Am. J. Hum. Genet. 49:443–449, 1991

A Common Lithuanian Mutation Causing Familial Hypercholesterolemia in Ashkenazi Jews

Vardiella Meiner,* Daniel Landsberger,* Neville Berkman,* Ayeleth Reshef,* Pesach Segal,† Harry C. Seftel,‡ Deneys R, van der Westhuyzen,§ Muhammad S. Jeenah,§ Gerhard A. Coetzee,§ and Eran Leitersdorf*

*Department of Internal Medicine B and Lipid Research Laboratory, Hadassah University Hospital, Jerusalem; †Diabetes and Lipid Unit, Sheba Medical Center, Tel Hashomer, Israel; ‡Department of Medicine, University of Witwatersrand, Johannesburg; and § Medical Research Council, University of Cape Town Research Unit for Cell Biology of Atherosclerosis, Department of Medical Biochemistry, University of Cape Town Medical School, Cape Town

Summary

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in the low-density-lipoprotein (LDL) receptor. Here we characterize an LDL-receptor founder mutation that is associated with a distinct LDL-receptor haplotype and is responsible for FH in 35% of 71 Jewish-Ashkenazi FH families in Israel. Sixty four percent (16/25) of the Ashkenazi patients who carry this mutant allele were of Lithuanian origin. The mutation was not found in 47 non-Ashkenazi FH families. This mutation was prevalent (8/10 FH cases) in the Jewish community in South Africa, which originated mainly from Lithuania. The mutation, a 3-bp in-frame deletion that would result in the elimination of Gly_{197}, has been previously designated FH-Piscataway. PCR amplification of a DNA fragment that includes the mutation in heterozygous individuals results in the formation of a heteroduplex that can be demonstrated by PAGE and used for molecular diagnosis.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disease with an estimated world prevalence of 0.2% (Goldstein and Brown 1989). Previous studies have shown that in several relatively isolated populations there is an increased prevalence of FH (Khachadurian and Uthman 1973; Seftel et al. 1980; Moorjani et al. 1989). Extensive molecular-genetic studies have revealed that in some populations specific low-density lipoprotein (LDL)–receptor mutations are common (Hobbs et al. 1987; Lehrman et al. 1987; Aalto-Setala et al. 1989; Leitersdorf et al. 1989*b*, 1990). Such findings support the concept that the higher prevalence of the disease in these populations is due to a founder effect and not to any as yet undetermined environmental selective forces.

Received February 5, 1991; revision received April 10, 1991.

Address for correspondence and reprints: Dr. Eran Leitersdorf, Department of Internal Medicine B and Lipid Research Laboratory, Hadassah University Hospital, i1–91120 Jerusalem, Israel.

0002-9297/91/4902-0023$02.00

A recent study (Seftel et al. 1989) has shown that there is an exceedingly high prevalence of FH in South African Jews. This Jewish community originated mainly from Lithuania during the massive emigration of eastern-European Jews at the turn of the nineteenth century. Here we describe a single LDL-receptor mutation that accounts for a majority of FH cases in South African Jews and for a large proportion of FH cases in the Israeli Ashkenazi population originating from Lithuania.

Material and Methods

Material

Restriction endonucleases, DNA polymerase I (Klenow fragment), and other enzymes were obtained from New England Biolabs (Beverly, MA), Boehringer Mannheim (Mannheim, Germany), and Pharmacia (Piscataway, NJ). DNA sequence analysis was done using Sequenase™ (United States Biochemical, Cleveland). Other chemicals were purchased from Sigma Chemical (St. Louis). *Thermus aquaticus* DNA poly-

merase I was obtained from Perkin Elmer–Cetus (Norwalk, CT). [α-^{32}P] dCTP (3,000 Ci/mmol) and [γ-^{32}P] ATP (7,000 Ci/mmol) were purchased from New England Nuclear (Boston). Biotrans™ nylon membranes were purchased from ICN Biochemical (Irvine, CA). Hoeschst dye 33258 was obtained from Polyscience (Warrington, PA). Oligonucleotides were synthesized by Biotechnology (Rehovot, Israel). DNA was amplified using the Programmable Thermal Controller (MJ Research). Plasma lipids were determined on a Vitatron model FPS-A autoanalyser (Avro Scientific Instruments, Sunnyvale, CA).

