

## REFERENCES

1. Innerarity TL, Mahley RW, Weisgraber KH, et al. Familial defective apolipoprotein B-100: a mutation of apolipoprotein B that causes hypercholesterolemia. *J Lipid Res* 1990; **31**: 1337-1349.
2. Rauh G, Keller C, Kormann B, et al. Familial defective apolipoprotein B-100: clinical characteristics of 54 cases. *Atherosclerosis* 1992; **92**: 233-241.
3. Tybjaerg-Hansen A, Humphries SE. Familial defective apolipoprotein B-100: a single mutation that causes hypercholesterolemia and premature coronary heart disease. *Atherosclerosis* 1992; **96**: 91-107.
4. Rubinsztein DC, Raal FJ, Seftel HC, Pilcher G, Coetzee GA, Van der Westhuyzen DR. Characterization of six patients who are double heterozygotes for familial hypercholesterolemia and familial defective apo B-100. *Arteriosclerosis and Thrombosis* 1993; **13**: 1076-1081.
5. Davignon J, Dufour R, Roy M, et al. Phenotypic heterogeneity associated with defective apolipoprotein B-100 and occurrence of the familial hypercholesterolemia phenotype in the absence of an LDL-receptor defect within a Canadian kindred. *Eur J Epidemiol* 1992; **8**: 10-17.
6. März W, Ruzicka C, Pohl T, Usadel KH, Gross W. Familial defective apolipoprotein B-100: mild hypercholesterolemia without atherosclerosis in a homozygous patient. *Lancet* 1992; **340**: 1362.
7. Ludwig EH, McCarthy BJ. Haplotype analysis of the human apolipoprotein B mutation associated with familial defective apolipoprotein B-100. *Am J Hum Genet* 1990; **47**: 712-720.
8. Hämmäläinen T, Palotie A, Aalto-Setälä K, Kontula K, Tikkanen MJ. Absence of familial defective apolipoprotein B-100 in Finnish patients with elevated serum cholesterol. *Atherosclerosis* 1990; **82**: 177-183.
9. Hosking JL, Bais R, Roach PD, Thomas DW. Hypercholesterolemia due to familial defective apolipoprotein B-100 in two Australian families (Correspondence). *Med J Aust* 1991; **155**: 572-573.
10. Friedlander Y, Dan EJ, Leitersdorf E. Absence of familial defective apolipoprotein B-100 in Israeli patients with dominantly inherited hypercholesterolemia and in offspring with parental history of myocardial infarction. *Hum Genet* 1993; **91**: 299-300.
11. Talmud P, Tybjaerg-Hansen A, Bhatnagar D, et al. Rapid screening for specific mutations in patients with clinical diagnosis of familial hypercholesterolemia. *Atherosclerosis* 1991; **89**: 137-141.
12. Hansen PS, Rüdiger N, Tybjaerg-Hansen A, Faergemann O, Gregian N. Detection of the apo-B-3500 mutation (glutamine for arginine) by gene amplification and cleavage with Msp 1. *J Lipid Res* 1991; **32**: 1229-1233.
13. Tybjaerg-Hansen A, Gallagher J, Vincent J, et al. Familial defective apolipoprotein B-100: detection in the United Kingdom and Scandinavia and clinical characteristics of the cases. *Atherosclerosis* 1990; **80**: 235-242.
14. Graadt van Roggen FJ, Van der Westhuyzen DR, Marais AD, Gevers W, Coetzee GA. Low density lipoprotein founder mutations in Afrikaner familial hypercholesterolemia patients: a comparison of two geographical areas. *Hum Genet* 1991; **88**: 204-208.
15. Henderson HE, Berger GMB, Marais AD. A new LDL receptor gene deletion in the South African population. *Hum Genet* 1988; **80**: 371-374.
16. Kotze MJ, Langenhoven E, Theart L, Loubser O, Micklem A, Oosthuizen CJJ. Recurrent LDL-receptor mutation causes familial hypercholesterolaemia in South African coloureds and Afrikaners. *S Afr Med J* 1995; **85**: 357-361 (this issue.)
17. Rubinsztein DC, Coetzee GA, Marais AD, Leitersdorf E, Seftel HC, Van der Westhuyzen DR. Identification and properties of the proline<sup>3500</sup>-leucine mutant LDL receptor in South Africans of Indian origin. *J Lipid Res* 1992; **33**: 1647-1655.
18. Leitersdorf E, Van der Westhuyzen DR, Coetzee GA, Hobbs HH. Two common low density lipoprotein gene mutations cause familial hypercholesterolemia in Afrikaners. *J Clin Invest* 1989; **84**: 954-961.
19. Kotze MJ, Langenhoven E, Warnich L, et al. The identification of two low-density lipoprotein receptor gene mutations in South African hypercholesterolaemics. *S Afr Med J* 1989; **76**: 399-401.
20. Study Group, European Atherosclerosis Society. The recognition and management of hyperlipidaemia in adults: a policy statement of the European Atherosclerosis Society. *Eur Heart J* 1988; **9**: 571-600.
21. Kotze MJ, Langenhoven E, Warnich L, Du Plessis L, Retief AE. The molecular basis and diagnosis of familial hypercholesterolemia in South African Afrikaners. *Ann Hum Genet* 1991; **55**: 115-121.
22. Rubinsztein DC. Monogenic hypercholesterolemia in Indians and familial defective apolipoprotein B-100. PhD Thesis, University of Cape Town, 1993.
23. Goldstein JL, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease*. 6th ed. New York: McGraw-Hill, 1989.

