Independent mutation of arginine$_{(3500)}$→glutamine associated with familial defective apolipoprotein B-100

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Abstract Familial defective apolipoprotein B-100 (FDB) is characterized by a decreased affinity of low density lipoprotein (LDL) to the LDL receptor resulting in a dominantly inherited increase of plasma LDL. It is postulated that FDB is caused by a G to A mutation at nucleotide 10,708 in exon 26 of the apoB gene creating a substitution of glutamine for arginine in amino acid 3500. The arginine$_{(3500)}$→glutamine mutation has been identified on the same haplotype of the apoB gene in several populations from North America and Europe, suggesting that it occurred on a single ancestral gene. Independent mutations were not observed. The purpose of this paper is to report on a family where individuals show a dominantly inherited increase of plasma LDL associated with an independent arginine$_{(3500)}$→glutamine mutation as determined by haplotype analysis using polymorphic markers of the apoB gene. The identification of these individuals is strong evidence that the arginine$_{(3500)}$→glutamine mutation is causative for the defective binding of apoB-100.—**Rauh, G., H. Schuster, C. K. Schewe, G. Stratmann, C. Keller, G. Wolfram, and N. Zöllner.** Independent mutation of arginine$_{(3500)}$→glutamine associated with familial defective apolipoprotein B-100. *J. Lipid Res.* 1993. **34:** 799–805.

Supplementary key words atherosclerosis • hypercholesterolemia • haplotypes • genetic mutation

Apolipoprotein B-100 (apoB-100), a protein of 4,536 amino acids, plays a central role in the metabolism of low density lipoprotein (LDL). LDL particles, which transport about two-thirds of plasma cholesterol, are catabolized primarily by the LDL receptor pathway (1). The interaction of LDL particles with the LDL receptor is mediated by apoB-100, the sole protein constituent of LDL (2). ApoB-100 is characterized by a high degree of genetic heterogeneity, which has been demonstrated in various immunochemical polymorphisms (3) and in a variety of published DNA sequences (4).

The role of apoB-100 in maintaining LDL homeostasis has been recently exemplified by the detection of familial defective apolipoprotein B-100 (FDB). FDB is characterized by defective binding of apoB-100 to the LDL receptor resulting in an accumulation of defective LDL particles in plasma to approximately a 2:1 ratio over normal LDL particles (5). Extensive sequence analysis of the apoB gene in affected family members revealed a mutation, which has not been described before (4): a G to A mutation at nucleotide 10,708 in exon 26 of the apoB gene creating a substitution of glutamine for arginine in amino acid 3500 (6). FDB is inherited in an autosomal dominant fashion, which is consistent with the fact that each LDL particle contains only one apoB-100 molecule (7).

FDB is one of the most common and widespread disorders resulting from a single-gene mutation. The arginine$_{(3500)}$→glutamine mutation was observed with similar frequency of approximately 1/500 in several populations of European origin: Austria (8), Australia (9), Denmark (10), Germany (11–13), Italy (14), North America (15), Sweden (10), and the United Kingdom (10). FDB was not identified in the Finnish population, which has a different ethnic origin (16). FDB is characterized by clinical features that are similar to familial hypercholesterolemia (FH) resulting from a defective LDL receptor, namely elevated LDL cholesterol, tendon xanthoma, and premature coronary artery disease (1, 17).

Questions concerning the origin of a mutation are amenable to haplotype analysis (18). Haplotype analysis using polymorphic markers of the apoB gene were performed in Austria (8), Germany (19), North America (20), and the United Kingdom (21). The arginine$_{(3500)}$→glutamine mutation was identified on the same haplotype, suggesting that this mutation occurred on a single ancestral gene. The purpose of this paper is to report on a family where individuals show a dominantly inherited increase of plasma LDL associated with an independent

Abbreviations: apoB-100, apolipoprotein B-100; ASO, allele specific oligonucleotide; FDB, familial defective apolipoprotein B-100; FH, familial hypercholesterolemia; HDL, high density lipoprotein; LDL, low density lipoprotein; PCR, polymerase chain reaction.

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arginine$_{(3500)}$→glutamine mutation as determined by haplotype analysis using polymorphic markers of the apoB gene (20, 22).

METHODS

Subjects

In previous studies we have detected 12 unrelated FDB heterozygotes in Munich (11, 19, 23). The identification of the propositus of this family resulted from further screening of 95 hypercholesterolemic individuals in Munich, whose total cholesterol levels were above the 95th percentile and whose triglyceride levels were below the 95th percentile of normal individuals from Munich (24). The ethnic origin of the family reported is German.

