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# Linkage study of the low-density lipoprotein-receptor gene and cholesterol levels in an Afrikaner family

## Quantitative genetics and identification of a minor founder effect

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### Summary

Overlap of clinical and biochemical characteristics between hypercholesterolaemia in members of the general population and familial hypercholesterolaemic (FH) individuals may lead to misdiagnosis. Quantitative analysis of family data may circumvent this problem. A way of looking for an association between plasma cholesterol levels and restriction fragment length polymorphism markers (RFLP) on the low-density lipoprotein (LDL) receptor gene by using reference cholesterol distributions was explored. Linkage, with a logarithm of the odds (LOD) score of 6,8 at  $\theta$  0, was detected between cholesterol levels and the LDL receptor in an extended Afrikaner family. Two RFLP-haplotypes, one previously found in a majority of Afrikaner FH homozygotes, and a second, *Stu I* -, *BstE II* +, *Pvu II* +, *Nco I* +, were associated with high cholesterol levels in this pedigree.

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Familial hypercholesterolaemia (FH), an autosomal dominant disease with a gene dosage effect, is characterised by raised plasma cholesterol levels, tendon xanthomas, and an increased risk of myocardial infarction at a young age.<sup>1</sup> Elevated cholesterol levels are the result of an inability of abnormal low-density lipoprotein (LDL) receptors to bind and internalise LDL, a major cholesterol-carrying particle in the blood.<sup>1</sup> At a prevalence of more than 1:100 among Afrikaners, FH is at least 5 times more prevalent in this group than has been described in other countries.<sup>2</sup> Evidence for founder effects includes: (i) shared ancestors in the majority of FH subjects (M. Torrington — unpublished data); (ii) a similar biochemical

defect revealed in LDL-receptor studies on a host of FH homozygotes;<sup>3</sup> and (iii) a single restriction fragment length polymorphism (RFLP) haplotype present in a majority of FH homozygotes.<sup>4,5</sup> In comparison, other haplotypes are known to be present in FH patients from other groups of the South African population (black, coloured and whites of non-Afrikaner origin (H. Henderson — personal communication).

The definitive diagnosis of heterozygous FH in individuals can be difficult. A dividing point of a plasma cholesterol level of 7 mmol/l between FH in the Afrikaner (as used by Seftel *et al.*)<sup>6</sup> and hypercholesterolaemia in members of the general population will neither exclude nor diagnose gene carriers every time.<sup>7</sup> For example, the population mean serum cholesterol level for women aged between 55 years and 64 years is 17,6 mmol/l.<sup>7</sup> Sex, age, population cholesterol background, population frequency of FH and pedigree are important parameters to consider in the probability of an individual having FH. Xanthomas, although virtually pathognomonic, are not always present. FH homozygotes, however, seem to identify themselves clearly, both at a clinical, plasma cholesterol and studies at a cellular level.<sup>3</sup>

Instead of using absolute criteria for diagnosis, it is proposed that quantitative diagnostic methods be explored. One way of doing this is explored in this article, i.e. the identification of linkage between an LDL receptor-associated RFLP haplotype and cholesterol level. The probable haplotype origin could be traced genealogically through 12 generations (see p. 289).<sup>8</sup>

### Methods

#### General principles

A prior assumption of FH segregating in a pedigree is made on the presence of early coronary heart disease, and/or xanthomas and a raised plasma cholesterol level in some individuals within the family. Cholesterol levels, adjusted to correct for age and sex, are then tested for linkage to the LDL-receptor locus using reference cholesterol phenotypic distributions for FH and for the general population.

#### Specific methods

The cholesterol levels of the individuals in this investigation were adjusted to that of the age group 25 - 34 years using the

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formula, adapted from Goldstein *et al.*:<sup>9</sup> observed plasma cholesterol level, minus mean level of plasma cholesterol in the particular age and sex group to which the individual belongs, plus the mean cholesterol level in the age group (25 - 34 years) of the same sex.

The age group 25 - 34 years was selected as the standard to adjust to since the reported difference between means and standard deviations for male and female are least in this age group when compared with other age groups.<sup>7</sup>

For the FH phenotypic reference distributions a previously well-characterised group of FH homozygotes and their obligate heterozygotic parents were used.<sup>3</sup> Plasma cholesterol levels were adjusted as described and the means and standard deviations are shown in Table I. For the general population published phenotype plasma cholesterol values for the age group 25 - 34 years were used.<sup>7</sup> Normal distributions of the adjusted plasma cholesterol values were assumed and standard deviations calculated. The age group < 15 years was treated as if belonging to the group 15 - 19 years; those > 64 years as 64 years.