Accepted 18 Apr 1994.

## Recurrent LDL-receptor mutation causes familial hypercholesterolaemia in South African coloureds and Afrikaners

M. J. Kotze, E. Langenhoven, L. Theart, O. Loubser, A. Micklem, C. J. J. Oosthuizen

Three low-density lipoprotein receptor (LDLR) gene mutations were previously shown to cause familial hypercholesterolaemia (FH) in up to 90% of affected Afrikaners. Association of each mutation with a single chromosomal background provided molecular genetic evidence that the proposed 'founder gene effect' was responsible for the high prevalence of FH among white Afrikaners. In this study we report the identification of the FH Afrikaner-2 (FH2) mutation, Val<sub>408</sub> to Met, in the so-called coloured population of South Africa, a people of mixed ancestry, with rapid non-radioactive methods for mutation detection. Haplotype analysis with polymorphisms on both sides of the FH2 mutation indicated that the identical LDLR gene mutations found in two different South African population groups were caused by independent events at a potential CpG mutational 'hot spot'. The allelic variation giving rise to the different chromosomal backgrounds of the FH2 mutation does not affect the properties of the abnormal LDLR protein product which causes FH in these subjects. This mutation is thus expected to cause the same severe form of FH in affected coloureds as was previously demonstrated in Afrikaners. Detection of mutant LDLR gene alleles in polymerase chain reaction products, directly after gel electrophoresis, now allows accurate presymptomatic diagnosis of the FH2 mutation in FH patients from two different South African population groups.

*S Afr Med J* 1995; **85**: 357-361.

Department of Human Genetics, University of Stellenbosch, Tygerberg, W. Cape

M. J. Kotze, PH.D.

E. Langenhoven, M.SC.

L. Theart, M.SC.

O. Loubser, M.SC.

A. Micklem, B.SC. HONS

C. J. J. Oosthuizen, PH.D.

Familial hypercholesterolaemia (FH) contributes significantly to the high mortality from coronary heart disease (CHD) in several South African populations. Since more than 150 different naturally occurring low-density lipoprotein receptor (LDLR) gene mutations have been described to date,<sup>1</sup> direct molecular diagnosis of FH is currently limited to genetically homogeneous populations, where the majority of individuals carry only a few disease related mutations. In such populations, like the South African Afrikaners<sup>2-4</sup> and Ashkenazi Jews,<sup>5</sup> specific founder gene defects can be diagnosed accurately. In the Afrikaner population it has been shown that three LDLR gene mutations cause FH in approximately 90% of affected individuals.<sup>6,7</sup> Although each founder-related mutation was found to be associated with a single chromosomal background in the Afrikaner patients analysed,<sup>4,8</sup> subsequent screening of Dutch FH patients for these mutations revealed a second haplotype associated with the FH Afrikaner-2 (FH2) mutation, Val<sub>408</sub> to Met.<sup>9</sup> In this study we identified the FH2 mutation on a third haplotype in two unrelated individuals of mixed ancestry, using direct non-radioactive molecular methods that are more convenient than those previously described, which involve the use of radioactivity and/or expensive restriction enzymes.<sup>6,7</sup>