Lipid determination

Blood samples were taken 12 h after the last meal. Lipid levels were determined following a period of at least 6 weeks without drug treatment or when the patients came to the lipid clinic for the first time and before therapy. Secondary hyperlipidemia was excluded in all subjects having normal levels of blood glucose and normal tests for hepatic, renal, thyroid, and pancreatic function. Cholesterol and triglycerides were determined by enzymatic assays (Boehringer, Mannheim, Germany, Kit No. 236 691). HDL-cholesterol was determined after precipitation of apoB-100-containing lipoproteins and LDL-cholesterol was calculated by the Friedewald formula when triglycerides were below 200 mg/dl. Lipoproteins were separated by preparative ultracentrifugation according to Havel, Eder, and Bragdon (25), if triglycerides were above 200 mg/dl.

Detection of arginine$_{(3500)}$→glutamine mutation by oligomelting technique

Genomic DNA was prepared from total blood cells by using a Triton X-100 lysis method (26). The arginine$_{(3500)}$→glutamine mutation was detected by amplifying a segment of genomic DNA spanning the site of the mutation in the apoB gene by polymerase chain reaction (PCR) (27), followed by hybridization with radioactive allele-specific oligonucleotide (ASO) probes. The conditions used were the same as in previous studies (11, 19).

Detection of arginine$_{(3500)}$→glutamine by DNA sequencing

Asymmetric amplification was performed to produce single stranded DNA (28). One μg of genomic DNA was amplified as described above with the modification that the primer 5'—AGGATCCTGCAATGTCAAGGTGTGAATTT—3'r was limited to 0.01 μM. Sequencing was performed on purified DNA (Geneclean II kit, Bio 101, La Jolla, CA) using ^{35}S dATP (Amersham International) and T7 DNA polymerase (Sequenase Version 2.0, U.S. Biochemical, Cleveland, OH) following the protocol as previously described (29). DNA was electrophoresed on 6% denaturing polyacrylamide gel. Gels were dried for 1 h at 80°C and exposed to X-ray films (Kodak X-Omat AR) for 48 h.

Determination of genotypes

The specific DNA segment was amplified by PCR (27). The conditions used for analysis of the *Xba* I, *Msp* I, and *Eco* RI restriction sites and the hypervariable region in the 3' end of the apoB gene (3'HVR) were the same as reported previously (19). Three known DNA markers (3'HVR36/34, 46/32, and 30/28) were a kind gift from Prof. S. E. Humphries, London. The nomenclature applied for the 3'HVR is that of Ludwig and McCarthy (20).

Haplotype analysis

Haplotypes for each individual were constructed by analysis of segregation of the polymorphic sites in all members of each pedigree (18, 30). Construction of the haplotypes was based on the assumption that there had been no recombination event within the apoB gene.

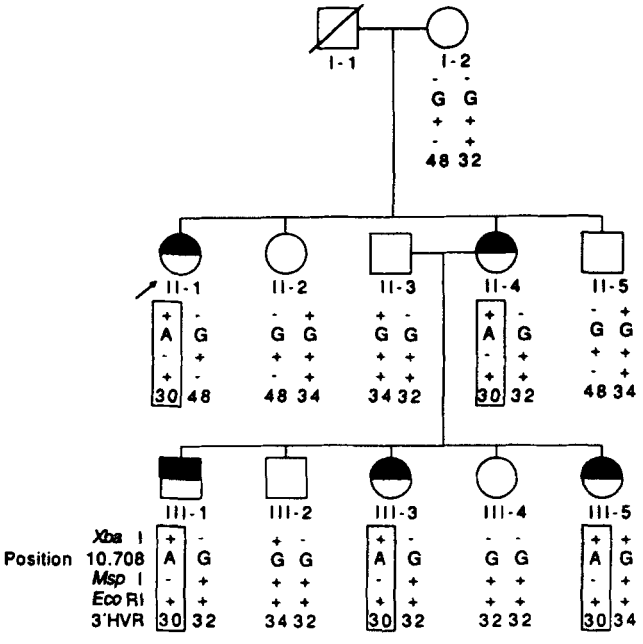

Fig. 1. Pedigree of FDB family. Haplotypes were constructed by analysis of segregation of following polymorphic sites: *Xba* I, *Msp* I, *Eco* RI, and the hypervariable region in the 3' end of the apoB gene (3'HVR). Construction of the haplotypes was based on the assumption that there had been no recombination event within the apoB gene. The number of the individual is shown below the symbol (according to Table 1). The haplotype of the mutant allele is characterized by following polymorphic sites: presence of *Xba* I restriction site, absence of the *Msp* I restriction site, presence of the *Eco* RI restriction site, and 30 repeats in 3'HVR; →, proband of FDB family; (+), presence of restriction site; (−), absence of restriction; (○, □), normal; (◓, ⬒), elevation of LDL-cholesterol.

