Linkage disequilibrium between a marker on the low-density lipoprotein receptor and high cholesterol levels

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Summary

We describe the presence of a linkage disequilibrium between high cholesterol levels in Afrikaner individuals and the common allele of the *Pvu* II restriction fragment polymorphism on the low-density lipoprotein (LDL) receptor gene. The frequencies of the common and the rare allele in a sample of the Afrikaner population were 0,654 and 0,346 (65 individuals) and 0,794 and 0,206 in the hypercholesterolaemic population (34 patients) ($P < 0,05$). This finding supports other evidence for a founder origin of the high frequency of familial hypercholesterolaemia among Afrikaners.

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Familial hypercholesterolaemia (FH) is an autosomally dominantly inherited form of hypercholesterolaemia with a heterozygote frequency of 1 in 100 (conservatively calculated) among Transvaal Afrikaners,[1,2] five times that reported from other Western countries.[3] A founder effect in the Afrikaner population has been suggested.[1,2,4] FH is characterized by high serum cholesterol and high low-density lipoprotein (LDL) cholesterol levels, tendon xanthomas, xanthelasma and an increased risk of myocardial infarction after the age of 35 years.[3] The homozygote frequency in South Africa is reported to be 1 in 30 000.[2] Survival for homozygously affected individuals is reported to rarely exceed 25 years.[3] Brown and Goldstein[5,6] have clearly demonstrated that the primary defect commonly resides in the LDL-receptor gene.

Afrikaners have been in South Africa for 12 - 15 generations. If a founder effect as suggested[1,2,4] is correct, the presence of a linkage disequilibrium between any marker on the LDL-receptor gene and FH could be expected in South Africa.

To test this hypothesis we looked at the frequency distribution of a bi-allelic restriction fragment length polymorphism (RFLP) on the LDL-receptor gene in a sample of Afrikaans-speaking individuals and in a sample of patients from the same population group with predominantly high cholesterol levels. Some of these patients have FH diagnosed by the usual criteria.

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Materials and methods

Sixty-five Afrikaans-speaking individuals, taken from laboratory staff, hospital staff and patients attending the general outpatient clinics of Tygerberg Hospital, were used to establish population frequencies for the respective alleles of the bi-allelic marker in the general population. No other data were obtained from these individuals.

Thirty-four (16 male, 18 female) Afrikaans-speaking individuals were used to establish the allele frequencies in the Tygerberg Hospital Lipid Clinic. The hospital serves mainly the Afrikaans-speaking population of the south-western Cape. Plasma cholesterol and triglyceride values and high-density lipoprotein cholesterol ratios were those determined by the Department of Chemical Pathology at Tygerberg Hospital on a routine basis. Individuals with only hypertriglyceridaemia were not included in the study. Where first-degree relationships were known to exist, only one member of a family was included in the study.

Human DNA was obtained from whole blood, as previously described:[7] 10 μg DNA from each individual was digested with the *Pvu* II restriction endonuclease (under manufacturer's conditions), electrophoretically separated by size on 0,6% agarose gel, washed in 0,25M HCl for 7,5 minutes to obtain efficient transfer of large DNA fragments,[8] denatured and transferred to nitrocellulose by Southern blotting.

The LDL-receptor gene probe pLDLR-2HH1 was donated by Dr D. W. Russell of Dallas and consists of a 1,9 kb fragment (base pairs 1573-3486) of the 3 end of the LDL-receptor cDNA clone.[9] Plasmid DNA was prepared and isolated by the alkaline lysis method.[10] The 1,9 kb base fragment was excised as described[10] and nick-translated to a specific activity of at least 10^8 cpm/μg probe DNA (using the nick translation kit and protocol supplied by Bethesda Research Laboratories).