**TABLE I. REFERENCE DISTRIBUTIONS: MEANS AND STANDARDS (mmol/l CHOLESTEROL)**

	Mean	Standard
Population <sup>7</sup>	5,72	1,31
FH heterozygotes (N = 19)	8,62	1,23
FH homozygotes (N = 19)	21,49	3,91

No transformation was done on triglycerides, since this was not used for analysis.

Genotype likelihoods for given phenotypes, and from the likelihoods of logarithm of the odds (LOD) scores,<sup>10</sup> were calculated to determine the most probable recombination fraction between the trait and the marker locus. This calculation was done on a personal computer with the LIPED genetic linkage program developed by Ott,<sup>11</sup> using RFLP and transformed cholesterol data from individuals, population RFLP frequencies and the parameters of the reference cholesterol distributions. A LOD score of more than 3 for a recombination fraction  $\theta$  less than 0,5 was accepted as evidence of linkage.<sup>10</sup> Closeness of linkage depended on where the LOD score peaked between  $\theta$  0 and 0,5.

RFLPs at the LDL receptor locus were detected using methods previously described by our group.<sup>4</sup> The two alleles for each DNA restriction enzyme used were called '−' or '+', where '−' and '+' denotes the absence or presence of a restriction site. Subjects were asked to fast for 12 hours before blood was drawn. Lipograms were performed by the Tygerberg Hospital chemical pathology laboratory.

**Results**

Relevant pedigree, clinical, biochemical and genetic data are shown in Fig. 1 and Table II.

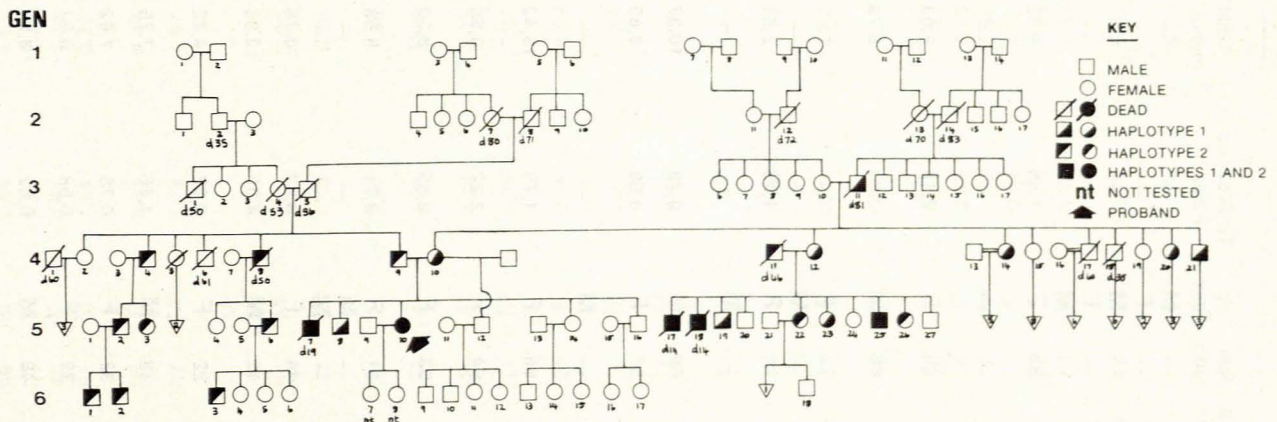
Four RFLPs were identified by DNA restriction enzymes, namely *Pvu* II,<sup>12</sup> *Stu* I,<sup>13</sup> *BstE* II<sup>14</sup> and *Nco* I.<sup>15</sup> The highest LOD score for a single enzyme was 3,5 at  $\theta$  0 for *Stu* I.

On examining the pedigree it was clear that only certain individuals were informative for *Stu* I. None of the 4 enzymes used in isolation were informative in all pedigree members. Therefore, assuming no recombination at an intragenic level, the haplotypes formed by the 4 RFLPs were used to recalculate the LOD score. Out of 16 possible combinations of the 4 RFLPs, 6 haplotypes have been identified in the general population (Fig. 2). Most pedigree members were informative for this allele system of 6 haplotypes. By using these haplotypes as markers the LOD score was recalculated and a score of 6,8 at  $\theta$  0 was obtained.