Recruitment of Patients and Families

The following inclusion criteria were used for recruitment of patients and families: Index cases were known Jewish patients with marked hypercholesterolemia (plasma LDL-cholesterol level above the 95th percentile for age and gender), with tendon xanthomas and a history of premature (age <60 years) ischemic heart disease (IHD), or with at least one first-degree relative with premature IHD. Detailed interviews of families up to four generations excluded cases from related families. The patients were allocated to the study from the lipid clinics of the following medical centers in Israel: The Hadassah University Hospital in Jerusalem, The Sheba Medical Center in Tel-Hashomer, The Beilinson Medical Center in Petah-Tikva, and The Rambam Medical Center in Haifa. South African patients attended the lipid clinics at the Johannesburg General Hospital or Groote Schuur Hospital in Cape Town. For each patient and family member a fasting blood sample was collected in 0.15% (w/v) EDTA and immediately shipped on ice to the Lipid Research Laboratory at the Hadassah Medical Center in Jerusalem for biochemical analysis and genomic DNA extraction. All clinical information, including data on the use of cholesterol-lowering medications, were recorded.

Biochemical Determinations

Plasma total triglyceride, cholesterol, and high-density lipoprotein (HDL)–cholesterol levels were determined using commercially available diagnostic kits (Boehringer Mannheim, Germany). Plasma LDL-cholesterol levels were calculated according to the Friedewald-Levy formula (Friedewald et al. 1972).

Determination of LDL-Receptor RFLPs and Construction of DNA Haplotypes

Genomic DNA was extracted from blood leukocytes (Hobbs et al. 1986) and diluted to a final concentration of approximately 0.1 mg/ml. Ten RFLPs were determined (Leitersdorf et al. 1989*a*). Eight sites (*Bsm*I, *Sph*I, *Spe*I, *Apa*LI-5′, *Pvu*II, *Nco*I, *Pst*I, and *Apa*LI-3′) were analyzed using the Southern blotting technique. Four different single-stranded DNA probes derived from the LDL receptor cDNA were used for probing the Southern blots. The probes were labeled by primer extension, using [α-^{32}P] dCTP and the Klenow fragment of DNA polymerase I. Hybridization conditions, washing, and autoradiography were as described elsewhere (Leitersdorf et al. 1989*a*). Two RFLPs (*Stu*I and *Ava*II) were determined by PCR (Saiki et al. 1988) and restriction analysis. The oligonucleotides used for the analysis of the *Stu*I site were as described elsewhere (Leitersdorf et al. 1989*a*). The oligonucleotides constructed for the analysis of the *Ava*II site were complementary to intron 12/exon 13 and exon 13/intron 13 sequences flanking this site and were as follows: AV1 (upstream) 5′-GCCTGTTTAGGACAAAGTATTTTGG-3′ and AV2 (downstream) 5′-CCCCCTTACCTCTTGGCTGGGTGAG-3′.

LDL-receptor haplotypes were constructed according to the cosegregation of RFLPs in extensive pedigrees as described elsewhere (Leitersdorf et al. 1989*a*). Cosegregation analysis was also done between the LDL-receptor haplotypes and hypercholesterolemia in each pedigree.

DNA Sequencing of Normal and Mutant LDL-Receptor Genes from an FH Heterozygote

During population screening (using PCR) for the identification of a common Afrikaner mutation (a single base substitution in exon 4 [Leitersdorf et al. 1989*b*]) an unexpected heteroduplex was detected by PAGE in an FH individual who was subsequently identified as being of Jewish-Ashkenazi origin. Following this initial observation, genomic DNA from another Jewish-Ashkenazi FH heterozygote was amplified using oligonucleotide primers D1a (upstream) (5′-ATAGGATCCCCAGCTGTGGGCCTGCGACAACG-3′) and D2a (downstream) (5′-ATAGCATGCGCCCATACCGCAGTTTTCC-3′) that are complementary to exon 4 and exon 4/intron 4 sequences of the LDL-receptor gene, respectively. D1a and D2a oligonucleotides were designed to include also 5′ extensions of nine nucleotides containing the consensus sequence for *Bam*HI and *Sph*I restriction endonucleases, respectively, and three additional nucleotides. Following amplification, the PCR products were column purified (PrimeErase®; Stratagene, La Jolla, CA), digested with *Bam*HI/*Sph*I restriction endonucleases,