## Materials and methods

### Analysis of the FH Afrikaner-2 mutation

DNA samples of individuals, previously typed by hybridisation with allele-specific oligonucleotides for the absence or presence of the FH2 mutation (Val<sub>408</sub> to Met, G to A mutation at base 1285 in exon 9 of the LDLR gene)<sup>6</sup> were used to standardise detection of the mutation with the amplification refractory mutation system (ARMS)<sup>10</sup> and single-strand conformation polymorphism (SSCP) method.<sup>11</sup> SSCP analysis was performed on polymerase chain reaction (PCR) products amplified with exon 9-specific oligonucleotides N2 (5'-GCTCCATCGCCTACCTCTTC-3') and A2 (5'-GCTCACCTGCAGATCATTCTCTGGG-3') as described previously.<sup>2</sup> Aliquots of the 172 bp amplified products (5 µl) were denatured with alkali (0.5M NaOH, 10 mM EDTA) at 42°C for 5 minutes, electrophoresed at 80 V overnight at room temperature in a non-denaturing 10% polyacrylamide gel supplemented with 5% glycerol, and stained with ethidium bromide (0.5 µg ml<sup>-1</sup>) for 10 minutes. Reactions for the ARMS analysis were performed in two separate tubes per sample, each containing one allele-specific oligonucleotide, 9.6 (5'-AGCCTCATCCCCAACCTGAGGACCG-3', normal allele) or 9.7 (5'-AGCCTCATCCCCAACCTGAGGACCA-3', mutant allele), in conjunction with a common distal primer A2 (see above). The underlined base was deliberately destabilised to ensure allele-specificity. Primers specific for exon 26 of the apolipoprotein (apo) B gene,<sup>12</sup> AB-1 (5'-GGAGCAGTTGACCACAAGCTTAGC-3') and AB-2 (5'-CAGGGTGGCTTTGCTTGTATGTTTC-3'), were included in all reactions and served to provide an internal control PCR product of 345 bp. Approximately 0.5 µg genomic DNA were used in the amplification reaction, with 5 pmol of each primer and one unit Taq DNA polymerase in a volume of 50 µl. Taq DNA polymerase, exclusively from Boehringer Mannheim, was used in the ARMS assay, since unacceptable

nonspecific bands were obtained when amplification was performed with Taq polymerase purchased from some other manufacturers. The four deoxynucleoside triphosphates were each added to 100 µM in 1 x Taq DNA polymerase buffer (Boehringer Mannheim). Reaction mixtures were overlaid with light mineral oil (Sigma, 50 µl), subjected to DNA denaturation at 94°C for 5 minutes and then to 30 cycles of amplification (93°C for 1 minute; 60°C for 1 minute; 72°C for 2 minutes). PCR products were analysed in 2% agarose gels.

### Subjects

The ARMS method described above was used to identify the FH2 mutation in 2 coloured and 28 Afrikaner FH patients unrelated to the second degree. A total of 24 family members were also screened for the FH2 mutation with either the ARMS or SSCP method. Patients attended the lipid clinic at Tygerberg Hospital, or were referred for DNA analysis of FH from other lipid clinics in South Africa, general practitioners or the Department of National Health and Population Development.

### Haplotype analysis

Haplotypic arrangements at 14 polymorphic sites within or closely linked to the LDLR gene were analysed: the ApaI restriction fragment length polymorphism (RFLP) in intron 3,<sup>13</sup> the TaqI RFLP in intron 4,<sup>14</sup> the SphI RFLP in intron 6,<sup>15</sup> the SmaI RFLP in intron 7,<sup>16</sup> the StuI RFLP in exon 8,<sup>17</sup> the guanine to adenine base change in exon 10,<sup>18</sup> the HincII RFLP in exon 12,<sup>19</sup> the BstEII RFLP in intron 12,<sup>20,21</sup> the Avall RFLP in exon 13,<sup>22</sup> the PvuII RFLP in intron 15,<sup>23</sup> the NcoI RFLP in exon 18,<sup>24</sup> the two MspI RFLPs in exon 18,<sup>25</sup> and the 3'-flanking PstI RFLP.<sup>26</sup> Genotypes at the polymorphic sites in introns 4 and 7, and exons 8, 10, 12, 13 and 18 were determined by PCR-based methods, while the others were analysed by Southern blot analysis.

FH-associated haplotypes for all subjects were deduced by segregation analysis, based on the assumption that there were no recombination events within families, or by homozygosity for the polymorphisms analysed.

