REVIEW ARTICLE

Monogenic primary hypercholesterolaemia in South Africa

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Abstract **Familial hypercholesterolaemia (FH) and familial defective apolipoprotein B-100 (FDB) are the two major causes of monogenic primary hypercholesterolaemia. In this review, FH and FDB are defined in relation to normal lipoprotein metabolism. In South Africa FH affects about 1% of Afrikaners, Jews and Indians, while FDB is probably a much rarer disorder. In Afrikaners, three 'founder' mutations are responsible for more than 80% of FH. The population genetics that created the exceptionally high frequency of FH and comparatively low frequency of FDB in various South African populations are described. The genetic organisation and itinerary of the normal low-density lipoprotein (LDL) receptor are reviewed, with particular emphasis on the structure-function relationships in the LDL receptor that have been clarified by the mutations found in South Africa. Finally, the clinical relevance of research into FH in South Africa is discussed.**

S Afr Med J 1994; **84:** 339-344.

Familial hypercholesterolaemia (FH) and familial defective apolipoprotein B-100 (FDB) are the two major causes of monogenic primary hypercholesterolaemia. Both of these autosomal dominant disorders have a prevalence of 1/500 in American and European populations and are among the commonest known Mendelian diseases.[1,2] Since FH is particularly common (> 1/100) in the Afrikaner,[3] Jewish[4] and Indian populations of South Africa (personal communication — H. C. Seftel, M. S. Asvat), an awareness of this disease on the part of medical practitioners is desirable.

The physiological consequences of these monogenic disorders are best appreciated in the context of normal lipoprotein metabolism. Intravascular lipoprotein metabolism involves interconversions between lipoprotein types, exchange of lipoprotein components and final cellular clearance. Two different pathways ensure lipoprotein metabolism in the blood.[1,5] The 'exogenous' pathway entails the transport of dietary lipid from the intestine to the rest of the body, while the 'endogenous' pathway is responsible for the transport of dietary and newly synthesised lipid from the liver to extrahepatic cells.

In the 'exogenous' pathway (Fig. 1), triglycerides and cholesterol are absorbed by the intestine where they are subsequently packaged into chylomicron particles which are secreted into the lymphatic system to enter the circulation via the thoracic duct. The triglycerides in these large lipoproteins are hydrolysed by lipoprotein lipase on the surface of the vascular endothelium. The resultant smaller chylomicron remnants are removed from the blood mainly by receptors on parenchymal liver cells. These receptors are probably distinct from the low-density lipoprotein (LDL) receptor since chylomicron remnant clearance is unimpaired in FH homozygotes with severe LDL-receptor deficiency.[6] The α_2 macroglobulin receptor, also known as the LDL receptor-related protein (LRP), might be the chylomicron remnant receptor.[7]

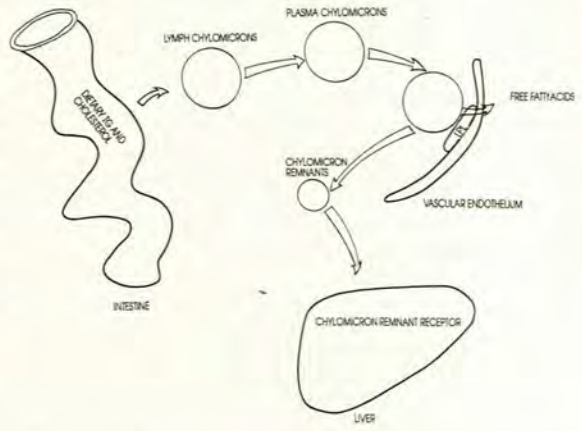

FIG. 1.
Schematic diagram of the exogenous lipoprotein pathway.

The 'endogenous' lipoprotein pathway involves the metabolism of lipoproteins secreted by the liver (Fig. 2). These triglyceride-rich particles are known as very-low-density lipoproteins (VLDL). The triglycerides of VLDL are lipolysed by lipoprotein lipase to yield smaller particles called intermediate-density lipoproteins (IDL). These IDL are either lipolysed further by lipoprotein lipase or hepatic lipase to form LDL or removed directly from the circulation by LDL receptors. LDL particles, which carry most of the plasma cholesterol, are removed from the circulation mainly by hepatic LDL receptors. Although approximately 70% of LDL catabolism is via the liver, which is the major exit route of cholesterol from the body, significant LDL clearance from the blood is mediated by the small intestine, skeletal muscle, kidney and spleen.