and subcloned into bacteriophage M13. Several dsM13 DNA clones were prepared, and each was digested with *Bam*HI/*Sph*I restriction endonucleases. At this stage, pairs of digested dsM13 DNA preparations were mixed, denaturated at 95°C, reannealed, and subjected to electrophoresis on 6% polyacrylamide gel. A heteroduplex DNA was identified from a single pair. Single-stranded M13 DNA was prepared from these two clones, and each was subjected to sequence analysis using the dideoxy chain-termination method (Sanger 1977).

Direct Detection of the Mutant Allele

We used PCR amplification and heteroduplex analysis for direct detection of an LDL-receptor mutation in FH heterozygotes. Oligonucleotides D1 (upstream) (5′-CCCCAGCTGTGGGCCTGCGACAACG-3′) and D2 (downstream) (5′-CGCCCATACCGCAG TTTTCC-3′) were used for amplification of the 3′ end of exon 4 of the LDL-receptor gene as described elsewhere (Leitersdorf et al. 1989*b*). The PCR protocol included 1 min of denaturation at 95°C and 5 min of annealing and extension at 68°C, using the *Thermus aquaticus* DNA polymerase enzyme for 35 cycles. Following amplification, the DNA was size fractionated on a 6% polyacrylamide gel. It was subjected either to autoradiography on a Kodak XAR-5 film for 3 h (in experiments where one of the oligonucleotides was end labeled using [γ-^{32}P] ATP and polynucleotide kinase) or to ethidium staining (in nonlabeled experiments).

Results

PCR was used to amplify the 3′ end of exon 4 of the LDL-receptor gene in a Jewish family of Lithuanian origin. When the amplified DNA was size fractionated by PAGE, in addition to the expected band of 220 bp (designated 217/220; fig. 1*d*), an extra band (fig. 1*d*, A) was observed in some cases. Haplotypes were constructed through analysis of the joint segregation of 10 RFLPs at the LDL-receptor gene locus (Leitersdorf et al. 1989*a*). The presence of an extra PCR fragment, haplotype 23 (*Bsm*I$^-$, *Sph*I$^+$, *Stu*I$^+$, *Ava*II$^+$, *Spe*I$^-$, *Apa*LI-5′$^+$, *Pvu*II$^-$, NcoI$^-$, *Pst*I$^+$, and *Apa*LI-3′$^+$), and hypercholesterolemia cosegregated in this family (fig. 1*b–d*) and in two other Jewish families of Lithuanian origin (results not shown).

In an attempt to explain the additional PCR band we fractionated the PCR products by using a denaturing urea-containing gel. In every case, only one band (approximately 220 nucleotides in length) was observed (fig. 1*e*). We interpret these results as showing that the additional band (fig. 1*d*, A) was a heteroduplex DNA fragment, consisting of a normal and a mutant strand containing either an insertion or a deletion.

