DELETION IN THE GENE FOR THE LOW-DENSITY-LIPOPROTEIN RECEPTOR IN A MAJORITY OF FRENCH CANADIANS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Abstract We found a large deletion (more than 10 kilobases) in the gene for the low-density-lipoprotein receptor in 63 percent of French Canadians with heterozygous familial hypercholesterolemia. The deletion also occurred in homozygous form in four of seven French Canadian homozygotes. The deletion removes the promoter and first exon of the gene, and it abolishes the production of messenger RNA for the low-density-lipoprotein receptor. The high frequency of this mutation is presumably related to a founder effect among the 8000 ancestors of present-day French Canadians, who have had relatively little crossbreeding with groups of other national origins.

This deletion has not been observed in any other ethnic group. It can be detected by analysis of genomic DNA from blood leukocytes, thus allowing direct diagnosis of familial hypercholesterolemia in a majority of affected French Canadians. (N Engl J Med 1987; 317: 734-7.)

THE examination of mutant human genes at a molecular level has revealed that inherited diseases are usually heterogeneous. Each disease can be produced by any of several mutations in a single gene or in a related set of genes. Heterogeneity of mutations within a single gene has been suggested by studies of the mutant proteins in the thalassemias and in glucose-6-phosphate dehydrogenase deficiency.[1] With the development of recombinant DNA techniques, this type of heterogeneity has been found to affect many mutant genes, including those responsible for the Lesch–Nyhan syndrome[2] and for hemophilia A.[3,4]

Genetic heterogeneity has created a problem in the application of recombinant DNA methods to diagnosis at the genomic level. Although a given patient may have a defined clinical syndrome, it is usually not feasible to screen his or her genomic DNA for all the mutations that are known to have occurred in the suspected gene. The field of suspicion must first be narrowed on the basis of subtle differences between the clinical effects of different mutations or through knowledge of the ethnic background of the patient. If a particular mutation accounts for most forms of the disease in one population, then it is possible to screen potentially affected persons from that population for that mutation.

One common disease in which genomic diagnosis has not been possible is familial hypercholesterolemia. This autosomal dominant disease occurs in 1 in 500 persons in most countries of the world. It produces a distinct syndrome involving hypercholesterolemia, tendon xanthomas, and myocardial infarction early in life.[5] So far, all patients with the classic genetic and clinical features of this syndrome have had evidence of a mutation in the gene for the low-density-lipoprotein (LDL) receptor, a cell-surface protein that mediates the removal of cholesterol-carrying LDL from blood. At least 12 different mutations have been recognized, and these are scattered throughout the 45-kilobase (kb) gene on chromosome 19.[6-9] This plethora of mutations makes it impractical to examine the DNA of all patients with hypercholesterolemia to determine whether they have a mutation in the LDL-receptor gene. All the mutations described to date are ones in which a messenger RNA (mRNA) for the LDL receptor is produced but the resultant receptor protein is defective. In one class of mutants, the null alleles, no detectable receptor protein is produced.[5,6]

In this paper, we report the elucidation of one mutation in the LDL-receptor gene that produces a null allele and accounts for most cases of familial hypercholesterolemia in one ethnic group. The mutation is a large deletion that removes the 5′ end of the LDL-receptor gene, which is necessary for the production of mRNA. The mutation was originally observed in four homozygotes from French Canada. Homozygotes represent the rare situation in which mutant LDL-receptor genes are inherited from both parents. These patients have a much more severe illness than heterozygotes, with plasma LDL levels more than six times higher than normal. They often, although not always, have myocardial infarctions during childhood. The large deletion that was initially observed in the French Canadian homozygotes was subsequently found in 63 percent of heterozygotes of French Canadian ancestry, thus creating the potential for genomic DNA diagnosis of mutations of the LDL receptor in this ethnic group.

METHODS

Materials

The restriction enzymes that were used for DNA blotting were obtained from New England Biolabs (Beverly, Mass.). The DNA polymerase I (Klenow fragment) used for probe preparation was obtained from Boehringer Mannheim (Indianapolis, Ind.). The [γ-^{32}P]cytidine triphosphate (3000 Ci per mmol) used for probe preparation was purchased from New England Nuclear (Boston). Biotrans nylon membranes were obtained from ICN Biomedicals (Irvine, Calif.). Hoechst dye 33258 was obtained from Polysciences (Warrington, Pa.).