After baking at 80°C until dry, the nitrocellulose filters were prehybridized for 5 minutes in a solution of 1% bovine serum albumin fraction V, 1 mM ethylene diamine tetra-acetic acid (EDTA), 7% sodium dodecyl sulphate (SDS), 0,5M $NaHPO_4$, pH 7,2. A '1M $Na\text{-}PO_4$' (pH 7,2) stock is composed of 0,5M Na_2HPO_4 and 4 ml 85% H_3PO_4/l (method modified from Church and Gilbert[11]). The filters were hybridized at 65°C for 8 - 24 hours in 10 ml of the same solution to give a minimum of 10^6 cpm/ml.[7] Post-hybridization washes consisted of two 5-minute washes at 65°C in a solution of 0,5% serum albumin fraction V, 160 mM $NaHPO_4$, pH 7,2, 1 mM EDTA, 5% SDS and six 5-minute washes at 65°C in a solution of 1 mM EDTA, 160 mM $NaHPO_4$, 1% SDS, the last wash being left for 20 minutes. The filters were then exposed to X-ray film for 1 - 4 days at −80°C.

Chi-square tests were performed to document that the populations investigated were or were not in Hardy-Weinberg equilibrium and to compare the chromosomal frequencies.

Results

Pvu II generates two bands of size 16,5 and 3,6 kb in the absence and 14,0, 3,6 and 2,6 kb in the presence of a variable restriction site detected with the LDL-receptor probe described (Fig. 1).[12,13]

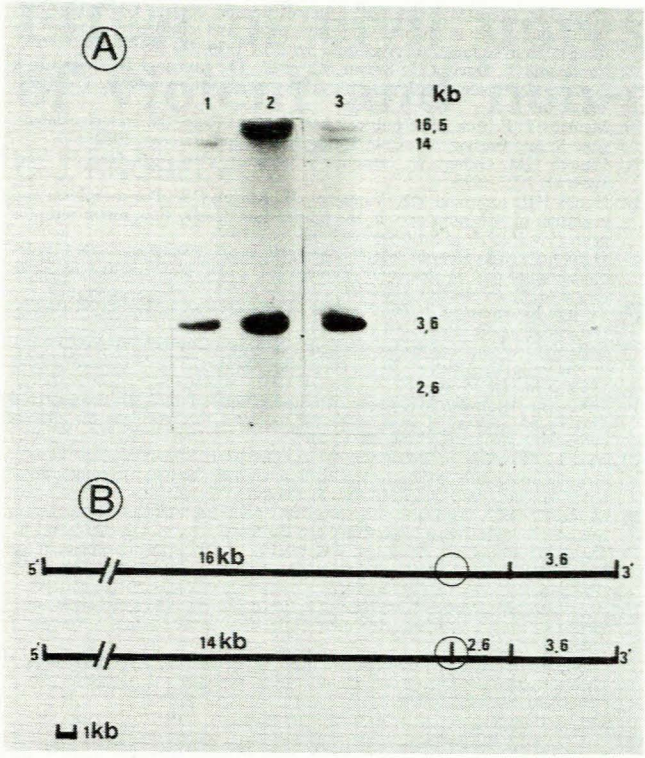

Fig. 1. *Pvu* II RFLP detected with LDL-receptor cDNA probe pLDLR-2HH1: (A) Southern blot analysis of the hybridization pattern detected for the (*i*) ++, (*ii*) −−, and (*iii*) +− genotypes; (B) diagram showing the relative positions of fragments detected on the LDL-receptor gene.[12,13] The circle shows the variable *Pvu* II restriction site.

The frequencies of the absence (−) and of the presence (+) of the polymorphic restriction site in the general Afrikaans-speaking population were 0,654 and 0,346 respectively; they differ from those reported in other populations.[12,13] The genotypes '−−', '−+', '++' are in a Hardy-Weinberg population equilibrium (Table I).[14]

The chromosomal frequencies of the alleles in the 34 hypercholesterolaemic individuals are 0,794 and 0,206 for the '−' and '+' allele respectively and the '−' is in a linkage disequilibrium compared with the general population. The hypercholesterolaemia phenotype is associated with the '−' allele more often than expected ($P < 0,5$). The genotype '−−', '+−' and '++' distribution is 20, 14 and 0 respectively for the 34 individuals and is not in a population equilibrium for this group.

