol/assay
AB - 2	50 pmol/µl	0.1 µl	5 pmol/assay
9.5 (distal primer)	50 pmol/µl	0.2 µl	10 pmol/assay
9.7 (mutant primer)	25 pmol/µl	0.4 µl	10 pmol/assay
Taq polymerase		0.2 µl	1 unit
DNA (denatured)		1.0 µl	

Run positive controls. Overlay with 2 drops of mineral oil. PCR cycles: 30 cycles 95°C for 35 secs, 60°C for 1 min, 72°C for 1 min. 1 cycle: 72°C for 10 mins. (Programme 14)

FH Afrikaner 2

Run 20 μ l of product (plus 3 μ l sucrose loading buffer) on a 12% acrylamide gel.

Only DNA with a mutant allele is amplified using the deliberately destabilised primer 9.7. Homozygosity or heterozygosity for this mutation can be confirmed by PCR with the common distal primer (9.5) and the normal allele specific primer (9.6). Product is formed in the heterozygote and not in the homozygote.

The apoB primers amplify a 345 bp product which acts as an internal control for the PCR in the absence of product for the normal allele of exon 9 of the LDL receptor.

Familial Binding Defective B₁₀₀

10 μ l of PCR product (plus 2 μ l sequencing loading buffer) is analysed on a long denaturing 10% (1% crosslinked) acrylamide gel.

Heteroduplex gel

9 g urea dissolved in 41.4 mls water

15 ml stock acrylamide

3.6 ml 10x TBE (0.6x)

0.4 ml 10% amps

40 μ l TEMED

40% stock acrylamide

80g acrylamide

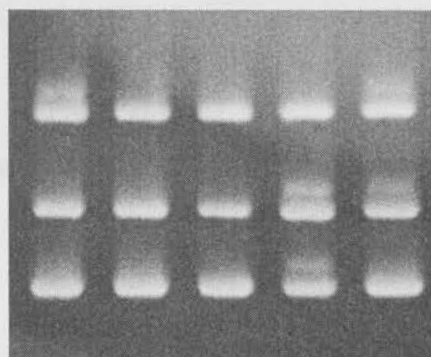
0.8g BIS in 200ml water

The gel is run at 350V between multiple loadings of PCR product and 200V overnight until the xylene cyanol band reaches the bottom of the gel. Stain with ethidium bromide and document the findings by a polaroid photograph, colour slide or Geldoc instrument.

Heteroduplex formation between normal and mutant alleles allow detection of mutations. The size of the heteroduplex can discriminate between the R3500W and R3500Q mutations.

Figure 1.

Hetero duplex formation of PCR product in assay for familialbinding defective apoB in 3 successive loadings of the gel. Double bands indicate the heteroduplex at position 4 in the first row, positions 4 and 5 in the middle row and positions 1 and 5 the top row.



REFERENCE

Kotze MJ, Theart L, Kallis M, Peeters AAV, Thiart R, Langenhoven E. Non-radioactive multiplex PCR screening strategy for the simultaneous detection of multiple low density lipoprotein receptor gene mutations. PCR Methods Appl 1995;4:352-356.

8. PCR ASSAY FOR 5' - EXON 4 MUTATION

INTRODUCTION

A mutation was found in a FH family at the Groote Schuur Hospital Lipid Clinic and was termed FH GSH3. The mutation is a 2bp deletion T2951 and C2952, resulting in a frameshift.

PROCEDURE

PRIMERS G106 20mer: 5'- GTGGTCTCGGCCATCCATCC-3'
 G 51 20mer: 5'- GGGGTCGTTGTCTGCAGGCCC-3'

CONDITIONS

	Stock concentration	Volume	Final concentration
Water		37.5 µl	
Buffer	10x	5.0 µl	
dNTP	2 mmol/L	2.5 µl	
G106 (H4A Kotze)		0.36 µl	5 pmol
G51 (4.3 Kotze)		0.42 µl	5 pmol
MgCl ₂	25 mmol/L	3.0 µl	1.5
Taq Polymerase	5 U/µl	0.2 µl	1U
DNA		1.0 µl	0.15 µg

Program 15: 1 cycle 95°C for 1 min, 10 cycles 95°C for 5 sec and 64°C for 30 sec and 72°C for 30 sec, 30 cycles at 95°C for 5 sec and 63°C for 30 sec, 72°C for 30 sec and 30°C for 10 min.