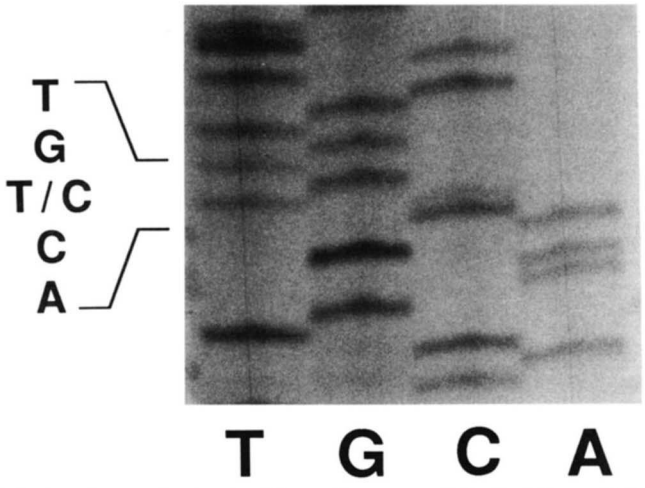

Fig. 2. Sequencing ladder of the antisense strand obtained from a FDB heterozygote. T in the antisense strand at nucleic acid 10,708 corresponds to A in the sense strand, confirming the G to A mutation at nucleotide 10,708. A, adenine; C, cytosine; G, guanine; T, thymine.

Evaluation of LDL-binding

Defective LDL-binding was evaluated using a modified proliferation assay first described by Frostegård et al. (31). U937 cells were grown in RPMI 1640 medium (Gibco, United Kingdom) supplemented with 10% heat-inactivated fetal calf serum (Gibco, United Kingdom). Serum-starved cells were seeded in multiwell plates (Becton Dickinson, USA) at a density of 100/μl and were kept in RPMI 1640 medium supplemented either with 1% serum of patients with the arginine$_{(3500)}$→glutamine mutation or with 1% serum of individuals lacking this mutation. After 4 days the cell number was determined by sixfold counts using a computerized cell counter analyzer system (CASY-I, Schärfe System, Reutlingen, Germany).

RESULTS

The family study detected among 11 family members, 6 normolipidemic individuals, and 5 hypercholesterolemic individuals (**Fig. 1**). The hypercholesterolemia was inherited in an autosomal dominant fashion and resulted from an increase of plasma LDL. A defect of the LDL receptor was excluded by tissue culture biochemistry (17) and by linkage analysis with polymorphic sites in the LDL receptor gene (data not shown).

The arginine$_{(3500)}$→glutamine mutation was present in all hypercholesterolemic individuals as determined by oligomelting technique, while in normocholesterolemic individuals the mutation was absent. The DNA segment in the vicinity of the mutation was sequenced in the propositus of the family (individual II-1) in order to confirm the G to A mutation at nucleotide 10,708. **Fig. 2** demonstrates a sequencing ladder of the antisense strand. T in the antisense strand at nucleic acid 10,708 corresponds to A in the sense strand.

In the propositus of the family (individual II-1) the defective LDL binding was determined by reduced proliferation of U937 cells. **Fig. 3** demonstrates the cell number after incubation in RPMI 1640 medium supplemented with 1% serum as a function of LDL cholesterol concentrations. Three unrelated FDB patients including the propositus II-1 are compared to eight individuals without the mutation. In the hypercholesterolemic individuals without this mutation, an LDL receptor defect was excluded by tissue culture biochemistry (17).

The cell number after incubation with serum from FDB patients is below the regression curve of the controls, indicating that LDL from these individuals induced a decreased growth of U937 cells compared to individuals without this mutation.

Table 1 summarizes the biochemical and clinical characteristics of all family members. Arcus lipoides, tendon xanthoma, plaques in the carotid artery as determined by duplex scan, and coronary artery disease as determined by coronary angiography were only observed in FDB heterozygotes. No data were available on the FDB heterozygote in the first generation (individual I-1), who was hypercholesterolemic and died of a myocardial infarction at age 52.