## Results

Fig. 1 shows the allelic differentiation obtained with the ARMS and SSCP methods respectively, directly after PCR amplification and gel electrophoresis of genomic DNA from normal individuals, heterozygotes and homozygotes for the FH2 mutation of the LDLR gene. When the ARMS method was used on the DNA of a normal individual, a product was derived only from the internal control primers and primers 9.6 and A2 (Fig. 1A, lane 1). No LDLR gene product (or very faint bands) was observed when primer 9.7, specific for the mutant allele, replaced primer 9.6 (lane 2). When DNA of a FH2 heterozygote was used in the two separate reactions, the expected 100 bp fragment was generated when either primer 9.6 or 9.7 was included in the reaction (Fig. 1A, lanes 3 and 4). Amplification occurred with DNA from a FH2 homozygote only when the mutant oligonucleotide was used (lane 6). Presence of the 345 bp apo B fragment (internal control) in all tubes indicated that amplification occurred in all the reactions. SSCP analysis was performed on the same

set of DNA samples, as well as on some additional samples, to test the mutation-detection efficiency of this method for the FH2 mutation. In Fig. 1B the G alleles in normal individuals could readily be distinguished from the A alleles in affected individuals.

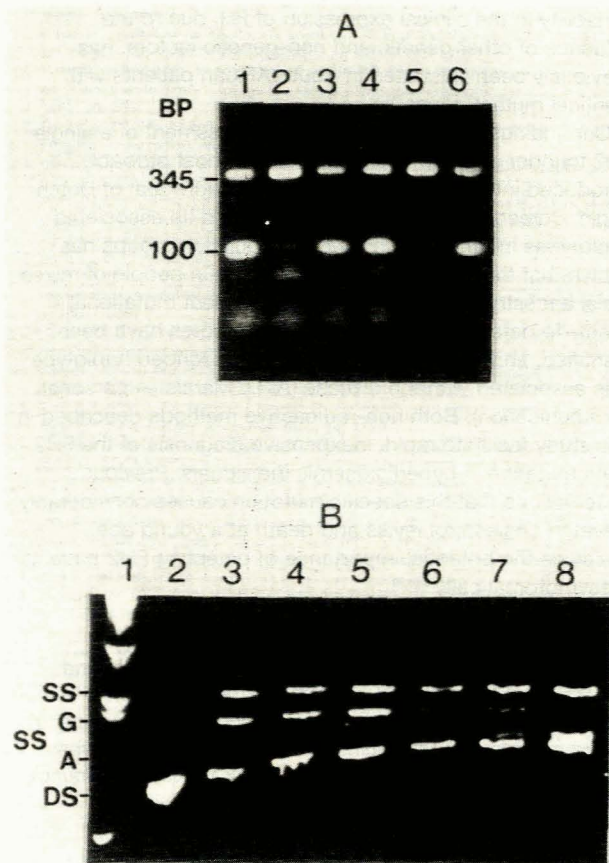


Fig. 1. Analysis of the FH2 mutation after PCR amplification and gel electrophoresis. (A) ARMS analysis of a normal control (lanes 1 - 2), a FH heterozygote (lanes 3 - 4) and a FH homozygote (lanes 5 - 6). ARMS primers were used as follows: lanes 1, 3 and 5, normal G-allele (9.6); lanes 2, 4 and 6, mutant A-allele (9.7). These were used in conjunction with a common distal primer (A2). Primers AB-1 and AB-2, producing PCR products of 345 bp, were used in each reaction as internal controls. PCR products were electrophoresed in 2% agarose gels. (B) Non-isotopic SSCP analysis of alkali-denatured PCR products of 172 bp on 10% acrylamide gels containing 5% glycerol. Lanes: (1) 1 kb DNA ladder, (2) undenatured DNA control, (3 - 5) DNA from normal controls, (6 - 7) DNA from FH patients heterozygous for the FH2 mutation and (8) DNA from a FH homozygote. (bp = base pairs; ss = single-stranded DNA; ds = double-stranded DNA.)

The above methods were used to identify 30 FH2 heterozygotes during an ongoing screening of South African patients for known LDLR gene mutations. Subsequent genotyping at 14 polymorphic sites showed that the FH2-associated haplotype in two unrelated coloured families differs from that in Afrikaners at 5 polymorphic sites framing the FH2 mutation. Table I compares the different polymorphic alleles associated with the FH2 mutation in the Afrikaner and coloured populations. None of the South African FH2 heterozygotes studied showed disease-association with the second haplotype described in Dutch patients.<sup>9</sup>

Table I. LDLR gene haplotypes associated with the FH2 mutation in two different South African population groups

Polymorphism	Location	Afrikaner	Mixed race
ApaI	Intron 3	+	+
TaqI	Intron 4	-	-
SphI	Intron 6	+	+
SmaI	Intron 7	-	+
StuI	Exon 8	+	+
-	Exon 10	A	A
HincII	Exon 12	+	+
BstEII	Intron 12	-	-
Avall	Exon 13	-	-
PvuII	Intron 15	-	+
NcoI	Exon 18	-	+
MspI	Exon 18	-	+
MspI	Exon 18	-	+
PstI	3' flanking	-	-

+ = presence of a restriction enzyme cutting site; - = absence of a restriction enzyme cutting site; A = adenine; G = guanine.