Genomic Southern Blotting Analysis

Genomic DNA was isolated from cultured fibroblasts or peripheral blood as described elsewhere[10] and digested for at least two hours with a fivefold excess of restriction enzyme in the presence of the

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Supported by a research grant (HL 20948) from the National Institutes of Health, a research grant (MA 5427) from the Medical Research Council of Canada, and the Fonds de la Récherche en Santé du Québec. Dr. Russell is the recipient of a Research Career Development Award (HL 01287) from the National Institutes of Health.

buffer suggested by the manufacturer. The DNA was quantified by a fluorescence assay[11] using Hoechst dye 33258, after which an aliquot of 5 μg was redigested with a twofold excess of enzyme. The DNA was fractionated on a 0.8 percent agarose gel and transferred to a Biotrans nylon membrane. A ^{32}P-labeled single-stranded probe derived from the LDL-receptor cDNA was prepared according to the method of Church and Gilbert,[12] hybridized with the filter at 42°C for 16 hours (5×10^6 cpm per milliliter) in 50 percent (vol/vol) formamide; 0.05 percent (wt/vol) each of bovine serum albumin, Ficoll 400, and polyvinylpyrrolidone 360; 0.9 M sodium chloride; 50 mM sodium phosphate; 5 mM EDTA (pH 7.4); 1 percent (vol/vol) sodium dodecyl sulfate; and 100 μg of denatured and sonicated salmon sperm DNA per milliliter. After hybridization, the filter was washed at 68°C for four hours in 75 mM sodium chloride, 7.5 mM sodium citrate, and 1 percent sodium dodecyl sulfate, and subjected to autoradiography.

Results

Table 1 summarizes the clinical data on four French Canadians with homozygous familial hypercholesterolemia whose fibroblasts were studied. Three of these homozygotes (Patients 49, 549, and 808) had previously been found to produce no detectable mRNA for the LDL receptor as determined by RNA blotting analysis.[7] The fourth patient (859) also had no detectable mRNA (data not shown). All the homozygotes had plasma cholesterol concentrations above 600 mg per deciliter (15.5 mmol per liter), tendon xanthomas, and planar cutaneous xanthomas.

Figure 1 shows a Southern blot of a restriction digest of genomic DNA from these four homozygotes. The DNA was digested with two restriction enzymes, *Kpn*I and *Xba*I. After electrophoresis and transfer to nylon membranes, the DNA fragments were visualized by incubation with a radioactive cDNA probe that hybridizes to exon 2 in the LDL-receptor gene. DNA from normal persons yields a single restriction fragment of approximately 9 kb (A in Fig. 1). DNA from all four French Canadian homozygotes lacked this normal band and instead showed a band of 15 kb (B in Fig. 1). The 9-kb band in the normal cells bridges an *Xba*I site in the first intron and a *Kpn*I site in the second intron. In the French Canadian homozygotes, there was a deletion of at least 10 kb that removed the *Xba*I site in intron 1 as well as an *Xba*I site upstream of exon 1. As a result, the double-enzyme digest gives rise to a 15-kb fragment that bridges the *Kpn*I site in intron 2 and an *Xba*I site that is far upstream.

Figure 2 shows a genomic Southern blot using DNA from two persons of French Canadian descent with heterozygous familial hypercholesterolemia who were not related to the homozygotes shown in Figure 1. After a double digest with *Xba*I and *Kpn*I, the DNA of one of the heterozygotes showed two bands — one normal restriction fragment of 9 kb and one abnormal restriction fragment of 15 kb. This subject was presumably heterozygous for the deletion shown in Figure 1. The other heterozygote had only a normal restriction fragment. This subject must have had a mutation of the LDL-receptor gene that was different from the deletion shown in Figure 1. For comparative purposes, on the same gel we also analyzed DNA from a normal subject and from one of the homozygotes who had the abnormal 15-kb band (Fig. 2).

To estimate the frequency of the 5′ deletion among French Canadian heterozygotes, we screened DNA samples from 84 subjects who were classified clinically as having heterozygous familial hypercholesterolemia (Table 2). These subjects were all living within 50 miles (80 km) of Montreal. Each of the subjects was from a different family unit, and none of the family units were known to be related at the level of first cousins. Of the 84 heterozygotes who were screened, 53 were found to have the 5′ deletion in heterozygous form. These data indicate that 63 percent of heterozygotes in French Canada have this particular mutation. We also found that several affected persons from the same family unit had the same deletion (data not shown).

Table 1. Clinical Data on French Canadians with Homozygous Familial Hypercholesterolemia.*

Patient No	Age (yr)	Sex	History of Consanguinity	Clinical Signs of Coronary Heart Disease		Comments
				Age at onset (yr)	Signs	
49	33	F	Yes	19	Angina pectoris and claudication	Ileal bypass at age 16; died at age 33 yr of malignant melanoma
549	3	M	No	3	None before sudden death at age 3 yr from arrhythmia; severe coronary atherosclerosis at autopsy	
808	4	F	No	—	None	
859	7	F	Yes	—	None	

*Each patient had the classic clinical and genetic findings of homozygous familial hypercholesterolemia, with a plasma cholesterol concentration above 600 mg per deciliter (15.5 mmol per liter), cutaneous plasma xanthomas, and premature coronary atherosclerosis as indicated.