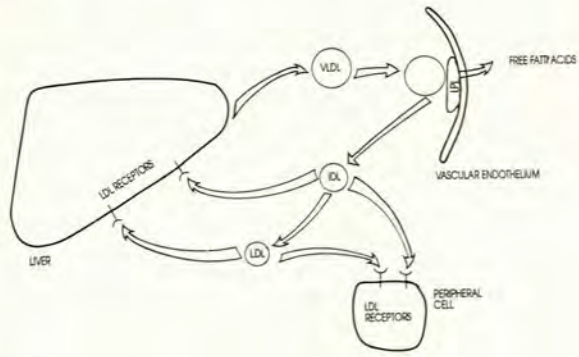

FIG. 2.
Schematic diagram of the endogenous lipoprotein pathway (VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein).

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Accepted 24 Mar 1993.

Monogenic disorders that result in impaired LDL metabolism

Impaired removal of LDL is the primary defect in the two related syndromes, FH and FDB.

FH is an autosomal dominant disorder that results from mutations at the LDL-receptor gene locus that affect either the number and/or function of the LDL-receptor protein.[8] This has two different effects on the endogenous lipoprotein pathway that cause hypercholesterolaemia. First, the rate of LDL removal is decreased and second, the production of LDL from IDL is increased as a result of impaired IDL clearance from the circulation. More than 50 different mutations that affect one or more vital facets of LDL-receptor function have been described.[8] The clinical severity of this disease is milder in patients with one mutant gene (heterozygotes) than in those with two (homozygotes). Heterozygotes express approximately 50% of the normal number of functional LDL receptors on their cells. Their plasma cholesterol levels are consequently about twice as high as normal and coronary heart disease is 25 times more common in heterozygotes than in unaffected persons. The situation in homozygotes who have two mutant alleles is even more severe: plasma cholesterol concentrations are raised up to five times the normal concentrations and coronary artery disease often manifests in childhood and frequently leads to death from a heart attack before the age of 25 years.[1,5] Xanthomata, xanthelasmata and corneal arci are visible manifestations of cholesterol deposition that are invariant in FH homozygotes and variable in FH heterozygotes.[1,5]

An alternative genetic disorder, described more recently, that causes pathologically decreased LDL clearance is FDB.[2] This disorder is caused by a glutamine for arginine substitution at codon 3500 of apo B. Apo B-100 is the sole apoprotein on LDL and is the ligand on the particle that is bound by the LDL receptor. The LDLs that carry the mutant apo B bind very poorly to LDL receptors and are cleared abnormally slowly from the circulation. FDB in its heterozygous state is clinically very similar to heterozygous FH.[9] In contrast to FH, FDB is caused by one mutation that probably occurred only once on an ancestral Caucasian chromosome.[2] Although the frequency of FDB in North American and European populations is approximately 1/500,[2] this mutation has not been detected in Finns[10] or in Japanese.[11]

FH in South Africa

FH has been a focus of research in South Africa ever since Seftel *et al.*[3] recognised it as a common disease among Afrikaners. The same researchers recognised later that FH was also prevalent among Indians and Jews in South Africa.[4] The frequency of FH in these populations is among the highest in the world. In Afrikaners and South African Jews, the high frequency is probably due to a 'founder effect'.[3,4]

The history of the settlement of the Afrikaners in South Africa serves as a classic example of the factors contributing to the so-called 'founder effect'. The majority of South Africa's present-day 2 million Afrikaners are descended from about 2 000 settlers of Dutch, German and French origin who arrived in South Africa in the late 17th and early 18th centuries. Given their small numbers and statistical chance, these settlers as a group had higher (or lower) frequencies of certain disease alleles than their original parent European populations. This was the result of chance over- or under-sampling of rare alleles. The odds of such occurrences are inversely proportional to the size of the settler population. This population maintained its particular genetic constitution as it expanded since, until recently, Afrikaners generally only married Afrikaners. These altered allelic frequencies were prone to further amplification or reduction by the random genetic drift to which small populations are prone. The Afrikaner population expanded far faster than its European counterparts by growing about 1 000-fold in 300 years.[3,8,12]

Three founder type mutations, FH Afrikaner-1, FH Afrikaner-2 and FH Afrikaner-3, have now been identified[13,14] and account for approximately 90% of the monogenic hypercholesterolaemia in this population.[12,15] All three of these mutations have been detected in the Netherlands.[16]