Following cloning of amplified DNA into bacterio-

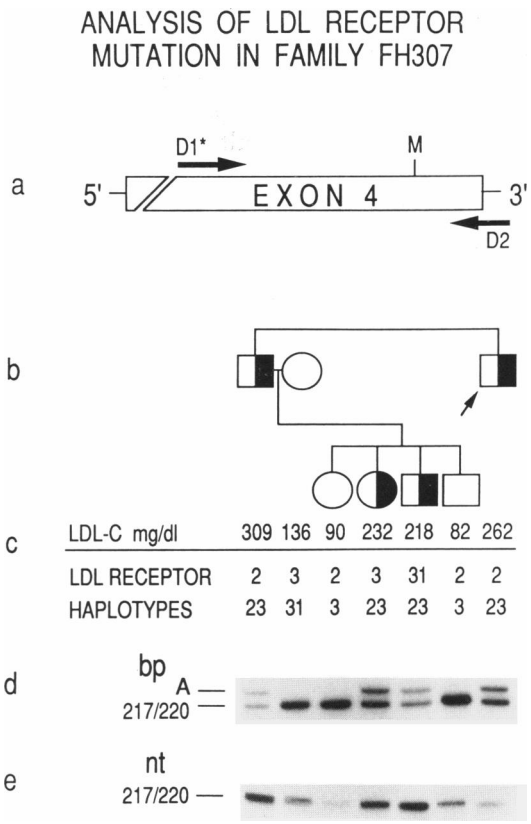

Figure 1 Analysis of LDL-receptor mutation in family FH 307. *a*, End-labeled oligonucleotide D1* and nonlabeled oligonucleotide D2, used for amplification of 3′ end of exon 4 of LDL-receptor gene. The site of the 3-bp deletion in the mutant gene (M) is shown. *b*, Two-generation pedigree, including seven individuals from family FH 307. The index case is marked with an arrow. Half-darkened pedigree symbols designate "affected" individuals (LDL-C levels above 95th percentile). *c*, LDL-C levels and LDL-receptor haplotypes for all family members. It is evident that haplotype 23 cosegregates with hypercholesterolemia in this family. *d*, Fragments after DNA amplification using oligonucleotide D1* and D2. The fragments were size fractionated on a 6% polyacrylamide gel, and the expected 220- and 217-bp bands for the normal and mutant gene, respectively, were visualized by autoradiography. In addition, an approximately 250-bp band (A) is also seen for the affected family members. This band represents a heteroduplex formed between the normal and the mutant LDL-receptor alleles. *e*, Analysis of PCR-amplified fragment from same family members, on 6% polyacrylamide–42% (W/V) urea denaturing gel showing only 217/220 nucleotide fragments. Band sizes were determined according to their migration relative to Phi-X DNA digested with *Hae*III.

phage M13, single-stranded DNA, which corresponded to both alleles of a heterozygous patient, was prepared. Sequence analysis revealed a 3-bp deletion in exon 4 in one of the LDL-receptor alleles (fig. 2). This mutation is expected to result in a deletion of Gly_{197} from a highly conserved cysteine-rich repeat of the binding domain of the receptor.

We subsequently screened index cases from 118 unrelated FH families, including 71 Jewish Ashkenazi families, in Israel (table 1). All patients had xanthomatosis, and their pretreatment plasma cholesterol levels were above the 95th percentile (for age and gender). Forty-three percent had IHD, a number that correlates well with the prevalence of IHD in FH (Goldstein and Brown, 1989). Their mean ± SD age was 50.2 ± 12.4 years, and 59% were males. According to the autosomal dominant inheritance of the disease, we would have expected an equal number of males and females in our sample. This introduces a bias to our analysis and probably results from the fact that some of the patients were referred from the clinics only after the occurrence of an ischemic event that tends to appear earlier in FH males.

Genomic DNA analysis using the PCR technique for detection of this LDL-receptor mutation was done on all patients. We have used unlabeled oligonucleotide primers for detection by ethidium staining of the gel bands. The analysis revealed that 35.2% (25/71) of the Ashkenazi patients in Israel carry this mutation and that none of the other 47 patients do (table 1). Sixty-four percent (16/25) of the Ashkenazi patients carrying this mutation could be traced back to their origin in Lithuania. Nine of the index cases with the mutation were born in Israel, and the others were immigrants from Lithuania (seven), Rumania (three), South Africa (two), Czechoslovakia (one), Germany (one), Poland (one), and Scotland (one). Screening for the mutation was also carried out on South African FH

