High frequency of the Lebanese allele of the LDLr gene among Brazilian patients with familial hypercholesterolaemia

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Abstract

We analysed the LDL receptor (LDLr) gene in 18 Brazilian patients with familial hypercholesterolaemia (FH) from 10 unrelated families. The combination of a direct search for the Lebanese allele of the LDLr gene by a PCR method and Southern blotting using cDNA probes allowed the identification of the gene defect in six out of 10 families. The Lebanese allele was found in five families and in one family the disease was caused by a 4 kb deletion in the 3' half of the LDLr gene. The results indicate an important contribution of the Lebanese allele to the prevalence of FH in the Brazilian population and suggest that it may also be the most common cause of FH in other mixed populations outside the Middle East.

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Familial hypercholesterolaemia (FH) is a common autosomal dominant disorder caused by a defect of the low density lipoprotein receptor (LDLr).[1] It affects about one in 500 subjects in western populations and is characterized by raised serum LDL cholesterol levels, tendon xanthomas, and premature coronary heart disease.[1,2] The analysis of the LDLr gene has led to the identification of more than 40 different mutations which include deletions, point mutations, and insertions.[3] Specific mutations have achieved high frequencies in certain populations, probably owing to a founder effect: Afrikaner-1 and Afrikaner-2 alleles in the Afrikaner population of South Africa, French Canadian-1 allele in the French Canadian population, Helsinki allele in the Finnish population, and Lebanese allele in Lebanese and Syrian populations.[3] The Lebanese allele was described and characterised by Lehrman *et al*[4] in four unrelated Arab patients with homozygous FH (the mutation accounted for all eight mutant alleles). Recently, Oppenheim *et al*[5] showed the presence of the Lebanese allele in five Israeli families with FH. No other study has been conducted to confirm the high incidence of the Lebanese allele in Arab populations, nor has this mutation been investigated in populations outside the Middle East.

In this paper we describe the results of the analysis of the LDLr gene by a direct search method for the Lebanese allele and Southern blotting in 18 Brazilian patients with FH.

Patients and methods

PATIENTS

We studied 18 Brazilian patients with FH (17 heterozygotes and one homozygote) from 10 unrelated and unselected families (table). All patients were followed up routinely at the University Hospital. The diagnosis of heterozygous FH was based on the following criteria: (1) total plasma and LDL cholesterol (LDL-c) levels above the 95th centile for age and sex, (2) triglyceride levels below 400 mg/dl

Clinical features and ethnic ancestry of the patients, and results of the PCR analysis.

Patient and family	Age (y)	Cholesterol (mmol/l)		Triglycerides (mmol/l)	Tendon xanthomas	Coronary heart disease*	Ethnic ancestry	Lebanese allele†
		Total	LDL-c					
1,A	36	10.4	7.9	2.7	No	No	Lebanese	+/−
2,A	40	8.4	6.5	2.6	No	No	Lebanese	+/−
3,A	37	10.8	8.7	1.7	No	No	Lebanese	+/−
4,B	57	7.4	5.5	1.6	No	Yes	Portuguese	−/−
5,C	64	13.7	11.2	2.7	No	Yes	Italian	−/−
6,D	38	8.5	6.9	2.0	No	No	Lebanese	+/−
7,E	58	9.6	7.5	3.1	No	Yes	Syrian	+/−
8,F	20	9.6	7.4	2.3	Yes	Yes	Lebanese	+/−
9,F	46	10.1	7.4	2.1	Yes	Yes	Lebanese	+/−
10,G	52	7.3	5.4	0.7	No	No	Portuguese	−/−
11,G	50	8.2	6.3	3.6	No	No	Portuguese	−/−
12,H	49	9.3	7.5	1.8	No	No	Italian	−/−
13,H	69	8.9	6.5	1.3	No	No	Italian	−/−
14,H	45	7.4	5.7	2.2	No	No	Italian	−/−
15,I	65	7.1	5.2	1.5	No	Yes	Italian	−/−
16,J‡	34	15.8	14.1	1.2	Yes	Yes	Lebanese	+/−
17,J	54	6.9	5.1	2.8	Yes	No	Lebanese	+/−
18,J	47	10.4	8.5	1.5	No	No	Lebanese	+/−

* Coronary heart disease confirmed by coronary arteriography.
† +/− = heterozygote for the Lebanese allele, −/− absence of the Lebanese allele.
‡ Clinical and laboratory data compatible with homozygous FH, all other patients were diagnosed as heterozygotes for FH.
LDL-c, low density lipoprotein cholesterol.

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(4·5 mmol/l), and (3) autosomal transmission. In addition to the criteria above, the homozygote had multiple tuberous and tendon xanthomas, several episodes of angina, and severe coronary atherosclerosis shown by coronary arteriography at the age of 26; the patient died of septicaemia at the age of 34. Fasting plasma cholesterol, high density lipoprotein cholesterol, and triglyceride levels were determined using commercially available kits (Boehringer Mannheim). The levels of LDL cholesterol were estimated according to the Friedewald equation.[6] The ethnic ancestry of each patient was ascertained by a questionnaire answered at the first clinical evaluation. All patients of Arab descent reported parents or grandparents born in Lebanon or Syria. The patients belonging to the other ethnic groups reported a remote European ancestry.