South African Jews of Eastern European (Ashkenazi) descent also have a particularly high incidence of FH (> 1/100).[4] The predominant mutation in this population is FH Piscataway.[17] The majority of this population are descendants of 40 000 Lithuanian immigrants who settled in South Africa between 1889 and 1910. In this case it is unlikely that a founder effect could have manifested in three or four generations from a relatively large starting population. Since this mutation also accounts for 35% of FH in Ashkenazi Israelis, it is more likely that a founder effect occurred in Lithuania. This mutation is particularly interesting since evidence of its founder origin is visible in many Ashkenazi Jewish families throughout the world.[17]

Another South African group that seems to have a particularly high incidence of FH (> 1/100) is the Indian population (personal communication — H. Seftel, M. Asvat). South African Indians are almost entirely descended from 150 000 immigrants who settled in South Africa between 1860 and 1911. The majority of these people were indentured labourers who originated in many different regions on the south and east coasts of India. A minority (less than 30 000) were traders and artisans who paid their own passage to South Africa. This group, known as the 'passenger Indians', came from areas in the Gujerat province on the west coast of the subcontinent. About 50% of these people came from the Surat and Valsad districts. The other major source of passenger Indians was Kathiowar on the Arabian Sea.[18] The Indian population of South Africa is of diverse cultural and religious origin; the majority (65%) of the population are Hindus (many of the castes are represented) and there are a significant number of Muslims (21%) and Christians (7,5%).

At least four different mutations cause FH in this population (unpublished results — D. C. Rubinsztein, G. A. Coetzee, D. R. van der Westhuyzen). One of these mutations, FH Zambia, was originally described in a Muslim Zambian of Gujerati origin.[19] This mutation has been identified in four unrelated families in South Africa[20] and in another Indian patient in the UK.[21] All of these people were Muslims of Gujerati descent. This suggests that this mutation might be very common in Gujerat, possibly as a result of a founder effect in that region of India. This mutation possibly spread all over the world wherever Muslim Gujeratis settled, in a manner analogous to the FH Piscataway mutation in Lithuanian Jews.

FH seems to be relatively rare in black South Africans.[22] This might be due to a decreased frequency of mutant genes in this population. Alternatively, because most rural blacks eat low fat diets and have relatively low cholesterol levels, FH might not be recognised readily. The hypothesised genetic protection against hypercholesterolaemia found in blacks[23] might also mask FH. However, two different black FH homozygotes have been identified; a Xhosa homoallelic homozygote, i.e. he inherited two identical

TABLE I.
LDL-receptor mutations in South Africa

Name	Population	Gene defect	Protein defect
FH Afrikaner-1[13,31]	Afrikaner, coloured	Codon 206 asp to glu exon 4	Slow processing; 2 populations of receptors, one with defective binding
FH Afrikaner-2[13,14,31]	Afrikaner, coloured	Codon 408 val to meth exon 9	Slow processing; rapid degradation
FH Afrikaner-3[14]	Afrikaner, coloured	Codon 154 asp to asn exon 4	Slow processing; decreased affinity for LDL at 37°C; increased precursor degradation
FH Cape Town-2[26]	Coloured	Deletion exon 7 and 8	Does not recognise LDL; rapid degradation in absence of β-VLDL is enhanced in the presence of β-VLDL
FH Piscataway[6,17]	Jewish	3 b.p. deletion of gly 197 exon 4	Slow processing
FH Zambia[20]	Indian	Codon 664 pro to leu exon 14	Slow processing; rapidly degraded
FH Durban-1[28]	Indian	Codon 69 asp to tyr exon 3	
FH Durban-2[28]	Indian	Codon 119 glu to lys exon 4	
FH Pietermaritzburg[35]	Indian	Null mutation, site unknown	No detectable receptor synthesis
FH Cape Town-1[19]	Xhosa	6 b.p. deletion asp-26 gly-27 exon 2	Slow processing
Pedi[25]	Pedi	3 b.p. deletion in repeat 1 of promoter	? Decreased synthesis

VLDL = very-low-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein.

mutant genes called FH Cape Town-1,[24] and a Pedi hetero-allelic homozygote who had two different mutations (both distinct from FH Cape Town-1[25]). Both patients died of complications of coronary heart disease.

The so-called coloured population of South Africa have all three of the Afrikaner founder mutations (unpublished results — F. Graadt van Roggen, M. Kotze). In addition, they also have the FH Cape Town-2 mutation,[26] now known to be one of the most common mutations found in the Netherlands.[16] The LDL-receptor mutations that have been identified to date in the various populations in South Africa are listed in Table I. The abnormalities in LDL-receptor expression that result from these mutations are also summarised and will be discussed below.