Run 15µl of product on a 8% polyacrylamide gel and the deletion can be seen.

REFERENCE

1. Primers Personal communication MJ Kotze.
2. Loubser O, Marais AD, Kotze MJ, Godenir N, Thiart R, Scholtz CL, de Villiers JNP, Hillermann R, Firth JC, Weich HFH, Maritz FJ, Jones S, van der Westhuyzen DR. Founder mutations in the LDL receptor gene provide evidence for Caucasoid admixture and contribute significantly to the familial hypercholesterolemia phenotype in an indigenous South African population of mixed ancestry. Clin Genet 1999;55:3340-345.

9. PCR ASSAY : EXON 14 (FH GUJERAT)

INTRODUCTION

The mutation P664L has been described from several regions of the world, including Norway and Zambia. The Zambian epithet came from investigating an immigrant Gujarati Indian family in Britain. Subsequently it has become known as FH Gujarati. It has been found frequently in South African Indians hailing originally from Gujarati and mainly confined to the Muslim faith. But one family of mixed ancestry has the mutation. (The Lebanese FH mutation C660X (C to A at 2043) can also be detected in this assay but conditions need to be optimised.)

PROCEDURE

PRIMERS : G29 26mer: 5'- AAAGAATTCAGTGCCAACCGCCTCAC-3'
G42 27mer: 5'- TATGTCCAGAAACAAGGCGTGTGCCAC-3'

CONDITIONS

	Stocks concentration	Volume	Final concentration
Water		35.3 µl	
Buffer	10x	5 µl	1x
dNTP	2 mmol/L	2.5 µl	100 µmol/L
OligoG29	20 pmol/µL	1 µl	20 pmol/assay
OligoG42	20 pmol/µL	1 µl	20 pmol/assay
MgCl ₂	25 mmol/L	3 µl	1.5 mmol/L
Promega Taq	5 U/µl	0.2 µl	1U
DNA		2 µl	0.3 µg

Program 4: 35 Cycles at 95°C for 35 secs and 60°C 3 mins, 1 cycle 95°C for 35 secs, 60°C for 6 min.

Mutation will form heteroduplex when run on a 1% cross-linked heteroduplex gel. Confirm the mutation with restriction enzyme digest. Cut 20µL of product with 1µL (10U) of PstI at 37°C overnight. Run on 12% acrylamide gel. Stain with ethidium bromide. This forms a 426bp fragment which is cut into 314 and 112bp.

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10. APOLIPOPROTEIN E GENOTYPING

GENERAL

Apolipoprotein E (apoE) is synthesised in the liver and monocytes and macrophages. It can transfer between lipoproteins and is found chiefly on HDL and VLDL. It seems to serve as a ligand for the LDL receptor on triglyceride-rich lipoproteins and their remnants. There are three naturally occurring isoforms, named according to their charges at isoelectric focussing: E2, E3, E4. Under physiologic conditions, apoE explains about 10% of the variance plasma cholesterol concentrations with ascending concentrations from apoE2 to E3 to E4 even though apoE2 is a poor ligand for the LDLR. The receptor-binding domain is between amino acids 128 and 148 of the 299 amino acids. This area is highly charged with arg and lys residues, and in the tertiary structure of the molecule arg158 loops back to provide an extra charge adjacent to this charged area of the molecule. Relative to apoE3 (cys112, arg158), apoE2 is cys112 and cys158 while E4 is arg112 and arg158. ApoE4 is a risk factor for Alzheimer's disease.

Under conditions that either increase the production of triglyceride-rich lipoproteins or decrease the clearance of triglyceride-rich lipoproteins, apoE becomes pivotal in lipoprotein metabolism. Poor apoE function will lead to the accumulation of remnants, or cholesterol-rich VLDL. This is type III hyperlipidaemia, a very atherogenic disorder which tends to set in during the 4th decade for men and 5th decade for women. The commonest setting for type III hyperlipidaemia is the concurrence of apoE2 homozygosity (about 1% of the population) with one of the types of stresses outlined above. In most instances thus, apoE2 is permissive for type III while not being wholly causal and the frequency of the disorder is about 0.01% of the population. Clearly absence of apoE and truncations of apoE may do the same. There are mutations in apoE that can cause autosomal dominant type III hyperlipidaemia, and all seem to occur in the ligand binding domain. While most seem to be point mutations, there is a notable insertion of a 7aa repeat in apoE Leiden.