## Discussion

Three founder-related LDLR gene mutations increased the prevalence of FH among Afrikaners to about 1 in 80,<sup>27</sup> compared with 1 in 500 in most other population groups. One of these, the so-called FH2 mutation, causes a severe phenotype<sup>28</sup> and accounts for FH in about 20% of Afrikaners.<sup>6</sup> In this study we applied non-radioactive ARMS and/or SSCP methods to screen for the FH2 mutation in hypercholesterolaemics who were referred to us for a DNA diagnosis of FH.<sup>29</sup> By utilising the exon 9-specific PCR amplification primers, described previously, for analysis of the FH2 mutation by differential oligonucleotide hybridisation,<sup>6</sup> we obtained reproducible results by direct visualisation of different alleles after electrophoresis in SSCP gels and ethidium bromide staining.<sup>30</sup> Direct analysis of the FH2 mutation, following allele-specific amplification by the ARMS method and gel electrophoresis, required synthesis of primers specific for the mutant and normal alleles for use in two separate PCR reactions. When screening FH heterozygotes we found a single PCR reaction, using only the mutant ARMS primers, to be adequate. The specificity of the ARMS primers was increased by the introduction of an additional mismatch near the 3' ends to avoid false-positive results. False-negative results were excluded by the co-amplification of a 345 bp PCR product of the apo B gene. The apo B gene products obtained in the reactions can be used simultaneously to screen hypercholesterolaemics for point mutations that cause familial defective apolipoprotein B-100,<sup>31</sup> by heteroduplex analysis in low cross-linking polyacrylamide gels.<sup>32</sup>

Haplotype analysis in the normolipidaemic and FH populations has previously shown the FH2 mutation to be associated with a single 10-RFLP arrangement in Afrikaners.<sup>6</sup> Subsequent screening for the 3 Afrikaner founder LDLR gene mutations in patients of Dutch descent resulted in the identification of the FH2 mutation in 1,6% of subjects.<sup>9</sup> Of the 16 FH2 heterozygotes detected in the Netherlands, 7 shared a LDLR gene haplotype with Afrikaner patients. The remaining 9 patients had the same FH2

mutation-associated haplotype, which was partly identical to that in Afrikaners and could have arisen from a single recombinational event. Genealogical and extended haplotype studies support the hypothesis that the FH2 mutation originated in the Netherlands and was introduced into South Africa by an early Dutch settler in the 17th century (J. C. Defesche — personal communication and unpublished results).

In this study the FH2 mutation was identified in 30 South African patients who had not previously been analysed in respect of the haplotype on which the mutation occurred.<sup>9</sup> To investigate the relationship between the FH2 mutations detected in both Afrikaner and coloured patients, we performed haplotype analysis using 14 polymorphic sites at the LDLR locus.<sup>13-26</sup> As expected, the chromosomal background originally described for the FH2 mutation<sup>9</sup> showed an association with the mutation in the Afrikaner patients, but a different disease-associated haplotype was identified in two unrelated coloured patients. These haplotypes differ on both sides of the mutation in exon 9: at the PvuII, NcoI and two MspI RFLPs on the 3' side and at the SmaI RFLP on the 5' side.

The above results suggest that independent mutational events in exon 9 of the LDLR gene gave rise to the FH2 mutation in two different South African populations. The fact that the single-base substitution in exon 9 involves a methylated CpG dinucleotide,<sup>33</sup> known to mutate frequently,<sup>34</sup> further supports this theory. The FH2 mutation could, however, also have had a common origin if the chromosomal background were changed by a crossover between the FH2 mutation in exon 9 and the PvuII polymorphism in intron 15, and a base pair change occurred at the SmaI site. This possible, but improbable, mechanism was excluded by haplotype analyses of normolipidaemic coloureds (data not shown). Since the heterozygosity of most RFLPs described earlier at the 5' end of the LDLR gene is very low,<sup>13,14</sup> conclusive evidence against the second mechanism's giving rise to the different FH2 mutation-associated haplotypes came from an analysis of the recently-described SmaI RFLP in intron 7.<sup>16</sup> The frequency of this excellent LDLR gene haplotype marker is high in FH and normal individuals from both population groups (data not shown). These results provide evidence against the possibility that the base pair change that creates the SmaI site in the 'coloured' haplotype arose on an ancient FH2 mutation-associated haplotype that underwent a single recombination event.