FDB in South Africa

FDB does not seem to be as frequent a cause of monogenic hypercholesterolaemia among South African Afrikaners, coloureds, Indians and blacks as it is in American and European populations (unpublished results — D. C. Rubinsztein, M. Kotze, G. A. Coetzee and D. R. van der Westhuyzen). A possible reason for the rarity of this disorder in Indians and blacks is that the gene is of Caucasian founder origin. Any FDB that arrived in the coloured gene pool from their European ancestors would have been diluted. Although FDB seems to be quite common in the Netherlands,[27] it is comparatively rare among Afrikaners. Perhaps this represents a negative founder effect where by chance FDB was underrepresented in South African settler populations.

The susceptibility of the LDL-receptor and apolipoprotein B genes to mutations is probably similar in all populations and neither FDB nor FH seems to confer obvious heterozygote disadvantages in affected individuals. Therefore, it is likely that random genetic drift and founder effects are major determinants of the frequency of primary monogenic hypercholesterolaemia in different populations. This is exemplified in South Africa, a country with many different recent founder populations, where FH in Afrikaners, Jews and Indians is five times more frequent than in most other populations and where FDB is comparatively rare.

Molecular biology of the South African LDL-receptor mutations

The genetic organisation and itinerary of the LDL receptor

The LDL-receptor gene is a highly mutagenic locus responsible for sustaining the high frequency of FH. It has twice as many 'Alu' repeats as the average human gene.[8] These Alu repeats, which are the commonest 'middle repetitive' DNA sequences in humans, are hotspots for recombination events. Seven of the eight large deletions in the LDL receptor that have been sequenced involve Alu repeats. The other mutagenic mechanism that seems to operate at this locus is the transition of cytosine to thymine at CpG dinucleotides containing methylated cytosines.[8] More than 44% of LDL-receptor point mutations can be accounted for by this process. The FH Afrikaner-2,[13,14] FH Zambia[19] and FH Durban-2[28] mutations are such cytosine-to-thymine transitions.

The 860 amino acid LDL receptor is a multi-domain protein with components of diverse evolutionary origin.[8] Its gene of 45 kB is found on the short arm of chromosome 19 and is transcribed into a 5,3 kB mRNA. The exons are arranged in modules where each exon or group of exons defines a distinct domain of the receptor (Fig. 3). Exon 1 codes for a short 5' untranslated region and a 21-amino acid signal sequence that aids in the co-translational translocation of the protein into the endoplasmic reticulum (ER). This region is cleaved off during the translocation process to yield a mature protein of 839-amino acid. Exons 2-6 code for the receptor's ligand-binding domain that consists of seven cysteine-rich 40-amino acid repeats homologous to the complement factor 9. Exons 7-14 code for a domain homologous to a region of the EGF-precursor molecule and exon 15 codes for 58-amino acids that are rich in attachment sites for O-linked sugars. Exon 16 and part of exon 17 code for the membrane-spanning domain and the rest of exon 17 and part of exon 18 code for the cytoplasmic tail of the receptor.

LDL-receptor proteins are synthesised on polyribosomes and are co-translationally inserted into the ER, where glycosylation starts to occur (Fig. 4). At this point the precursor receptor has an apparent MW of 120 kDa. After transport to the Golgi where the sugars are modi-

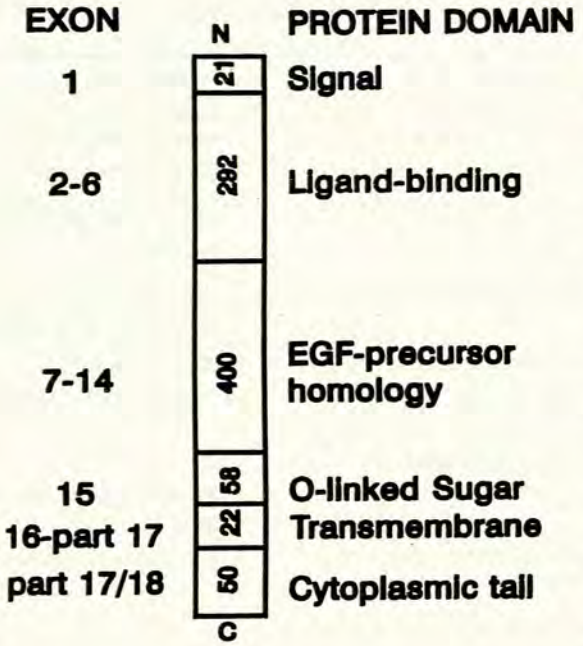

FIG. 3.
Schematic diagram showing the relationship between the exon arrangement and the protein domains of the LDL receptor.