Discussion

Previous studies of patients with familial hypercholesterolemia have disclosed a large variety of mutations at the LDL-receptor locus on the short arm of chromosome 19.[5-9] Except in one instance, no two families have been shown to have the same mutation. The one exception has occurred in several Arab families from the Middle East, some members of which were homozygotes. Four of these homozygotes, from four different families (three from Lebanon and one from Syria), were found to have a nonsense mutation at amino acid 660.[9] The finding of this mutation in several Lebanese homozygotes suggested that it may account for the high frequency of familial hypercholesterolemia previously observed in Lebanon,[13] but it has not been possible to study a large number of heterozygotes in the Lebanese population to determine whether this is the case. Familial hypercholesterolemia also occurs with a high frequency

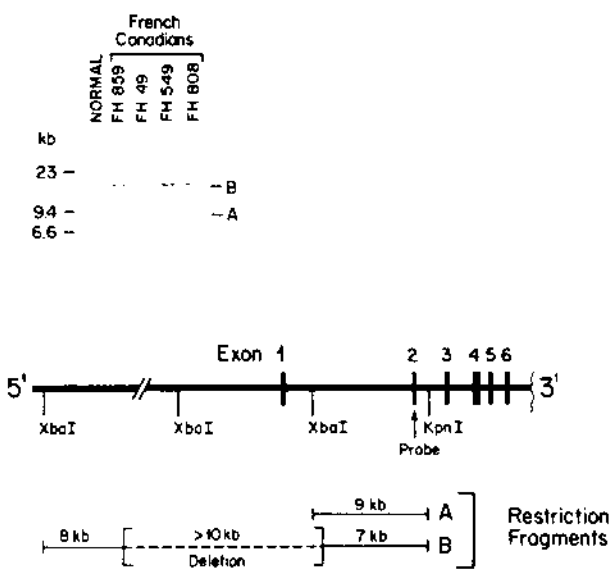


Figure 1. Southern Blot Hybridization of Genomic DNA from Four French Canadians with Homozygous Familial Hypercholesterolemia.

Total genomic DNA from each person was digested with *Kpn*I and *Xba*I, subjected to electrophoresis on a 0.8 percent agarose gel, transferred to a nylon membrane, and hybridized with a single-stranded ^{32}P-labeled cDNA probe corresponding to exon 2, as described in Methods. The filter was exposed to Kodak XAR-5 film for 36 hours at −70°C with an intensifying screen. The positions to which *Hind*III fragments of bacteriophage λ DNA migrated and their sizes in kilobases are indicated on the left side of the figure. A restriction map of the relevant region of the LDL-receptor gene is shown below the blot, with the *Kpn*I and *Xba*I sites noted. The location of the probe used for this Southern blot is indicated.

among Afrikaners in South Africa, but the nature and number of molecular defects in the LDL-receptor gene in this population have not yet been defined.

In this paper, we report a mutation that deletes at least 10 kb from the 5′ end of the LDL-receptor gene. This mutation is extremely common among French Canadians with familial hypercholesterolemia and has thus far not been observed in any other ethnic group. The mutation occurred in homozygous form in four French Canadian homozygotes from four different families. In addition to the four subjects shown in Table 1, we have also studied three other French Canadian homozygotes (data not shown). One of these subjects had one copy of the 5′ deletion, and the other two did not have the 5′ deletion. Thus, the 5′ deletion accounted for 9 of 14 mutant alleles (64 percent) among French Canadian homozygotes (Table 2). The 5′ receptor mutation was also found in 63 percent of unselected heterozygotes from French Canada. Taken together, these two estimates indicate that the 5′ deletion accounts for nearly two thirds of all the mutations among French Canadians in the Montreal area (Table 2).

The present French Canadian population is believed to be descended from approximately 8000 immigrants from northern and western France who settled in Canada between 1608 and 1763.[14] Immigration of French people to Canada essentially ceased after the British victory in the French and Indian War in 1763.[14] There has apparently been relatively little crossbreeding between French Canadians and groups of other national origins within Canada.[14] It seems likely that the high frequency of the 5′ deletion among French Canadians stems from a founder effect. Inasmuch as careful genealogic records of French Canadian families exist,[14] it may be possible to search for a common ancestor among the families that are shown to have this gene.

From a practical viewpoint, the present data should allow the diagnosis of familial hypercholesterolemia with use of Southern blotting of genomic DNA in most heterozygotes in French Canada. Among persons from this ethnic group presenting with hypercholesterolemia, an examination of their leukocyte DNA with an *Xba*I–*Kpn*I restriction endonuclease digest should provide a diagnosis in about 60 percent. The inheritance of the mutant gene can then be traced in other family members, thus allowing earlier therapy with diet and drugs.