DNA ANALYSIS

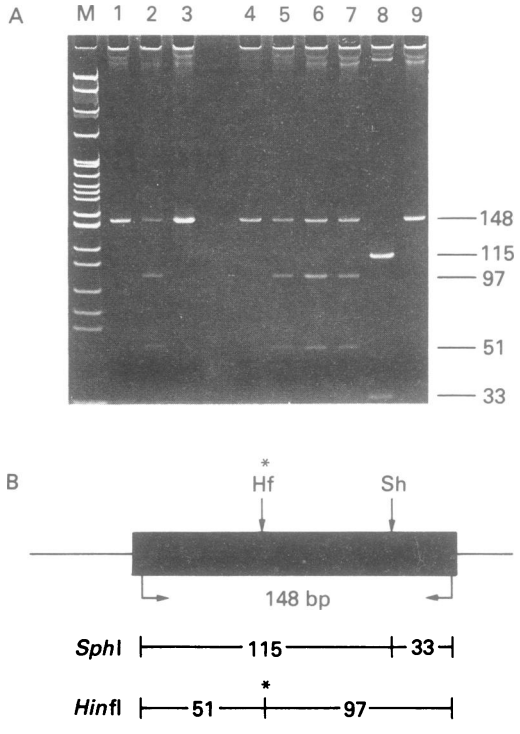
Genomic DNA was isolated from peripheral blood cells by phenol/chloroform extraction.[7] The DNA from the homozygous patient was isolated from a paraffin embedded liver fragment that was obtained at necropsy.[8]

Polymerase chain reaction (PCR)

To detect the Lebanese allele we used the primers 5'-GAACTGGTGTGAGAGGACCA-3' and 5'-TGTGAGGCAGCTCCTCATGT-3' designed for the selective amplification of exon 14 of the LDLr gene. The oligonucleotides were synthesised on an Applied Biosystem 391 oligonucleotide synthesiser, based on the cDNA sequence previously reported.[9] The PCR was performed in 25 µl reaction volume containing 100 ng of genomic DNA, 10 mmol/l Tris-HCl pH 8·5, 50 mmol/l KCl, 1·5 mmol/l $MgCl_2$, 0·01% gelatin, 200 µmol/l of each dNTP, 0·25 µmol/l of each primer, and 1 U *Taq* DNA polymerase; 35 cycles of amplifications were carried out (94°C for 30 seconds, 55°C for 10 seconds, and 72°C for one minute). For the amplification of DNA obtained from the liver tissue we used 2 U *Taq* DNA polymerase and a slight modification of amplification cycles (94°C for 30 seconds, 55° for 50 seconds, and 72°C for one minute). All other parameters were as above. The specificity of the amplified 148 bp segment was confirmed by digestion with the enzyme *Sph*I, which generates two fragments (115 and 33 bp) (figure). The Lebanese allele was identified after digestion with the enzyme *Hin*fI, since the mutation generates a new *Hin*fI site in exon 14 of the LDLr gene (figure).

Southern blotting

Degradation of the DNA obtained from the fixed liver tissue precluded its analysis by this method. For all other samples, 10 µg DNA were digested with *Bam*HI, *Eco*RI, or *Xba*I restriction enzymes, separated on 1% agarose gel, and blotted onto Gene Screen Plus membranes (DuPont).[7] The filters were hybridised to fragments of full length human LDLr cDNA (obtained from clone pLDLR3, purchased from American Type Culture Collection, Rockville, Maryland) at 42°C for 16 to 48 hours, and washed at 65°C for one hour. The probes were labelled with [α-^{32}P]dCTP by the random primer labelling method.[7] The membranes were exposed to *x* ray film (X-Omat AR, Kodak) with intensifying screens at −70°C from one to seven days.



(A) Amplified DNA separated on 6% polyacrylamide gel. Lane M, molecular weight marker (pBR322/MspI), lanes 1 and 9, undigested PCR products, lanes 2-7, amplified DNA from patients 3–8 respectively, digested with HinfI, lane 8 PCR product digested with SphI. (B) Schematic representation of exon 14 of the LDLr gene. The primers and restriction enzymes sites, and the expected fragment sizes are indicated. Sh = monomorphic SphI site; Hf = mutant HinfI site.

Results

With the two combined approaches (PCR and Southern blotting) we were able to identify the mutant allele in six out of 10 families. The Lebanese allele was identified in nine patients (eight heterozygotes and one homozygote) from five families (figure) and was associated with Arab ancestry in all cases (table). The analysis performed on the DNA sample from the paraffin embedded liver tissue (patient 16) was repeated three times and the heterozygous presence of the Lebanese allele was confirmed in all experiments. Furthermore, analysis of DNA samples from two other affected members of the same family confirmed the presence of the Lebanese allele.

Southern blotting of the DNA sample from patient 5 showed a heterozygous 4 kb deletion in the 3' half of the LDLr gene (data not shown). All other samples showed a normal hybridisation pattern.

Based on the clinical and laboratory data, the homozygous patient may be regarded as a compound heterozygote for the Lebanese allele and an unidentified mutant.

Discussion

This is the first report of a systematic search for the Lebanese allele as a cause of FH outside the Middle East. The Arab contribution to the composition of the Brazilian population is negligible, although exact figures are not available.[9] In the present study, we found Arab ancestry in five out of 10 unselected families with FH, always associated with the presence of the Lebanese allele (table). These results indicate an important contribution of this ethnic group to the prevalence of FH in Brazil, far in excess of the proportional contribution of persons of Arab ancestry to the Brazilian population. This would be explained by the higher frequency of FH in Arabs when compared to the other ethnic groups that form the majority of the Brazilian population (whites of European ancestry and blacks[10]). We may speculate that similar results may be found in other mixed populations.

In addition, the results corroborate the findings of Lehrman *et al*[4] and Oppenheim *et al*,[5] which suggest a high frequency of this mutation in the Arab population.

The PCR method allowed easy and rapid detection of the Lebanese allele in FH patients, and was applied successfully even to material obtained in unsatisfactory conditions like those described for the homozygous patient, who had died six years before this study. This approach may be used to determine the contribution of the Lebanese allele to the prevalence and pathogenesis of FH in other countries with large populations of Arab origin, such as the USA, France, Germany, and Great Britain.

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