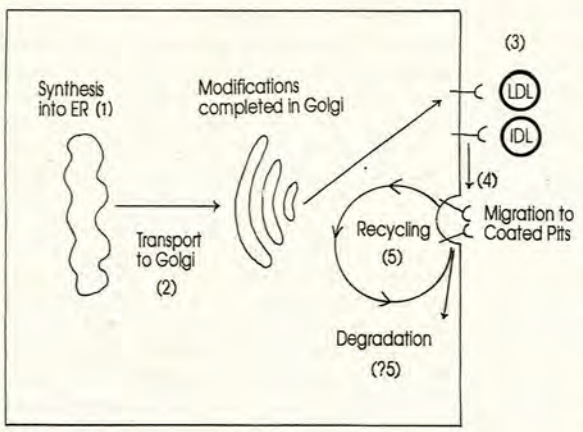

FIG. 4.
Schematic diagram of the itinerary of the LDL receptor (IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; ER = endoplasmic reticulum). Numbers in brackets indicate the class of mutation that results from interruption of the indicated process.

fied, the receptor attains its mature form of apparent MW 160 kDa. This process normally takes 15-30 minutes. From the Golgi the receptor is transported to the cell surface where it can bind lipoproteins that bear its ligands, apolipoprotein B-100 and/or E. Receptors are normally concentrated in specific regions of the plasma membrane called coated pits, that mediate endocytosis. Following their uptake, the pH in endocytic vesicles is lowered by a proton-driven pump. In the compartment for the uncoupling of the receptor and ligand (CURL), the pH is sufficiently low for the receptor and ligand to dissociate. The ligand is delivered to lysosomes where its apolipoproteins are degraded and the cholesterol esters are hydrolysed. The liberated free cholesterol is transported out of the lysosome. It has many regulatory consequences, including the stimulation of intracellular cholesterol esterification and the down-regulation of LDL receptor and *de novo* cholesterol synthesis.[1,5]

The unoccupied receptors return from the CURL to the cell surface where they can undergo many further rounds of endocytosis. Each round trip takes about 12 minutes.[1] The receptors are degraded with a half-life of 10 - 12 hours, probably in a non-lysosomal compartment.[28]

The diverse mutations in the LDL-receptor have been grouped into 5 classes depending on the resulting disturbance in function.[8] Many mutations result in disturbances of more than one facet of normal LDL receptor function and can therefore be placed in more than one class.

Structure-function relationships in the LDL receptor that have been clarified by the South African mutations

Class 1 mutations produce no detectable receptors. These mutations often result from large deletions of the gene's promoter that produce no detectable mRNA, e.g. the most common of the French-Canadian founder mutations.[8] Most of the control of LDL-receptor expression is thought to be at the transcriptional level.[8] One of the mutations in the South African Pedi is possibly an interesting case of a class 1 mutation in that 3 b.p. are deleted from the promoter in a region where the positive transcription factor, Sp1, is thought to bind.[25] Studies are under way to establish the consequences of this mutation on transcription. Class 1 mutations can also be caused by nonsense mutations or deletions that create premature stop codons.

Class 2 mutations result in receptors that are either slowly transported from the ER to the Golgi or retained in the ER. This manifests in a delay in conversion of the precursor receptor (120 kDa) to its mature form (160 kDa).[8] This phenomenon is probably due to the interaction of the mutant proteins with 'gate-keeper' proteins that prevent transport of malfolded proteins out of the ER. The FH Cape Town-1[24] mutant receptor that has a deletion of 2 amino acids in the first ligand-binding domain repeat is a classic example of such a mutation. Since the deletion of the entire first repeat had no effect on protein transport,[30] it seems that each ligand-binding repeat functions as a semi-independent module. The small deletion in FH Cape Town-1 probably disrupts the protein folding within the first repeat to cause a class 2 mutation. A similar phenomenon is found in FH Piscataway,[8] a deletion of 1 amino acid in the 4th ligand-binding domain repeat. This phenomenon is also found in many of the South African mutations, including the 3 Afrikaner founder mutations[13,31] and FH Zambia.[19,20] A delay in receptor transport alone, as opposed to a complete block, is not sufficient to cause a decreased pool of cell surface receptors. A decreased surface receptor number will only occur if there is impaired synthesis or increased degradation of precursor or mature receptors. Normally, no precursor degradation occurs and there is complete conversion of all the precursors to the mature forms.[5] The FH Afrikaner-3 receptor is an example of a mutation that results in slowed transport and degradation of precursors (the mature receptor has normal stability) (unpublished results — F. Graadt van Roggen, G. A. Coetzee, D. R. van der Westhuyzen). In this case, the long retention of precursors in the ER markedly increases the proportion of receptor precursors that are degraded and hence the number of receptors that reach the cell surface.