By inspection it was clear that in this pedigree two haplotypes, a common one previously shown to be present in a majority of Afrikaner FH homozygotes (since extended to 4 RFLPs, haplotype No. 1, Fig. 2<sup>4</sup>) and a second haplotype (haplotype No. 2, Fig. 2) are segregating. Individuals 4.9, 4.11 and some of their affected sibs (Fig. 1) carry the latter haplotype. This second haplotype has also been identified in a single family in the previously described host of homozygotes (kindred 2)<sup>4</sup> and in another Cape family. A common ancestral link between individuals 4.9 and 4.11 in the pedigree (Fig. 1), kindred 2<sup>4</sup> and the other Cape family could be traced to an immigrant(s) who came to the Cape 12 generations ago in 1692 (see Torrington and Brink<sup>8</sup>).

**Discussion**

In this study a quantitative way of testing linkage was explored, and close linkage was detected between the LDL-receptor locus and cholesterol levels in an extended pedigree in which



**Fig. 1.** The segregation of haplotypes 1 and 2 is shown. It generally coincides with high (> 7 mmol/l) adjusted plasma cholesterol levels as can be verified from Table II. An exception is individual 6.1 whose adjusted cholesterol level is 6,98 mmol/l. However the latter person inherited haplotype 2 and therefore FH. Note that unrelated individuals 4.9 and 4.11 share the same RFLP haplotype. The latter's haplotype could be deduced from the known haplotypes of his children and spouse. Of interest are individuals 5.10 and 5.25 who inherited both haplotypes 1 and 2. They also have both the highest absolute and adjusted cholesterol levels in the pedigree. This agrees with the inheritance of two genes (a double dose) at the same locus causing hypercholesterolaemia.



TABLE II. SUMMARY OF CHOLESTEROL, CLINICAL AND HAPLOTYPE DATA OF PEDIGREE

Pedigree position*	Age (yrs)	Sex	Triglyceride (mmol/l)	Cholesterol			Tendon xanthoma	Myocardial infarction	Haplotype		Comment
				Total (mmol/l)	Adjusted cholesterol levels (mmol/l)	HDL total cholesterol			S B P N	S B P N†	
2.2	—	M	—	—	—	—	—	—	—	—	d, 35, heart disease
2.7	—	F	—	—	—	—	—	—	—	—	d, 80, heart disease
3.1	—	M	—	—	—	—	—	—	—	—	d, 50, heart disease
3.4	—	F	—	—	—	—	—	—	—	—	d, 53, heart disease
3.5	—	M	—	—	—	—	—	—	—	—	d, 56, heart disease
3.10	85	F	1,55	6,55	5,11	0,24	—	—	(+ ---)	—	—
3.11	—	—	—	—	—	—	—	—	(+ ---)	—	—
4.3	63	F	2,18	6,03	5,52	0,20	—	—	(+++)	—	Deduced haplotype, d, 81, heart disease
4.4	65	M	0,91	8,79	8,17	0,16	—	+	+ ---+	—	58, MI
4.5	—	F	—	—	—	—	—	—	—	—	—
4.6	—	M	—	—	—	—	—	—	—	—	—
4.7	71	F	1,35	7,51	5,87	0,19	—	—	+ - + +	—	d, 61, heart disease
4.8	—	M	—	—	—	—	—	—	(+ + + +)	—	—
4.9	69	M	0,79	10,37	9,75	0,08	+	—	(?)	—	Deduced haplotype, d, 50, heart disease
4.10	57	F	0,58	8,89	7,25	0,11	—	—	- + + +	—	—
4.11	—	M	—	—	—	—	—	—	+ ---+	—	—
4.12	56	F	1,79	15,42	13,78	0,09	—	—	(+ + - +)	—	Deduced haplotype d, 46, heart disease
4.14	64	F	2,22	9,56	7,92	0,14	—	—	(- + + +)	—	—
4.15	53	F	2,65	8,09	6,98	0,12	—	—	+ ---+	—	—
4.16	51	F	3,71	8,58	—	0,09	—	—	+ ---+	—	—
4.17	—	M	—	—	—	—	—	—	—	—	—
4.18	—	M	—	—	—	—	—	—	—	—	d, 40, heart disease
4.20	44	F	0,93	10,76	10,40	0,12	—	—	+ ---+	—	d, 35, heart disease
4.21	42	M	4,08	12,95	12,51	0,07	—	—	+ ---+	—	—
5.1	22	F	0,67	4,42	4,83	0,25	—	—	+ ---+	—	—
5.2	38	M	1,46	7,75	7,31	0,14	—	—	+++ +	—	—
5.3	32	F	0,75	7,62	7,62	0,16	—	—	+++ +	—	—
5.5	39	F	0,68	6,91	6,55	0,20	—	—	+ - + +	—	—
5.6	48	M	0,82	8,99	8,32	0,10	+	—	+ - + +	—	—
5.7	—	M	—	—	—	—	—	—	+ - + +	—	—
5.8	30	M	1,22	11,99	11,99	0,09	—	—	+ - + +	—	d, 19, SD
									+ ---+	—	—