It should also be possible to use the Southern blotting method to screen unselected persons of French Canadian ancestry to determine the frequency of the mutation in the population at large. Given the large number of patients with this single mutation, it seems likely that the total incidence of familial hypercholesterolemia among French Cana-

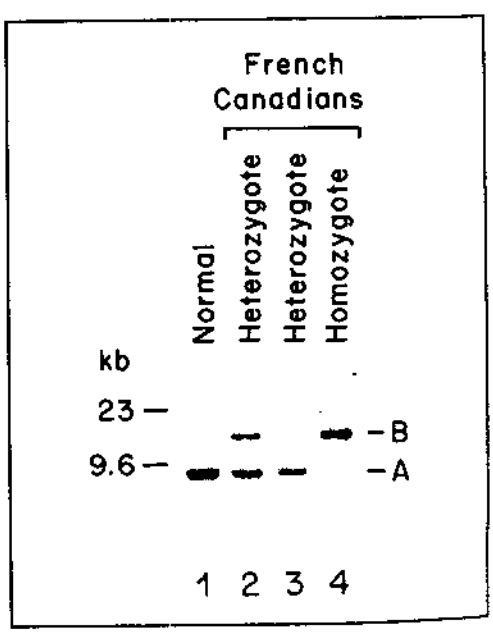


Figure 2. Southern Blot Hybridization of Genomic DNA from Two Persons of French Canadian Descent with Heterozygous Familial Hypercholesterolemia.

Total genomic DNA from each person was subjected to Southern blot hybridization as described in the legend to Figure 1. The DNA in Lane 1 is from a normal person and shows the normal band (A). The DNAs in Lanes 2 and 3 are from two unrelated heterozygotes of French Canadian descent. The DNA in Lane 4 is from a patient who is homozygous for the French Canadian deletion and thus has two copies of the abnormal band (B).

Table 2. Frequency of the 5' Deletion in French Canadian Heterozygotes and Homozygotes with Familial Hypercholesterolemia.*

	Heterozygotes	Homozygotes
No. of persons screened	84	7
No. of mutant genes screened	84	14
No. with deletion	53	9
Mutations attributed to deletion (%)	63	64

*Each subject was from a different family with dominantly inherited familial hypercholesterolemia — i.e., no two first-degree relatives from the same family were studied.

dians may be higher than the incidence of 1 in 500 observed in most countries.

The mutation we observed in French Canadians offers some insight into the nature of the variable severity of atherosclerosis among heterozygotes and homozygotes with familial hypercholesterolemia. The French Canadian homozygotes have the most complete deficiency of LDL receptors yet described, in that their mutant gene cannot produce any LDL-receptor mRNA or protein. Despite the completeness of this mutation, there is a variable prognosis. One of the French Canadian homozygotes listed in Table 1 (Patient 49) did not have symptomatic coronary artery disease until she was 17 years old. She was treated with an ileal bypass and a variety of drugs, but her serum cholesterol level remained in the range of 600 to 1200 mg per deciliter (15.5 to 31 mmol per liter). Nevertheless, she lived until the age of 33, when metastatic malignant melanoma developed. She had unstable angina at the time of her death. No autopsy was performed.

On the other hand, Patient 549 died suddenly at the age of three years, apparently of a myocardial infarction.[15] At autopsy, he had severe coronary atherosclerosis with 99 percent stenosis of the proximal right and left main coronary arteries and of the left anterior descending coronary artery. Thus, even in the presence of the extremely high circulating levels of LDL that result from a complete deficiency of LDL receptors, there can be a 30-year difference in the age at death. Important local or systemic factors must determine the resistance of the coronary arteries to high levels of LDL. Most persons seem to be in the high-susceptibility category, as indicated by the high frequency of symptomatic coronary disease in children with homozygous familial hypercholesterolemia,[5] but some seem to be relatively resistant.

Most of the mutations in familial hypercholesterolemia are less severe than the one observed in French Canadians,[5,16] leading to the production of a diminished number of apparently normal receptors or of a normal number of receptors that have a diminished ability to bind LDL. Patients with these "leaky" mutations have been reported to have somewhat lower blood cholesterol levels, better responsiveness to therapy, and less coronary heart disease than those whose fibroblasts produce no LDL receptors.[5,17,18] Given the fact that the clinical outcome can vary dramatically even among patients who are homozygous for the deletion found in the French Canadians, it is clear that more investigative work will be needed before we can determine whether the prognosis for a homozygote who has no receptors will always be worse than that for a homozygote with defective receptors.

We are indebted to Shellie Craig, Scott Clark, and Evan Tobin for excellent technical assistance; to Edith Womack and Deborah Darne for invaluable help in growing cultured cells; and to Helène and Madeliene Roy for able assistance in screening and obtaining samples from the patients with familial hypercholesterolemia.

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