The historic ethnic origin of the FH2 mutation found in coloureds was not investigated further. Whites, Asians and blacks have contributed to the gene pool of the present-day coloured population of South Africa.<sup>35</sup> We do believe that, as more FH patients in more population groups both in and outside South Africa are genotyped at the disease locus, further light will be shed on the origins of this mutation.

Although the properties of the abnormal LDLR are not affected by the polymorphic sites that allow differentiation of the chromosomal background for the FH2 mutation in the two South African patient groups, the presence of a PvuII site in coloureds may result in a more favourable genetic background for the phenotypic expression of FH. This neutral PvuII RFLP in intron 15, that is probably in linkage disequilibrium with a functionally important sequence

change in the LDLR gene, shows significant association with cholesterol level variation in healthy individuals.<sup>36,37</sup> The effect associated with the PvuII rare allele (+) is to lower total and LDL-cholesterol levels, and its relatively higher frequency in individuals over the age of 65 years suggests that the allele may be associated with increased fitness for survival.<sup>37</sup> Variability in the clinical expression of FH, due to the influence of other genetic and non-genetic factors, has previously been illustrated in South African patients with identical mutant genes.<sup>38,39</sup>

Our findings confirm the previous assessment of a single FH2 founder gene among Afrikaners,<sup>2,4,8</sup> most probably introduced into South Africa by a single individual of Dutch origin. Screening for the FH2 mutation and its associated haplotypes in other South African population groups has shown that this mutation is also common in people of mixed racial ancestry as a result of an independent mutational event. To date, 4 coloured FH2 heterozygotes have been identified, and in all of them the newly-described haplotype was associated with the disease (A. D. Marais — personal communication). Both non-radioactive methods described in this study facilitate rapid, inexpensive diagnosis of the FH2 gene mutation in hyperlipidaemic individuals. Previous observations that this specific mutation causes considerably elevated cholesterol levels and death at a young age underline the potential importance of detecting FH2 patients presymptomatically.<sup>9,28,39</sup>

We thank Professors G. A. Coetzee and D. R. van der Westhuizen for helpful discussions, Drs D. Rubinsztein and J. C. Defesche for the kind gifts of exon 18- and intron 4-specific oligonucleotides, R. Titus for technical assistance and J. Brusnick for critical reading of this manuscript. This work was supported by the South African Medical Research Council, the University of Stellenbosch and the Cape Provincial Administration.

#### REFERENCES

1. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat* 1992; **1**: 445-466.
2. Kotze MJ, Langenhoven E, Warnich L, et al. The identification of two low-density lipoprotein receptor gene mutations in South African familial hypercholesterolaemia. *S Afr Med J* 1989; **76**: 399-401.
3. Kotze MJ, Warnich L, Langenhoven E, Du Plessis L, Retief AE. An exon 4 mutation identified in the majority of South African familial hypercholesterolaemics. *J Med Genet* 1990; **27**: 298-302.
4. Leitersdorf E, Van der Westhuizen DR, Coetzee GA, Hobbs HH. Two common low density lipoprotein receptor gene mutations cause familial hypercholesterolemia in Afrikaners. *J Clin Invest* 1989; **84**: 954-961.
5. Meiner V, Landsberger D, Berkman N, et al. A common Lithuanian mutation causing familial hypercholesterolemia in Ashkenazi Jews. *Am J Hum Genet* 1991; **49**: 443-449.
6. Kotze MJ, Langenhoven E, Warnich L, Du Plessis L, Retief AE. The molecular basis and diagnosis of familial hypercholesterolaemia in South African Afrikaners. *Ann Hum Genet* 1991; **55**: 115-121.
7. Graadt van Roggen JF, Van der Westhuizen DR, Marais AD, Gevers W, Coetzee GA. Low density lipoprotein receptor founder mutations in Afrikaner familial hypercholesterolemic patients: a comparison of two geographical areas. *Hum Genet* 1991; **88**: 204-208.
8. Kotze MJ, Langenhoven E, Retief AE, Seftel HC, Henderson HE, Weich HFH. Haplotypes identified by 10 DNA restriction fragment length polymorphisms at the human low density lipoprotein receptor gene locus. *J Med Genet* 1989; **26**: 255-259.
9. Defesche JC, van Diermen DE, Lansberg PJ, et al. South African founder mutations in the low-density lipoprotein receptor gene causing familial hypercholesterolemia in the Dutch population. *Hum Genet* 1993; **92**: 567-570.
10. Newton CR, Graham A, Heptinstall LE, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 1989; **17**: 2503-2516.
11. Orita M, Sasaki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989; **5**: 874-879.
12. Cladaras C, Hadzoupolo-Cladaras M, Nolte RT, Atkinson D, Zannis VI. The complete sequence and structural analysis of human apolipoprotein B-100: relationship between apoB-100 and apoB-48 forms. *EMBO J* 1986; **13**: 3495-3507.