The genotype was determined directly from an ethidium bromide-stained agarose gel as described above. **Fig. 4** demonstrates the five different 3'HVR alleles observed in this family. All 3'HVR alleles consist of an even number of repeats (3'HVR30, 32, 34, 36, and 48).

Haplotypes based on polymorphic sites in the vicinity of the arginine$_{(3500)}$→glutamine mutation could be constructed unambiguously for each member of the pedigree. Recombinations within the apoB gene were not observed.

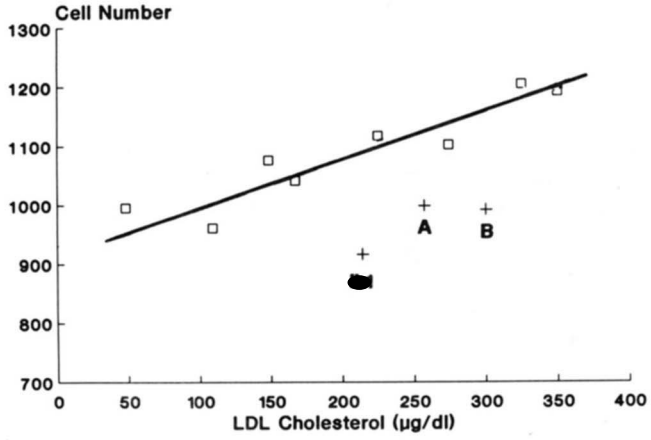

Fig. 3. Cell number of U 937 cells after incubation in RPMI 1640 medium supplemented with 1% serum as a function of LDL cholesterol concentrations. Three unrelated FDB patients (propositus II-1, A, B) are compared to eight individuals without the mutation. The cell number after incubation with serum from FDB patients is below the regression curve of the controls; (□), individuals without the arginine$_{(3500)}$→glutamine mutation; (+), FDB patients.

TABLE 1. Biochemical and clinical characteristics of all family members

Patient	Gender	Age	Amino Acid 3500	Lipid Levels				Arcus	Xanthoma	Plaques Carotid Artery	Coronary Artery Disease
				TC	TG	LDL-C	HDL-C				
		yr				*mg/dl*					
I-1	M	52[a]	NA	411	122	NA	NA	NA	NA	NA	NA
I-2	F	89	Arg	209	78	122	61				
II-1	F	62	Gln	406	154	307	68	+	+	+	+
II-2	F	65	Arg	212	106	137	64				
II-3	M	63	Arg	241	139	136	47				
II-4	F	60	Gln	332	86	263	52	+	+	+	+
II-5	M	55	Arg	245	121	159	46				
III-1	M	38	Gln	387	182	291	42	+	+		
III-2	M	33	Arg	209	188	137	48				
III-3	F	30	Gln	336	76	274	47				
III-4	F	29	Arg	242	89	149	58				
III-5	F	28	Gln	281	189	209	36				

HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NA, not available; TC, total cholesterol; TG, triglyceride; +, presence of a specific clinical feature. The 95th percentile total cholesterol as determined in the Munich population ranges from 231 mg/dl (age group 20–30 years) to 285 mg/dl (age group 60–70 years) (24).

[a]Died of a myocardial infarction at age 52.

Using cosegregation analysis, we followed the autosomal dominant inheritance of the mutant allele, i.e., the allele carrying the arginine$_{(3500)}$→glutamine mutation. The haplotype of the mutant allele is characterized by the following polymorphic sites (Fig. 1): *Xba* I$^+$/*Msp* I$^-$/*Eco* RI$^+$/3'HVR30.

DISCUSSION

The arginine$_{(3500)}$→glutamine mutation associated with FDB (6) is the only known mutation of the apoB gene that can virtually abolish the receptor binding activity of LDL (32). The first evidence of defective binding of LDL came from turn-over studies (33); later the defective binding was demonstrated using cultured human fibroblasts (5). Haplotype analyses performed in Austria (8), Germany (19), North America (20), and the United Kingdom (21) demonstrated the arginine$_{(3500)}$→glutamine mutation on the same chromosomal background (**Table 2**). The haplotype with the mutation is characterized by following polymorphic sites: 5' HVR14/SP$^+$/*Apa* LI$^+$/*Hinc* II$^-$/*Pvu* II$^-$/*Alu* I$^-$/*Xba* I$^-$/*Msp* I$^+$/*Eco* RI$^-$/3'HVR48. In studies using less polymorphic sites or in FDB heterozygotes where the haplotype was not unequivocally deduced from the combination of genotypes, the results were consistent with the combination of genotypes, with one exception: an FDB heterozygote from North America demonstrated 3'HVR46. This has been attributed to unequal crossing-over or slippage during DNA replication within the 3'HVR itself, which is known from other hypervariable loci in the human genome (34). Therefore, all reported haplotypes are consistent with the notion that the arginine$_{(3500)}$→glutamine mutation occurred on 5'HVR14/SP$^+$/*Apa* LI$^+$/*Hinc* II$^-$/*Pvu* II$^-$/*Alu* I$^-$/*Xba* I$^-$/*Msp* I$^+$/*Eco* RI$^-$/3'HVR48.