Table II shows the results of lipid analysis, the family history and presence of the tendon xanthomas in the 34 patients.

TABLE I. GENOTYPE DISTRIBUTION AND ALLELE FREQUENCY OF *Pvu* II RFLP IN THE AFRIKANER POPULATION IN GENERAL AND IN INDIVIDUALS WITH HIGH CHOLESTEROL LEVELS

	No. of individuals			Frequency	
	−	+	++	−	+
General population ($N = 65$)	29	27	9	0,653	0,346
Hypercholesterolaemic population ($N = 34$)	20	14	0	0,794	0,206

$\chi^2 = 6,4$ (1 df); $P < 0,05$.

TABLE II. CHARACTERISTICS OF 34 PATIENTS ATTENDING THE LIPID CLINIC

	Findings
Age range (yrs)	14 - 66
Male/female ratio	16 : 18
Percentage	47 : 53
Total serum cholesterol (mmol/l)*	4,73 - 14,3
No. of patients with total cholesterol > 6 mmol/l	33
HDL/total cholesterol ratio (%) (range)	7 - 25
No. of patients with HDL/total cholesterol ratio < 20%	32
Serum triglyceride (mmol/l) (range)	0,11 - 10,7
No. of patients with positive family history (%)†	16 (47)
No. of patients with tendon xanthomas (%)	18 (53)

*Tygerberg Hospital Chemical Pathology Department routine tests.
†Family history denotes the presence of a myocardial infarct or sudden death before age 55 years in a first-degree relative.
HDL = high-density lipoprotein.

Discussion

There are three different causes of a linkage disequilibrium between a marker and a phenotype in a population: (*i*) the marker is the cause of the phenotype; (*ii*) the phenotype or the marker is a new mutation, not coupled, but has not reached a population equilibrium yet; and (*iii*) the marker or the phenotype is a newly introduced mutation, closely coupled and unlikely to be dissociated in successive generations. The *Pvu* II markers looked at occur elsewhere as normal variations in populations,[12,13] and the first possibility is therefore excluded as a cause of the linkage equilibrium that we detected between the '−' allele and hypercholesterolaemia in the selected study group. The fact that Afrikaners have been in South Africa for 12 - 15 generations makes the second possibility unlikely, since the markers and the hypercholesterolaemia phenotype should have reached a population equilibrium by now. This leaves a third possibility. A gene or genes causing hypercholesterolaemia coupled to the '−' allele has been introduced in the Afrikaner population.

The prevalence of FH among Afrikaners is high and a founder effect has been suggested.[1,2,4] The response to a cholesterol-lowering diet is usually poor among these individuals[15] and therefore they may cluster in specialized lipid clinics in contrast to patients who respond well to diet.

The defect is heterogeneous and has been classified into five classes on the basis of receptor studies.[16] The Afrikaner FH might be homogeneous and of the so-called receptor-defective type described by Van der Westhuyzen *et al.*[17]

The presence of the linkage disequilibrium between a marker on the LDL-receptor gene and high cholesterol levels in patients attending a lipid clinic serving a predominantly Afrikaans-speaking community is therefore not unexpected. At the same time it also provides additional evidence of a founder effect.

The availability of more RFLPs coupled to the LDL-receptor gene would aid in making a predictive diagnosis about the presence or absence of FH in individuals in 90% or more of families by doing classic linkage studies.[18] A new RFLP is described elsewhere in this issue.[19] Haplotypes (markers with several alleles) for the defect would also be established and enable researchers to address the question whether one or more abnormal LDL-receptor genes are present

in the Afrikaner population. Founders could be identified and genetic counselling done more effectively.[20] Family studies are in progress.

It should in addition be possible to investigate the question why receptor-defective FH is clinically so variable and, generally speaking, less severe than other described defects[21] by correlating the vertical transmission of defective genes in families with clinical and blood lipid studies.