Class 3 mutants exhibit impaired ligand binding.[8] Experiments on artificially created mutant receptors

suggested that integrity of repeats 2 to 7 in the ligand-binding domain and exon 7 in the EGF-precursor homology domain was necessary for LDL (apo B) binding, while only repeat 5 was critical for β-VLDL (apo E) binding.[30,32] This is exemplified by FH Cape Town-2 (where exons 7 and 8 are deleted),[26] which normally recognises antibodies and apo E-containing lipoproteins but does not bind LDL. An interesting variation on this theme was discovered in the FH Afrikaner-1 mutation that results in at least 2 different mutant protein subpopulations being produced from 1 mutant gene that has a point mutation in exon 4. The one subpopulation that constitutes about 30% of the receptors normally recognises apolipoprotein B-100 on LDL, while the other subpopulation does not bind LDL at all.[31,33] These 2 populations of receptors possibly result from differently folded forms of the same primary amino acid sequence. Previously, class 3 mutations were thought of as homogeneous populations of receptors with impaired LDL binding.

The cytoplasmic tail of the LDL receptor contains specific amino acids that are important determinants for the clustering of receptors in coated pits. Mutations of these amino acids that result in receptors that bind ligand but do not get internalised to any significant extent are called class 4 mutations.[8] No South African FH patient with this rare type of mutation has been identified.

In 1990, a fifth class of mutation was defined. These were recycling-defective receptors that failed to separate from bound ligand after being internalised in endosomes. Such receptors are trapped in the cell in the presence of ligand and are unable to return to the cell surface. These receptors are rapidly degraded as a result.[8] The FH Cape Town-2 mutation,[26] a deletion of two exons in the EGF-precursor-like domain found in coloureds, demonstrates this phenomenon. However, certain mutations such as FH Afrikaner-2[31] and FH Zambia[19,20] have been identified that exhibit increased degradation even in the absence of ligand. These receptors are therefore not being degraded primarily as a consequence of impaired dissociation from their ligands. The instability of these receptors is of critical importance since the resulting low steady-state pool of receptors is the cause of FH in these patients.

Clinical relevance of FH research in South Africa

FH is found in more than 1/100 Afrikaners,[3] Jews[4] and Indians in South Africa (personal communication — H. C. Seftel, M. S. Asvat). The polymerase chain reaction that rapidly amplifies desired target DNA sequences has revolutionised the diagnosis of many genetic diseases and has facilitated the design of easy and reliable methods of screening for these FH mutations at the DNA level (e.g. ref. 12). There are suggestions that the severity and prognosis of FH among Afrikaners vary according to the type of mutation: heterozygotes with the FH Afrikaner-1 mutation that produces a significant number of normal functional receptors seem to be less affected than those with the FH Afrikaner-2 mutation whose receptors are so rapidly degraded in the mature form that there are practically no mutant receptors found on the cell surface. These findings complement an earlier study that showed that FH homozygotes, whose LDL-receptor alleles expressed some functional LDL receptors, had lower LDL-cholesterol levels, better therapeutic responses and less severe coronary atherosclerosis than homozygotes whose alleles produce no receptors.[1] In addition, there have been recent reports that the response of individuals to the lipid-lowering drug, simvastatin, is also partly influenced by the specific mutation in FH heterozygotes.[34] The ease of screening for these mutations can thus be applied in a specific, detailed diagnostic setting and for genetic counselling and prenatal diagnosis, especially where there is a risk of a homozygote child being born (e.g. in Afrikaners or in Muslim Indians where consanguinity is common).

Monogenic diseases are normally considered to be exceptionally rare medical curiosities of interest only to academics. In South Africa, however, FH has a significant impact on the health of Afrikaners, Indians and Jews. Due to the high frequencies of FH in these populations the prevalence of FH in South Africans in general is about 1/250. Therefore this disease must be considered when dealing with hypercholesterolaemic individuals or patients with premature coronary artery disease.

We would like to thank the South African Medical Research Council, the University of Cape Town, the Stella and Paul Loewenstein Trust and Old Mutual (South Africa) for financial support.

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