We observed the arginine$_{(3500)}$→glutamine mutation on a different haplotype: *Xba* I$^+$/*Msp* I$^-$/*Eco* RI$^+$/3$^-$HVR30. There are two alternatives to explain this finding: an independent arginine$_{(3500)}$→glutamine mutation occurred on haplotype *Xba* I$^+$/*Msp* I$^-$/*Eco* RI$^+$/3'HVR30 or the arginine$_{(3500)}$→glutamine mutation occurred once on hap-

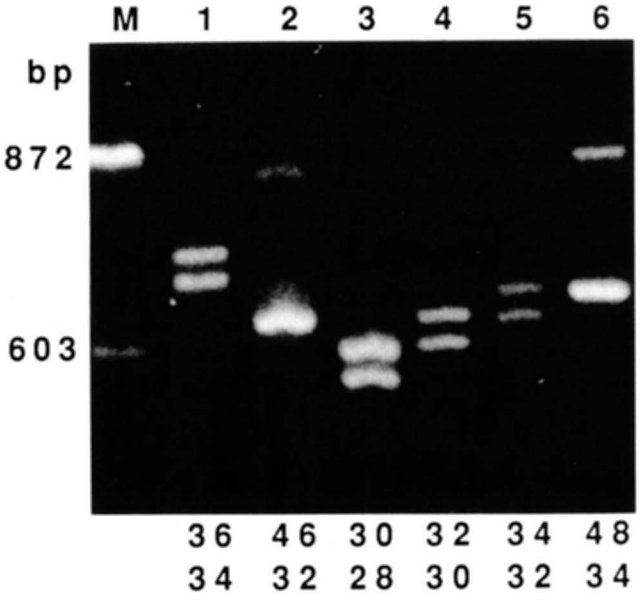

Fig. 4. Polymorphic bands of the hypervariable region in the 3' end of the apoB gene (3'HVR). The polymorphic bands were analyzed on an ethidium bromide-stained agarose gel. All 3'HVR alleles consisted of an even number of repeats. The size of the 3 'HVR allele from FDB patients (lanes 4–6) was identified by comparison with DNA size standards (lane M) and three known markers (lanes 1–3). Lane M contains DNA size standards from ϕX174 digested with *Hae* III (872 bp corresponding to 3'HVR48, 603 bp corresponding to 3'HVR30). Lanes 1, 2, and 3 contain typed markers (3'HVR36/34, 46/32, and 30/28). The nomenclature applied for the 3'HVR is that of Ludwig and McCarthy (20).

lotype *Xba* I⁻/*Msp* I⁺/*Eco* RI⁻/3'HVR48 and subsequently spread by two recombination events in the same exon to *Xba* I⁺/*Msp* I⁻/*Eco* RI⁺/3'HVR30.

Recombination events have not been observed in the apoB gene. Recently Dunning et al. (35) observed complete allelic association between the antigen group (x/y) and polymorphism at sites encoding amino acids 2712 and 4311 in 688 chromosomes where phase could be determined unambiguously. This indicates that there has been no recombination event within a distance of approximately 7,000 base pairs. This finding argues against the hypothesis that the arginine$_{(3500)}$→glutamine mutation spread by two recombination events from haplotype *Xba* I⁻/*Msp* I⁺/*Eco* RI⁻/3'HVR48 to haplotype *Xba* I⁺/*Msp* I⁻/*Eco* RI⁺/3'HVR30.

We therefore favor the hypothesis of independent arginine$_{(3500)}$→glutamine mutation on haplotype *Xba* I⁺/*Msp* I⁻/*Eco* RI⁺/3'HVR30. The occurrence of an independent mutation is consistent with the finding that the CG dinucleotide at amino acid 3500 is a potential hotspot for mutation. The CG dinucleotide is known to be frequently associated with point mutations in other genes, like the hemoglobin gene cluster and the factor VIII gene (36). The vast majority of CG mutations are CG→TG or CG→CA mutations. This is consistent with methylation-induced deamination of 5-methylcytosin (37).