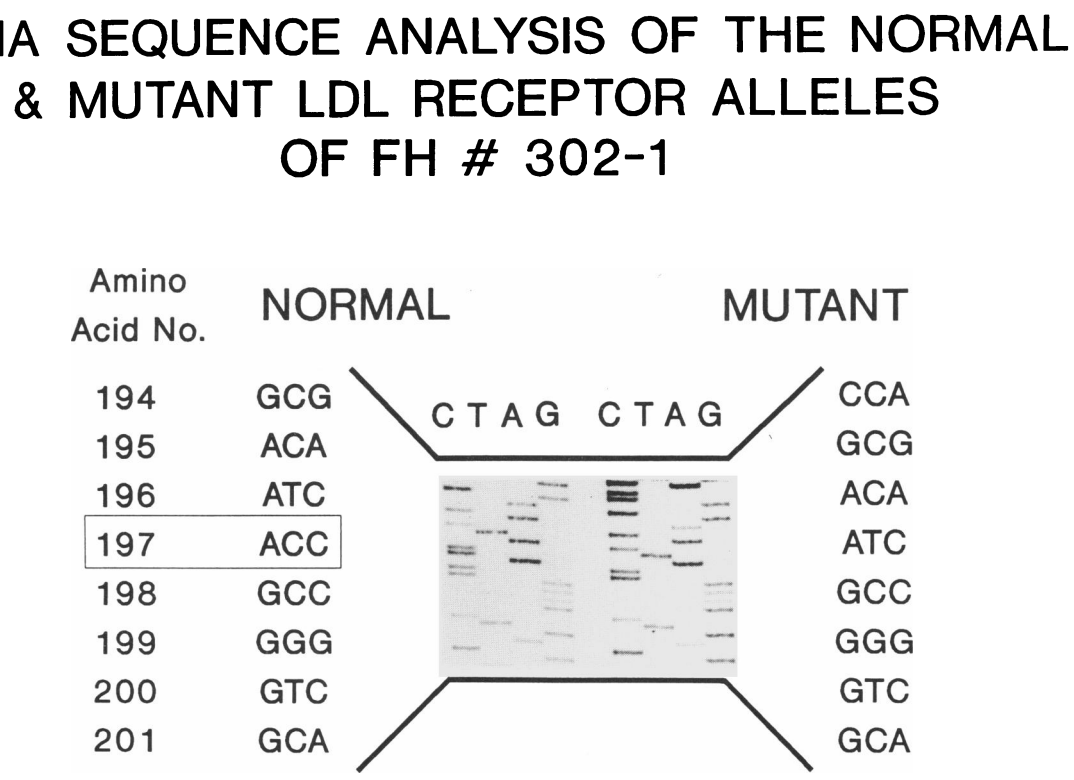

Figure 2 DNA sequence analysis of normal and mutant LDL-receptor alleles of FH 302–1. Genomic DNA was amplified using oligonucleotides D1a and D2a that are complementary to exon 4 and exon 4/intron 4 sequences of the LDL-receptor gene. These oligonucleotides include also the consensus sequences for *Bam*HI and *Sph*I restriction enzymes, respectively. After amplification the PCR products were digested with *Bam*HI and *Sph*I and subcloned into the polylinker site of bacteriophage M13. Sequence analysis of the noncoding strand of both alleles (see Material and Methods) was performed using the dideoxy chain-termination method. A 3-bp deletion that is expected to result in the elimination of Gly_{197} is evident in the mutant gene.

Table 1

Clinical Characteristics of 118 patients with Marked Hypercholesterolemia (LDL-C above 95th percentile) and Xanthomatosis

Group	No. of Patients	Mean ± SD age (years)	No. (%) Males	No. (%) IHD
Ashkenazi with Gly_{197} deletion	25	50.8 ± 11.4	16 (64)	11 (44)
Other Ashkenazi.....	46	53.1 ± 10.8	24 (52)	18 (39)
Others (non-Ashkenazi)	47	47.2 ± 13.8	30 (64)	22 (47)
Total	118	50.2 ± 12.4	70 (59)	51 (43)

Jewish patients. The mutation was prevalent, being found in eight of the 10 unrelated FH heterozygotes analyzed.

Discussion

The mutation described accounts for 21.2% (25/118) of the index cases with FH in our sample and for 35.2% (25/71) of the Ashkenazi patients. Sixty-four percent (16/25) of the Ashkenazi patients who have this mutant allele were traced back to their origin from Lithuania. Since these cases were recruited from the four major referral centers in Israel, it is expected that this cohort is representative of the Israeli population.

Sequencing of PCR-amplified genomic DNA from an FH heterozygote revealed a 3-bp deletion in exon 4 of the LDL-receptor gene (fig. 2). The mutation is expected to result in the elimination of a single amino acid from the highly conserved binding domain of the LDL receptor. Literature search revealed that an identical mutation, designated FH-Piscataway, was previously reported among the Dallas collection of FH homozygotes and was characterized at the molecular level and classified as a class II (impaired transport and processing) mutation (Hobbs et al. 1990). This mutation probably leads to an improperly folded binding domain of the LDL receptor, an improper folding due to abnormal spacing between conserved cysteine residues and hence to a block in transport of receptor precursor molecules to the cell surface. Extremely low expression of functional receptors encoded by this mutant allele is therefore expected.