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Nuus en Kommentaar/News and Comment

Verswakte bestuursvermoë — vergeet nie die ander medisyne nie

Die meeste dwelms wat die sentrale senustelsel beïnvloed, het ook die potensiaal om 'n motorbestuurder se vermoë te verswak. In hierdie groep wek alkohol steeds die meeste kommer, aangesien dit verreweg die grootste oorsaak van motorongelukke is. Maar namate al hoe meer middels soos bensodiasepien in gebruik geneem word, moet die kollig ook op ander dwelms gerig word.

'n Konsensuspaneel van die nasionale instituut vir dwelmmisbruik in die VSA (*JAMA* 1985; **254:** 2618) het hierop ingegaan en o.m. gevind dat die misbruik van psigoaktiewe middels aan die toeneem is en dat beter chemiese ontledingstegnieke nodig is om verswakte bestuurvermoëns by motorryers te bewys. Daar bestaan nog nie 'n tegniek om dwelms soos marijuana (dagga) of antihistamiene en bensodiasepiene doeltreffend in die liggaam te peil nie en daar is min twyfel dat navorsing in hierdie rigting versnel moet word.

Die eerste oorweging vir sulke navorsing moet egter gaan oor die invloed van marijuana en bensodiasepiene, want die is die algemeenste dwelms in gebruik. 'n Bykomstige studie is ook nodig om die uitwerking van dwelms in kombinasie met alkohol te bepaal.

Die paneel sê dat alhoewel die inligting oor die uitwerking van alkohol op motorbestuurders al langer as 'n eeu beskikbaar is, hierdie kennis ver van volledig is en sekere aspekte beslis verder ondersoek moet word. Realistiese definisies met betrekking tot die uitwerking van ander dwelms op die bestuursvermoë van 'n motorryer is gegrond op ons ondervinding met alkohol, maar die grense van bestuursvermoë is nog nie bepaal nie. Eers as dít gedoen is, kan die ware werking van ander dwelms benewens alkohol gepeil word. Baie faktore soos liggaamsgewig, genetiese en omgewingsinvloede sal in die studie in gedagte gehou moet word en metodes om werklike toestande waarin die motorryer hom bevind te simuleer, sal waarskynlik die beste resultate in dié toetse lewer.

Hepatocellular carcinoma by perinatal transmission

Hepatocellular carcinoma, one of the most intractable tumours to treat, may well also be the commonest in the world. Its relation to hepatitis B virus (HBV) infection and the subsequent HbsAg carrier-state is now quite clear. The viral infection may be acquired from tattooing, ritual circumcision, scarification by local healers, or less frequently by blood transfusion or sexual intercoure.

However, there is another possibility, namely infection of an infant immediately after birth from a mother who is a carrier of the HBV surface antigen. Trounce *et al.* (*Q J Med* 1985; **57:** 791) report the very unusual case of a 9-year-old boy born of Chinese parents in England and adopted by an English couple at an early age who presented with primary hepatocellular carcinoma in a non-cirrhotic liver. There was no past history of hepatitis or of blood transfusion and he had not left England since his adoption. His serum contained hepatitis B surface antigen and 'E' antibody and the authors conclude that it is highly probable that this boy acquired his HBV infection from his mother by perinatal transmission.

The risk of transmission of HBV from mother to baby is estimated to be 95% if mother is an HBeAg carrier, 70 - 90% if clinical hepatitis B occurs in the last trimester of pregnancy, and 20% if mother is an HBsAg carrier but negative for both HBeAg and anti-HBe.

Doctors in the UK are likely to discover more of these cases as time goes by, with the large Third World community in the country. The authors of the report believe that all mothers in high HBsAg-carrier groups should be screened during pregnancy and if positive their infants should be given immunobulin to induce passive immunity straight away, preferably in the delivery room. Active vaccination should also be instituted at birth. Now that both passive and active immunoprophylaxis is possible, the eventual eradication of this very dangerous HBV infection has become a possibility, but obviously not for many years to come.