The present results for the arginine$_{(3500)}$→glutamine mutation are similar to those reported for the hemoglobin gene cluster: most point mutations are associated with a single haplotype (38), only a few point mutations were observed on different haplotypes (39). The finding for the arginine$_{(3500)}$→glutamine mutation may be contrasted with the most common mutation leading to a defect in the adenosine deaminase where the mutation occurs on different haplotypes (40).

The identification of a family with a dominantly inherited increase of plasma LDL resulting from independent arginine$_{(3500)}$→glutamine mutation is another line of evidence that this mutation is causative for the defective binding of apoB-100. However, the final proof requires identification of the functional defect in expressed full-length apoB-100 gene constructs, differing only in amino acid 3500. *

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REFERENCES

1. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
2. Kane, J. P., and R. J. Havel. 1989. Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins. *In* The Metabolic Basis of Inherited Disease. 6th edition. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw Hill, New York. 1139–1164.
3. Brequet, G., R. Bütler, E. Bütler-Brunner, and A. Sanchez-Mazas. 1990. A worldwide population study of the Ag-system haplotypes, a genetic polymorphism of human low density lipoprotein. *Am. J. Hum. Genet.* **46:** 502–509.
4. Yang, C. Y., Z. W. Gu, S. A. Weng, T. W. Kim, S. H. Chen, H. J. Pownall, P. M. Sharp, S. W. Liu, W. H. Li, A. M. Gotto, and L. Chan. 1989. Structure of apolipoprotein B-100 of human low density lipoproteins. *Arteriosclerosis.* **9:** 96–108.
5. Innerarity, T. L., K. H. Weisgraber, K. S. Arnold, R. W. Mahley, R. M. Krauss, G. L. Vega, and S. M. Grundy. 1987. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. *Proc. Natl. Acad. Sci. USA.* **84:** 6919–6923.
6. Soria, L. F., E. H. Ludwig, H. R. G. Clarke, G. L. Vega, S. M. Grundy, and B. J. McCarthy. 1989. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc. Natl. Acad. Sci. USA.* **86:** 587–591.

TABLE 2. Haplotype associated with FDB in this case and in cases observed in various populations

Population[a]	Number of FDB Heterozygotes[b]	5'HVR (5' Ex 1)	SP (Ex 1)	ApaLI (Ex 4)	HincII (In 4)	PvuII (In 4)	AluI (Ex 14)	XbaI (Ex 26)	Gln$_{(3500)}$ (Ex 26)	MspI (Ex 26)	EcoRI (Ex 29)	3'HVR (3' Ex 29)
North America (16)	11 (6)	14	+	+	–	–	–	–	+	+	–	48
	1	14	+	+	–	–	–	–	+	+	–	46
United Kingdom (17)	8 (3)		+			–		–	+	+	–	48
Germany (18)	11 (5)							–	+	+	–	48
Austria (8)	2	14	+	+	–	–	–	–	+	+	–	48
									X	X		
Germany (this case)	1							+	+	–	+	30

AluI, ApaLI, EcoRI, HincII, MspI, PvuII, and XbaI, restriction fragment length polymorphisms caused by a single base substitution; Ex, exon; 3' HVR, hypervariable region in the 3' end of the apoB gene caused by a varying number of TG dinucleotide repeats; 5' HVR, hypervariable region in the 5' end of the apoB gene caused by a varying number of 15 base pair repeats; In, intron; SP, signal peptide polymorphism caused by an insertion or deletion of a 9-base pair fragment; +, presence of polymorphic site or Gln$_{(3500)}$; –, absence of polymorphic site. X denotes the location of the two recombination events in exon 26, if the mutation occurred only once.

[a]Number in parentheses denotes the reference.

[b]Number in parentheses denotes the number of unrelated FDB heterozygotes, where the haplotype was determined unequivocally; in the remainder of individuals, the genotype of the individual was consistent with this haplotype.