5.9	39	M	0,89	4,63	4,19	0,22	—	—	—	—
5.10	32	F	0,54	17,58	17,58	0,05	+	—	+ ---	Clinical homozygote
5.11	40	F	0,44	4,73	4,37	0,28	—	—	—	—
5.12	41	M	1,24	6,29	—	0,14	—	—	—	—
5.14	39	F	0,31	4,99	4,63	0,27	—	—	—	—
5.16	37	M	0,74	4,97	—	0,23	—	—	—	—
5.17	—	M	—	—	—	—	—	—	—	d, 14, heart disease
5.18	—	M	—	—	—	—	—	—	—	d, 14, heart disease
5.19	27	M	0,99	7,20	7,20	0,10	—	—	+ ---	—
5.21	34	M	1,46	5,10	5,10	0,15	—	—	—	—
5.22	30	F	0,60	7,89	7,89	0,15	—	—	—	—
5.23	22	F	2,66	9,60	10,01	0,13	—	—	—	—
5.24	25	F	0,70	5,38	5,79	0,28	—	—	—	—
5.25	17	M	0,25	17,08	19,39	0,03	+	—	—	Clinical homozygote
5.26	20	F	0,45	10,09	11,03	0,11	—	—	—	—
6.1	6	M	0,36	5,69	6,98	0,20	—	—	—	—
6.2	8	M	0,56	7,34	8,63	0,15	—	—	—	—
6.3	11	M	0,7	8,39	9,68	0,12	—	—	—	—
6.4	8	F	0,46	4,76	5,40	0,29	—	—	—	—
6.5	13	F	0,61	4,58	5,22	0,25	—	—	—	—
6.6	16	F	—	—	—	—	—	—	—	—
6.7	8	F	0,55	6,92	—	0,24	—	—	—	—
6.8	6	F	0,55	7,25	—	0,24	—	—	—	—
6.9	8	M	0,38	4,06	—	0,23	—	—	—	—
6.11	7	F	0,69	4,29	—	0,19	—	—	—	—
6.12	14	F	0,60	4,94	—	0,26	—	—	—	—
6.14	14	F	0,44	4,50	—	0,31	—	—	—	—
6.15	16	F	0,59	5,02	—	0,24	—	—	—	—
6.16	11	F	0,74	5,43	—	0,23	—	—	—	—
6.17	8	F	0,81	3,07	—	0,24	—	—	—	—
6.18	8	M	0,57	3,51	4,80	0,33	+	—	—	—

\*Refers to individuals as they appear in pedigree in Fig. 1.

†S B P N denotes haplotypes of variable restriction sites identified by the enzymes *Stu* I, *Bst* E II, *Pvu* II, *Nco* I in the order that they appear on the LDL-receptor gene. (— = absence; + = presence of a restriction site).  
 HDL = high-density lipoprotein; d = died; numeral = age (yrs); MI = myocardial infarction; SD = sudden death.