Having confirmed the diagnosis of type III by ultracentrifugation, it is thus of interest to proceed to genotyping. Should the subject not be homozygous for apoE2, the possibility of an unusual mutation in apoE should be investigated. The genotyping is done by amplifying the segment spanning from aa 92 to 176 and then restricting it with HhaI which will allow discrimination of the 3 isoforms by its cutting action or lack thereof at positions 112 and 158. At the UCT Lipid Clinic there is a remarkable prevalence of the E145arg-cys mutation which can be detected by an Fnu4HI, Ital or Bbv restriction analysis. Once this mutation has been excluded, it is worth checking the uncut fragment size for significant deletions or insertions, and thereafter the fragment should be sequenced.

PROCEDURE FOR GENOTYPING E2,3,4

	Stock concentration	Volume	Volume
		Boehringer	Promega
Water		32.8 µl	29.8 µl
Buffer	10X	5.0 µl	5.0 µl
dNTP	2 mmol/L	2.5 µl (100 µmol/L)	2.5 µl
G23	20 µmol/L	2.5 µl (1 µmol/L)	2.5 µl
G24	20 µmol/L	2.5 µl (1 µmol/L)	2.5 µl
DMSO		2.5 µl (5%)	0.2 µl
Taq polymerase	5 units/µl	0.2 µl (1 unit)	0.2 µl
DNA (denatured)		2.0 µl	2.0 µl
MgCl ₂	25 mmol/L	0	3.0 µl (final 1.mmol/L)

Note that the Boehringer buffer contains Mg while the Promega buffer does not and thus needs Mg supplement in assay. Run positive controls of apo E2, E3 and E4 homozygotes.

Overlay with 2 drops of mineral oil. PCR cycles: 1 cycle 95 for 5mins, for 35 cycles: 94.5 for 45 secs, 60 for 1 min.

Hha 1 digestion

Run 10µl of PCR product on 1% agarose gel to check whether PCR worked.

Transfer 30 ul of product to Eppendorf tube.

Add 1µl (10 units) Promega Hha 1 enzyme to tube and incubate in 37°C waterbath overnight.

Spin in microfuge 2 secs and add 4 uls sucrose loading buffer.

Run on 12% polyacrylamide gel.

Stain with ethidium bromide for 10 mins. Photograph with Polaroid camera at on UV viewing box or record in GelDoc UV mode. A colour slide can also be taken with a 50mm lens and 1/60 second.

The nucleotide substitutions that result in arg-cys interchanges at positions 112 and 158 also alter Hha 1 cleavage sites. Each genotype can be distinguished by unique combinations of Hha 1 fragment sizes in all homozygous and heterozygous combinations. The 7 aa repeated by insertion of 122-129 at 122 in apoE yields a larger fragment than normal.

	2/2	3/3	4/4	2/3	2/4	3/4
91	—	—		—	—	—
83	—			—	—	
72			—		—	—
48		—	—	—	—	—
35		—	—	—	—	—

PROCEDURE FOR APOE R145C

The enzyme FnuH1 and isoschizomer Ital both cut at GCNGC , yielding from 5' to 3', fragments of 25,7,40,3,18,40,33,9 and 69bases. In the R145C the fragments are 25,7,40,3,18,42,69bp. BbvI cuts at GCAGC yielding from 5' to 3' fragments 25,108,33,78 bases in the wild type and in R145C the fragments are 25,108,111 from 5' to 3'. The latter process gives a clearer distinction between heterozygotes and normal types.

Prepare the same amplified fragments as above. The procedure of choice is to use the enzyme BbvI (New England Biolabs) for which the buffer is the same as Taq polymerase if using Ital the product needs to be dried by centrifugation under vacuum until just dry (about 3 hours). This removes dimethylsulphoxide which can impair enzyme activity. Reconstitute the pellet with 30µl dH₂O. For the BbvI transfer 30µl of product to eppendorf tubes and cut with 2 units of enzyme overnight at 37°C. Run on a 12% PAGE for 4 hrs at 110V. Document the gel as above.

The homozygote R145C has 3 bands: a doublet (111 and 108bases) and a small fragment (25 bases). An heterozygote has 5 bands: a doublet at high molecular mass, a band at 78 and a band at 33 as well as 25bases. A non-R145C subject has 4 bands commencing with a single band at high molecular mass, and bands 78, 33 and 25bases.