We have designed a rapid screening method for the detection of this mutation in FH heterozygotes, using the PCR (Saiki et al. 1988). The PCR product is expected to include equal amounts of amplified genomic DNA originating from the normal and the mutant LDL-receptor alleles. Annealing of the two heterologous DNA strands creates a heteroduplex that can be detected by PAGE because of its slower electrophoretic mobility. It is not visible on agarose gels, a finding in agreement with a previous observation by Nagamine et al. (1989). As expected, the heteroduplex dissociates following denaturation (fig. 1*e*). A similar phenomenon following PCR amplification has been recently described for the detection of a 4-bp insertion mutation leading to Tay-Sachs disease (Triggs-Raine and Gravel 1990).

The discovery of this FH mutation in Ashkenazi Jews of Lithuanian origin raises some important issues in population genetics. Previous studies (Bonne-Tamir et al. 1979) have shown that the Ashkenazi Jews are a uniform group with respect to blood groups, isoenzymes, and the histocompatibility antigens, in contrast to European populations with whom they have lived. The Jewish community of Lithuania dates back to 1388 (Ankori 1979). They lived in a relative cultural and environmental isolation until the turn of the nineteenth century, when many of them emigrated to South Africa, Great Britain, Australia, Latin America, North America, and Palestine (Gar 1971). The predominance of the FH-Piscataway mutation in the Ashkenazi Jews in Israel and South Africa, together with the high frequency of FH in South Africa, is suggestive of a founder effect in these populations. However, since 40,000 Lithuanian immigrants who came to South Africa between 1880 and 1910 gave rise to most of the South African Ashkenazi population, we feel that it is unlikely that a founder effect would have manifested itself in South Africa, given such a large initial founder population in only three or four generations. The same argument applies to Israel, where hundreds of thousands of eastern-European Jews settled during this century. It therefore seems likely that the mutation may have originated and been subjected to earlier expansion in Lithuania. Its predominance in the immigrant populations in Israel and in South Af-

rica is probably due to multiple sources of introduction of this gene into these populations in a short time period. Our genealogical analysis reveals worldwide distribution of this "Lithuanian" mutation, in contradistinction with the other reported LDL-receptor founder mutations that are confined to specific geographical distributions.

Recent studies have revealed that mutations leading to several autosomal recessive disorders are identical in both Ashkenazi Jews and non-Jews. For example, an increased prevalence of a specific mutation in the beta-glucosidase gene was found in Ashkenazi patients with Gaucher disease type 1, although the same mutation was also found in non-Jews (Theophilus et al. 1989). In addition, it has also been established that a common mutation underlies the adult form of Tay-Sachs disease (G_{M2} gangliosidosis) in Ashkenazi-Jews and non-Jews (Navon et al. 1990). In the present study we have screened for a specific LDL-receptor mutation in non-Ashkenazi FH patients including Jews from other ethnic origins (i.e., Sephardic and Oriental) and in several Christian, Moslem, and Druze FH patients, and we have not yet found a single case. Among Jews, therefore, the mutation appears to be limited to the Ashkenazi Jews.

The currently described mutation is expected to have a significant impact on plasma lipoprotein levels and cardiovascular morbidity in the world's Jewish-Ashkenazi communities. This issue is currently under investigation.

Acknowledgments

We thank Drs. Y. Stein and S. Eisenberg for helpful discussions and Drs. M. S. Brown, J. L. Goldstein, and D. W. Russell for providing the DNA probes required for the analysis of the LDL-receptor gene RFLPs. Professor G. Brook from the lipid clinic of the Rambam Medical Center, Dr. Beigel from the Lipid Unit of the Beilinson Medical Center in Israel, and Dr. A. D. Marais from the Lipid Clinic, Groote Schuur Hospital, Cape-Town, are thanked for their cooperation and help in recruitment of patients to the study. Osnat Eliav and Rubina Levitzki are thanked for their technical assistance. This study was supported in part by The Chief Scientist's office, Israel Ministry of Health (grant 1556 to E.L.), The South African Medical Research Council, and the University of Cape Town.