7. Elovson, J., J. C. Jacobs, V. N. Schumaker, and D. L. Puppione. 1985. Molecular weights of apoprotein B obtained from human low-density-lipoprotein (apolipoprotein B-P1) and from rat very-low density lipoprotein (apoprotein B-PIII). *Biochemistry.* **24:** 1569–1578.
8. Friedl, W., E. H. Ludwig, M. E. Balestra, K. S. Arnold, B. Paulweber, F. Sandhofer, B. J. McCarthy, and T. L. Innerarity. 1991. Apolipoprotein B gene mutations in Austrian subjects with heart disease and their kindred. *Arterioscler. Thromb.* **11:** 371–378.
9. Hosking, J. L., R. Bais, P. D. Roach, and D. W. Thomas. 1991. Hypercholesterolemia due to familial defective apolipoprotein B-100 in two Australian families. *Med. J. Aust.* **155:** 572–573.
10. Tybjærg-Hansen, A., J. Gallagher, J. Vincent, R. Houlston, P. Talmud, A. M. Dunning, M. Seed, A. Hamsten, S. E. Humphries, and N. B. Myant. 1990. Familial defective apolipoprotein B-100: detection in the United Kingdom and Scandinavia, and clinical characteristics of ten cases. *Atherosclerosis.* **80:** 235–242.
11. Schuster, H., G. Rauh, B. Kormann, T. Hepp, S. Humphries, C. Keller, G. Wolfram, and N. Zöllner. 1990. Familial defective apolipoprotein B-100: comparison with familial hypercholesterolemia in 18 cases detected in Munich. *Arteriosclerosis.* **10:** 577–581.
12. Rauh, G., C. Keller, H. Schuster, G. Wolfram, and N. Zöllner. 1992. Familial defective apolipoprotein B-100: a common cause of primary hypercholesterolemia. *Clin. Invest.* **70:** 77–84.
13. Rauh, G., H. Schuster, B. Kormann, F. Spengel, H. Schuster, G. Wolfram, and N. Zöllner. 1992. Familial defective apolipoprotein B-100: clinical characteristics of 54 cases. *Atherosclerosis.* **92:** 233–241.
14. Corsini, A., S. Fantappie, A. Granata, F. Bernini, A. L. Catapano, R. Fumagalli, L. Romano, and C. Romano. 1989. Binding-defective low-density lipoprotein in family with hypercholesterolemia. *Lancet.* **i:** 623.
15. Innerarity, T. L., R. W. Mahley, K. H. Weisgraber, T. P. Bersot, R. M. Krauss, G. L. Vega, S. M. Grundy, W. Friedl, J. Davignon, and B. J. McCarthy. 1990. Familial defective apolipoprotein B-100: a mutation of the apolipoprotein B that causes hypercholesterolemia. *J. Lipid Res.* **31:** 1337–1349.
16. Hämäläinen, T., A. Palotie, K. Aalto-Setälä, K. Kontula, and M. J. Tikkanen. 1990. Absence of familial defective apolipoprotein B-100 in Finnish patients with elevated serum cholesterol. *Atherosclerosis.* **82:** 177–183.
17. Keller, C., K. Harders-Spengel, F. A. Spengel, A. Wieczorek, G. Wolfram, and N. Zöllner. 1981. Serum cholesterol levels in patients with familial hypercholesterolemia confirmed by tissue culture. *Atherosclerosis.* **39:** 51–59.
18. Botstein, D., R. L. White, M. Snolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32:** 314–331.
19. Rauh, G., H. Schuster, J. Fischer, C. Keller, G. Wolfram, and N. Zöllner. 1991. Familial defective apolipoprotein B-100: haplotype analysis of the arginine$_{(3500)}$→glutamine mutation. *Atherosclerosis.* **88:** 219–226.
20. Ludwig, E. H., and B. J. McCarthy. 1990. Haplotype analysis of the human apolipoprotein B mutation associated with familial defective apolipoprotein B-100. *Am. J. Hum. Genet.* **47:** 712–720.
21. Myant, N. B., J. J. Gallagher, B. L. Knight, S. N. McCarthy, J. Frostegård, J. Nilsson, A. Hamsten, P. J. Talmud, and S. E. Humphries. 1991. Clinical signs of familial hypercholesterolemia in patients with familial defective apolipoprotein B-100 and normal low density lipoprotein receptor function. *Arterioscler. Thromb.* **11:** 691–703.
22. Boerwinkle, E., S. S. Lee, R. Butler, V. N. Schumaker, and L. Chan. 1990. Rapid typing of apolipoprotein B DNA polymorphisms by DNA amplification: association between Ag epitopes of human apolipoprotein B-100, a signal peptide insertion/deletion polymorphism, and a 3' flanking DNA variable number of tandem repeats polymorphism of the apolipoprotein B gene. *Atherosclerosis.* **81:** 225–232.
23. Rauh, G., H. Schuster, J. Fischer, C. Keller, G. Wolfram, and N. Zöllner. 1991. Identification of a heterozygous compound individual with familial hypercholesterolemia and familial defective apolipoprotein B-100. *Klin. Wochenschr.* **69:** 320–324.
24. Henze, K., A. Wallmüller-Strycker, M. Bauer, C. Barth, G. Wolfram, and N. Zöllner. 1981. Cholesterin und Triglyceride im Serum einer Münchener Bevölkerungsgruppe: Beziehungen zu Alter und Geschlecht. *J. Clin. Chem. Clin. Biochem.* **19:** 1013–1019.
25. Havel, R., H. Eder, and J. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
26. Kunkel, L. M., K. D. Smith, S. H. Boyer, D. S. Borgaonkar, S. S. Wachtel, O. J. Miller, W. R. Breg, H. W. Jones, and J. M. Rary. 1977. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc. Natl. Acad. Sci. USA.* **74:** 1245–1249.
27. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. G. Higuchi, T. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science.* **239:** 487–491.
28. Kreitman, M., and L. F. Landweber. 1989. A strategy of producing single-stranded DNA in the polymerase chain reaction: a direct method for genomic sequencing. *Gene. Anal. Tech.* **6:** 84–88.
29. Schuster, H., S. Richter, G. Stratmann, C. Keller, G. Wolfram, and N. Zöllner. 1992. Identification of a silent point mutation in the LDL-receptor gene by direct DNA sequencing. *Klin. Wochenschr.* **69:** 517–521.
30. Rauh, G., H. Schuster, B. Müller, S. Schewe, C. Keller, G. Wolfram, and N. Zöllner. 1990. Genetic evidence from 7 families that the apolipoprotein B gene is not involved in familial combined hyperlipidemia. *Atherosclerosis.* **83:** 81–87.
31. Frostegård, J., A. Hamsten, M. Gidlund, and J. Nilsson. 1990. Low density lipoprotein-induced growth of U937 cells: a novel method to determine the receptor binding of low density lipoprotein. *J. Lipid Res.* **31:** 37–44.
32. Innerarity, T. L., M. E. Balestra, K. S. Arnold, R. W. Mahley, G. L. Vega, S. M. Grundy, and S. G. Young. 1988. Isolation of defective receptor-binding low density lipoproteins from subjects with familial defective apolipoprotein B-100. *Arteriosclerosis.* **8:** 551a (Abstr.)
33. Vega, G. L., and S. M. Grundy. 1986. In vivo evidence for reduced binding of low density lipoproteins to receptors as a cause of primary moderate hypercholesterolemia. *J. Clin. Invest.* **78:** 1410–1414.
34. Jeffreys, A. J., N. J. Royle, V. Wilson, and Z. Wong. 1988. Spontaneous mutation rates to new length alleles at tandem repetitive hypervariable loci in human DNA. *Nature.* **332:** 278–281.
35. Dunning, A. M., H-H. Renges, C-H. Xu, R. Peacock, R. Brasseur, G. Laxer, M. Tikkanen, R. Bütler, N. S. Saha, A. Hamsten, M. Rosseneu, P. Talmud, and S. E.