UCT PROCEDURE FOR APOE-R145C

Mismatch assay devised with Prof. H Henderson, Red Cross Children's Hospital.

PRIMERS	G129 Normal:	5' ATC GCT GAG GAG CCG CTT TCG 3'
	G128 Mutant:	5' ATC GCT GAG GAG CCG CTT TCA 3'
	G23 Opposite:	5' TA AGC TTG GCA CGG CTG TCC AAG GA 3'

PROCEDURE

	Stock concentration	Volume	Final concentration
Water		28.3	
Buffer	10x	5	1x
dNTP	2 mmol/L	2.5	100 μ mol/L
G23	20 μ mol/L	2.5	1 μ mol/L
G128(M) or G129(N)	20 μ mol/L	2.5	1 μ mol/L
DMSO		5	10%
MgCl ₂	25 mmol/L	2	1 μ mol/L
Taq	5 units/ μ l	0.2	1 unit
DNA		2	

Program: 1 cycle 95°C for 35sec

35 cycles of 94.5°C for 45sec, 60°C for 60sec, 72°C for 60sec

1 cycle 70°C for 3min

10% PAGE with sucrose load buffer. Repeated load in same lane for mutant and normal PCR (he/ho).

Ethidium stain. Videodensitometer.

LISTING OF APO-E MUTATIONS (in sequence)

The normal variants are listed, followed by deletions and insertions. The rest of the list is according to mutations in sequence of the molecule but excluding polymorphism at 112. The functional defect may not relate to the first specified mutation as it may be outside the binding domain for heparan sulphate proteoglycans and lipoprotein receptors. IEF included when given in publication.

Name	Mutation	112	IEF	Dysβ	Comment
Isoform E3	R158	C	3	No	Wild type
Isoform E4	R158	R	4	No	Original gene. Hypercholesterolaemia, Alzheimer's.
Isoform E2	R158C	C	2	Reces	Hypochol; HSPG bind. influences phenotype
	-491A>T			No	modulates response to fibrates & statins
	-219G>T			No	Increased MI if T allele
	A>G intron3			Dom?	Penultimate nucleotide at splice junction
E null	delG30, FsX60			Reces	He- has low apoE
	del-L149			Dom	Spanish, strong penetrance
E Leiden	Repeat 121-129		R	3	Dom Early onset
	E3K			No	Atherosclerosis, Japan
	E3K, E13K		5	?	Mixed hyperlipidaemia
Frankfurt	E3K, R84P		5	No	IHD, dominant
Philadelphia	E13K, R145		5	Dom	USA, white 13AAG>GAG, 145CGT>TGT
Freiburg E4	L28P	R	4-	No	Normal variant, slightly acid, CTG>CCG
Freiburg E3	T42A		3-	No	Normal variant, slightly acid, ACA>GCA
	P84R	R	4	No	Normal variant CCG>CGG USA white
	A99T, A152P		3	No	USA, GCG>ACG, GCC>CCC
	G127D		R	1	Dom Finland. Hypertriglyceridaemia
	R134Q			No	Normal variant, CGG>CAG. Dutch
Heidelberg	R136C			Dom	
	R136			Reces	Quebec
Christchurch	R136S			2	Dom Spanish population
	R142C		R	3	Dom
	R142L				
	R145C		C	2	Dom SA Black, white, mixed
Kochi	R145H			3-	Dom? Japan. Slightly more acidic
	R145P				LP glomerulopathy
Harrisburg	K146E			1	Dom
"Dutch"	K146Q			2	Dom SA
	L146N, R147W			Dom	Strong early penetrance
Baden	R180C		C	1	No HyperTG. HaeI site. Dom. lipid domain. German
Washington	W210X			3	Dom [ApoE] 4%, 24kda, TGG>TAG, new Mae site.
Fukuoka	R224Q, R158C			2	?
Oegstgeest	V236E, R158C			2	?
Suita	E244K, E245K			7	Dom Japan, GAC>GAG, AAC>AAG.
	R251G		R	?	Dutch.
Muenster	L252E	R	1		Hypercholesterolaemia
Doetinchem	R274H		R	4	Normal variant TGC>CGC Dutch
Amsterdam	S296R			4 +	Normal variant, slightly more basic

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