References

Aalto-Setala K, Helve E, Kovanen PT, Kontula K (1989) Finnish type of low density lipoprotein receptor gene mutation (FH-Helsinki) deletes exons encoding the carboxyl-terminal part of the receptor and creates an internalization-defective phenotype. J Clin Invest 84:499–505

Ankori Z (1979) Origins and history of Ashkenazi Jewry (8th to 18th century) In: Goodman RM, Motulsky AG (eds) Genetic diseases among Ashkenazi Jews. Raven, New York, pp 19–46

Bonne-Tamir B, Ashbel S, Kenett R (1979) Genetic markers: benign and normal traits of Ashkenazi Jews. In: Goodman RM, Motulsky AG (eds) Genetic diseases among Ashkenazi Jews. Raven, New York, pp 59–76

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 18:499–502

Gar J (1971) Lithuania. In: Berman MM, Carlebach A (eds) Judaica encyclopedia. Keter, Jerusalem, pp 362–390

Goldstein JL, Brown MS (1989) Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1215–1250

Hobbs HH, Brown MS, Goldstein JL, Russell DW (1986) Deletion of exon encoding cysteine-rich repeat of LDL receptor alters its binding specificity in a subject with familial hypercholesterolemia. J Biol Chem 261:13114–13120

Hobbs HH, Brown MS, Russell DW, Davignon J, Goldstein JL (1987) Deletion in the gene for the low-density lipoprotein receptor in a majority of French Canadians with familial hypercholesterolemia. N Engl J Med 317:734–737

Hobbs HH, Russell DW, Brown MS, Goldstein JL (1990) The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet 24:133–170

Khachadurian AK, Uthman SB (1973) Experience with the homozygous cases of familial hypercholesterolemia. Nutr Metabol 15:132–140

Lehrman MA, Schneider WJ, Brown MS, Davis CG, Elhammer A, Russell DW, Goldstein JL (1987) The Lebanese allele at the LDL receptor gene locus: nonsense mutation produces truncated receptor that is retained in the endoplasmic reticulum. J Biol Chem 262:401–410

Leitersdorf E, Chakravarti A, Hobbs HH (1989*a*) Polymorphic haplotypes at the LDL receptor locus. Am J Hum Genet 44:409–421

Leitersdorf E, Tobin EJ, Davignon J, Hobbs HH (1990) Common low-density lipoprotein receptor mutations in the French Canadian population. J Clin Invest 85:1014–1023

Leitersdorf E, van Der Westhuyzen DR, Coetzee GA, Hobbs HH (1989*b*) Two common low density lipoprotein gene mutations cause familial hypercholesterolemia in Afrikaners. J Clin Invest 84:954–961

Moorjani S, Roy M, Gagne C, Davignon J, Brun D, Toussaint M, Lambert M, et al (1989) Homozygous familial

hypercholesterolemia among French-Canadians in Quebec Province. Arteriosclerosis 9:212–216

Nagamine CM, Chan K, Lau Y-FC (1989) A PCR artifact: generation of heteroduplexes. Am J Hum Genet 45:337–339

Navon R, Kolodny EH, Mitsumoto H, Thomas GH, Proia RL (1990) Ashkenazi-Jewish and non-Jewish adult G_{M2} gangliosidosis patients share a common genetic defect. Am J Hum Genet 46:817–821

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Seftel HC, Baker SG, Jenkins T, Mendelsohn D (1989) Prevalence of familial hypercholesterolemia in Johannesburg Jews. Am J Med Genet 34:545–547

Seftel HC, Baker SG, Sandler MP, Forman MB, Joffe BI, Mendelsohn D, Jenkins T, et al (1980) A host of hypercholesterolemic homozygotes in South Africa. Br Med J 281:633–636

Theophilus B, Latham T, Grabowski GA, Smith FI (1989) Gaucher disease: molecular heterogeneity and phenotype-genotype correlations. Am J Hum Genet 45:212–225

Triggs-Raine BL, Gravel RA (1990) Diagnostic heteroduplexes: simple detection of carriers of a 4-bp insertion mutation in Tay-Sachs disease. Am J Hum Genet 46:183–184