Humphries. 1992. Two amino acid substitutions in apolipoprotein B are in complete allelic association with the antigen group (x/y) polymorphism: evidence for little recombination in the 3' end of the human gene. *Am. J. Hum. Genet.* **50:** 208–221.

36. Cooper, D. N., and H. Youssoufian. 1988. The CpG dinucleotide and human genetic disease. *Hum. Genet.* **78:** 151–155.

37. Coulondre, C., J. H. Miller, P. J. Farabaugh, and W. Gilbert. 1978. Molecular basis of base substitution hot spots in *Escherichia coli*. *Nature.* **274:** 775–780.

38. Antonarakis, S. E., H. H. Kazazian, and S. H. Orkin. 1985. DNA polymorphism and molecular pathology of the human globin gene clusters. *Hum. Genet.* **69:** 1–14.

39. Antonarakis, S. E., S. H. Orkin, H. H. Kazazian, S. C. Goff, C. D. Boehm, P. G. Waber, J. P. Sexton, H. Ostrer, V. F. Fairbanks, and A. Chakravarti. 1982. Evidence for multiple origins of the β^E-globin gene in Southeast Asia. *Proc. Natl. Acad. Sci. USA.* **79:** 6608–6611.

40. Market, M. L., C. Norby-Slycord, and F. E. Ward. 1989. A high proportion of ADA point mutations associated with a specific alanine-to-valine substitution. *Am. J. Hum. Genet.* **45:** 354–361.