13. Geisel J, Weisshaar B, Oette K, Doerfler W. A new ApaI restriction fragment length polymorphism in the low density lipoprotein receptor gene. *J Clin Chem Clin Biochem* 1988; **26**: 429-433.
14. Yamakawa K, Okafuji T, Iwamura Y, Russell DW, Hamaguchi H. TaqI polymorphism in the human LDL receptor gene. *Nucleic Acids Res* 1987; **15**: 7659.
15. Leitersdorf E, Chakravarti A, Hobbs HH. Polymorphic DNA haplotypes at the LDL receptor locus. *Am J Hum Genet* 1989; **44**: 409-421.
16. Jensen LG, Jensen HK, Kjeldsen M, et al. A new, highly informative SmaI polymorphism in intron 7 of the low density lipoprotein receptor (LDLR) gene. *Clin Genet* 1994; **45**: 52-53.
17. Kotze MJ, Retief AE, Brink PA, Weich HFH. A DNA polymorphism in the human low-density lipoprotein receptor gene. *S Afr Med J* 1986; **70**: 77-79.
18. Warnich L, Kotze MJ, Langenhoven E, Retief AE. Detection of a frequent polymorphism in exon 10 of the low-density lipoprotein receptor gene. *Hum Genet* 1992; **89**: 362.
19. Leitersdorf E, Hobbs HH. Human LDL receptor gene: HincII polymorphism detected by gene amplification. *Nucleic Acids Res* 1988; **16**: 7215.
20. Steyn LT, Pretorius A, Brink PA, Bester AJ. RFLP for the human LDL receptor gene (LDLR): BstEII. *Nucleic Acids Res* 1987; **15**: 4702.
21. Kotze MJ, Langenhoven E, Retief AE. Improved visualisation of the BstEII RFLP of the human LDL receptor gene by co-digestion. *Nucleic Acids Res* 1987; **15**: 10067.
22. Hobbs HH, Esser V, Russell DW. Avall polymorphism in the human LDL receptor gene. *Nucleic Acids Res* 1987; **15**: 379.
23. Humphries SE, Horsthemke B, Seed M, et al. A common DNA polymorphism of the low-density lipoprotein (LDL) receptor gene and its use in diagnosis. *Lancet* 1985; **1**: 1003-1005.
24. Kotze MJ, Langenhoven E, Dietzsch E, Retief AE. A RFLP associated with the low-density lipoprotein receptor gene (LDLR). *Nucleic Acids Res* 1987; **15**: 376.
25. Geisel J, Weisshaar B, Oette K, Mechtel M, Doerfler W. Double MspI RFLP in the human LDL receptor gene. *Nucleic Acids Res* 1987; **15**: 3943.
26. Funke H, Klug J, Frossard P, Coleman R, Assman G. PstI RFLP close to the LDL receptor gene. *Nucleic Acids Res* 1986; **14**: 7820.
27. Jooste PL, Benade AJS, Rossouw JE. Prevalence of familial hypercholesterolaemia in three rural South African communities. *S Afr Med J* 1986; **69**: 548-551.
28. Kotze MJ, De Villiers WJS, Steyn K, et al. Phenotypic variation among familial hypercholesterolemics heterozygous for either one of two Afrikaner founder low density lipoprotein receptor mutations. *Arterioscler Thromb* 1993; **13**: 1460-1468.
29. Kotze MJ, Langenhoven E, Theart L, Marx MP, Oosthuizen CJJ. Report on a molecular diagnostic service for familial hypercholesterolemia in Afrikaners. *Genet Couns* 1993; **5**: 15-22.
30. Yap EPH, McGee JO'D. Nonisotopic SSCP detection in PCR products by ethidium bromide staining. *Trends Genet* 1992; **8**: 49.
31. Soria LF, Ludwig EH, Clarke HRG, Vega GL, Grundy SM, McCarthy BJ. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc Natl Acad Sci USA* 1989; **86**: 587-591.
32. Kotze MJ, Langenhoven E, Peeters AV, Theart L, Oosthuizen CJJ. Detection of two point mutations causing familial defective apolipoprotein B-100 by heteroduplex analysis. *Mol Cell Probes* 1994; **8**: 513-518.
33. Rideout WM, Coetzee GA, Olumi AF, Jones PA. 5-Methylcytosine is an endogenous mutagen in the human LDL receptor and P53 genes. *Science* 1990; **247**: 1288-1290.
34. Cooper DN, Youssoufian H. The CpG dinucleotide and human genetic disease. *Hum Genet* 1988; **78**: 151-155.
35. Nurse GT, Weiner JS, Jenkins T. *The Peoples of Southern Africa and their Affinities*. Oxford: Clarendon Press, 1985: 218-224.
36. Pedersen JC, Berg K. Normal DNA polymorphism at the low density lipoprotein receptor (LDL-R) locus associated with serum cholesterol levels. *Clin Genet* 1988; **34**: 306-312.
37. Humphries S, Coviello DA, Masturzo P, Balestreri R, Orecchini G, Bertolini S. Variation in the low density lipoprotein gene is associated with differences in plasma low density lipoprotein cholesterol levels in young and old normal individuals from Italy. *Arterioscler Thromb* 1991; **11**: 509-516.
38. Kotze MJ, Davis HJ, Bissbort S, Langenhoven E, Brusnick J, Oosthuizen CJJ. Intrafamilial variability in the clinical expression of familial hypercholesterolemia: importance of risk factor determination for genetic counselling. *Clin Genet* 1993; **43**: 295-299.
39. Kotze MJ, Langenhoven E, Kriek JA, Oosthuizen CJJ, Retief AE. DNA screening of hyperlipidemic Afrikaners for familial hypercholesterolemia. *Clin Genet* 1992; **42**: 43-46.