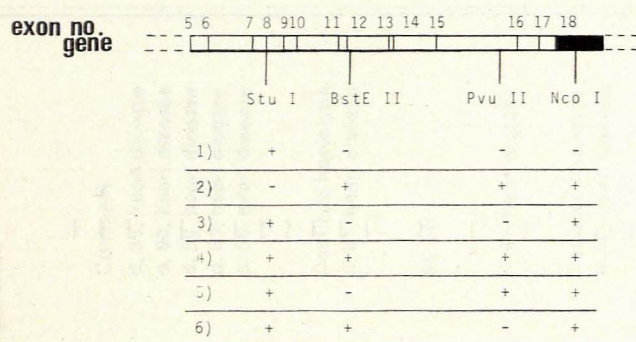


Fig. 2. Position of, and haplotypes formed by, variable restriction sites for enzymes used. Shown horizontally is a linear diagram of the 3' end of the LDL receptor gene. Dark areas with numerals 5 - 18 at the top indicate exons.<sup>17</sup> Shown below the restriction map are haplotypes 1 - 6 detected in the general population. Haplotypes 1 and 2 segregate with FH in this study.

some members have typical FH. The identification of a linked RFLP haplotype allows founder members to be identified.<sup>8</sup>

Instead of making the diagnosis of FH in specific individuals, a previous assumption of FH segregating in the pedigree was made. A bi- or tri-modal distribution of cholesterol level was to be expected if this assumption was true. However, the effect of a defective LDL receptor can be masked by factors such as sex, age, population lipid profile and other unknown elements. An adjustment to a uniform age and sex was therefore necessary. In a small data set it can be difficult to differentiate between a skewed distribution and a bi- or tri-modal distribution. For this reason, a reference distribution was created. In the presence of strong evidence of close linkage (an LOD score of 6,8 at  $\theta$  0), it can be accepted that the assumption of a tri-modal distribution is correct and that the LDL receptor locus plays a causal role.<sup>10</sup> This does not exclude the possibility that a gene closely linked to the LDL receptor may be causing the raised cholesterol levels. The latter possibility is, however, unlikely. No gene associated with cholesterol metabolism that is close to the LDL receptor gene has been described. The previous probability of finding another cholesterol-associated gene in this way has been calculated<sup>16</sup> as 0,005.

A criticism that may be levelled is that the reference data are not ideal. For example, published South African population lipid data do not include figures for people aged 2 - 15 years and over 65 years. Another possible criticism is that the 14 families, members of which were used to create the FH reference groups, were not closely related but taking data from all 19 available homozygotes from these 14 families means that 5 sibling pairs were used. However, FH homozygotes are rare in the population and this is the only host of Afrikaner homozygotes and their parents that is well defined.<sup>3</sup> The data are probably valid only for this population group at present. Changes in lifestyle and diet may change the assumptions about the reference distributions in the future.

Risk associated with FH may have been different in the past. It should be noted that members of generations 2 and 3 in respect of haplotype 1 attained ages well above those generally observed in this form of FH. Factors other than defects at the LDL receptor-gene locus may have contributed to this. Quite a few early deaths were, however, recorded in previous generations for the line of descent of haplotype No. 2, so there may perhaps be a difference in severity between the two genotypes.

It is felt that the method advocated in this article could be useful in establishing the presence of FH — by FH a LDL receptor defect is assumed — in families. It should be especially useful in families in which a suspicion of the presence of

FH exists but pathognomonic signs, such as xanthomas or a FH homozygote (most families), are not present.

Another way of addressing the problem of diagnosing FH is using a semi-quantitative approach with percentiles. However, the 90th percentile for plasma cholesterol levels is not effective enough.<sup>7</sup> If FH does occur at 1:100 in the Afrikaner group and if plasma cholesterol levels are mostly above the 90th percentile it still means that only 1:10 above this percentile will have FH. It should be possible — using a Bayesian approach that takes into account percentiles, population frequency of polymorphisms, FH frequency and pedigree structures — to develop effective diagnostic formulas for the Afrikaner group or other genetically defined groups.

Since FH among Afrikaners — as a result of the hypothesised founder effects — is expected to be the result of a small number of mutations, it is expected that routine tests that detect the exact mutations in this group will become available and that the use of linked markers will not then be necessary. However, FH as a result of other mutations also exists in other population groups in this country and presents a challenge in diagnosis. Some Afrikaner FH families do not belong to the founder groups. In addition, other factors that play a role in lipid metabolism have to be elucidated. For example, as is being done elsewhere,<sup>17</sup> the role that different apolipoproteins play in South African populations can be examined at a genetic level. The techniques of genetic epidemiology and molecular genetics should prove invaluable in addressing the problem of heart disease in the family context.

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