Accepted 24 May 1994.

## Supplemented low-protein diets — are they superior in chronic renal failure?

M. G. Herselman, E. C. Albertse, C. J. Lombard, C. R. Swanepoel, F. S. Hough

Twenty-two patients with chronic renal failure were randomly assigned to a conventional low-protein diet containing 0,6 g protein/kg/day or a very-low-protein diet containing 0,4 g protein/kg/day supplemented with essential amino acids; they were followed up for 9 months. There were no significant changes in body mass index, arm muscle area, percentage body fat, serum albumin and transferrin levels in any of the groups; neither was there any difference between the groups in respect of these parameters. Renal function, as measured by the reciprocal of serum creatinine over time, stabilised in both groups during intervention, with no significant difference between the groups. There was however no correlation between changes in renal function and changes in blood pressure, or dietary intake of protein, phosphorus, cholesterol, polyunsaturated and saturated fatty acids. There were also no significant changes and no significant differences between the groups in serum levels of parathyroid hormone and alkaline phosphatase, urine cyclic adenosine monophosphate, tubular reabsorption of phosphate, and the theoretical renal threshold for phosphate.

The results of this study suggest that the supplemented very-low-protein diet was not superior to the conventional low-protein diet in terms of its effect on protein-energy status, renal function and biochemical parameters of renal osteodystrophy.

*S Afr Med J* 1995; **85**: 361-365.

Research has shown substantial support for the thesis that dietary intervention slows the progression of chronic renal failure.<sup>1-3</sup> Although renal function in earlier studies was based on changes in serum creatinine ( $S_{cr}$ ), the protective effect of a low-protein (LP) diet was confirmed by Ihle *et al.*<sup>4</sup> who used

Departments of Human Nutrition and Endocrinology and Metabolism, University of Stellenbosch, Tygerberg, W. Cape

M. G. Herselman, PH.D.

F. S. Hough, F.C.P., M.MED., M.D.

Nutrition Consultant, PO Box 204, Groenkloof, 0027

E. C. Albertse, PH.D.

Division of Biostatistics, Medical Research Council, Parowvallei, W. Cape

C. J. Lombard, PH.D.

Renal Unit, Department of Medicine, Groote Schuur Hospital and University of Cape Town

C. R. Swanepoel, M.R.C.P.