

High prevalence of familial defective apolipoprotein B-100 in Switzerland

André R. Miserez,¹ Roland Laager, Nicole Chiodetti, and Ulrich Keller

Departments of Research and Internal Medicine, University Hospital, Petersgraben 4, CH-4031 Basel, Switzerland

Abstract Familial defective apolipoprotein B-100 (FDB) is caused by a single G-to-A substitution at nucleotide 10,708 leading to an arginine to glutamine change at amino acid 3,500 of the apolipoprotein B-100 and thus, a reduced binding of the apolipoprotein B to the low density lipoprotein (LDL) receptor. In the present study, the prevalence of FDB in Switzerland was estimated, on the one hand, from a sample of 728 healthy volunteers whose origin was spread out over the entire German, French, and Romansh speaking parts of the country, and, on the other hand, from 142 unrelated Swiss families with primary hypercholesterolemia comprising 520 individuals. Using polymerase chain reaction (PCR)-based methods, three individuals were identified with the point mutation in the sample of volunteers, equivalent to a prevalence of approximately 1/240 (90% confidence interval: 1.51×10^{-3} – 1.03×10^{-2}). The frequency of FDB in the sample of hypercholesterolemic subjects was 7/142, yielding a prevalence of approximately 1/190 extrapolated to the general population (90% confidence interval: 2.63×10^{-3} – 9.17×10^{-2}). The combined prevalence based on both samples was 1/209. Thus, the investigated point mutation was highly prevalent in Switzerland and appeared to be more frequent than in other populations studied hitherto. Furthermore, the presence of the mutation was not necessarily associated with an elevation of serum cholesterol levels, particularly in young individuals. While in the non-affected volunteers cholesterol levels increased between the age of 19 and 23 years by 0.22 mmol/l or by 5.6% ($P = 0.001$), this phenomenon was even more pronounced in individuals with FDB. The three volunteers with the point mutation demonstrated an increase in total cholesterol concentrations by 1.30 mmol/l or by 25% within 2 years, suggesting that, in the early twenties, cholesterol concentrations increase markedly from normal to elevated levels. ■ Considering the estimated high prevalence and the relative ease of PCR-based tests, screening for FDB may become a standard procedure in patients with suggested familial forms of hypercholesterolemia. — Miserez, A. R., R. Laager, N. Chiodetti, and U. Keller. High prevalence of familial defective apolipoprotein B-100 in Switzerland. *J. Lipid Res.* 1994. 35: 574–583.

Supplementary key words point mutation • hypercholesterolemia • compensatory mechanisms • genetics • age of the mutation • polymerase chain reaction (PCR) • restriction fragment length polymorphisms (RFLPs)

Defective binding of low density lipoprotein (LDL) due to a specific structural defect of the apolipoprotein B-100

was recently identified in patients with moderately increased LDL cholesterol concentrations (1–3). This metabolic disorder was designated familial defective apolipoprotein B-100 (FDB). A markedly reduced (approximately 32% of the normal) binding of LDL particles isolated from FDB patients to LDL receptors of cultured fibroblasts and a decrease of the clearance of LDL in turnover studies has been demonstrated in affected individuals (1). FDB is caused by a single base substitution (G-to-A) at nucleotide 10,708 in exon 26 of the apolipoprotein B-100 gene (chromosome 2) creating an arginine to glutamine substitution at the codon for amino acid 3,500 of the mature apolipoprotein (4). The point mutation is transmitted as an autosomal codominant trait (2); clinical features in FDB patients may be similar to those in patients with familial hypercholesterolemia (FH) (5). The prevalence of FDB has been estimated to be 1/500–1/700 in different populations (6–8). Individuals with FDB have been detected in Caucasians from the United States (2, 6, 9), Canada (6, 9), the United Kingdom (7), Denmark (7), Austria (6, 10), Germany (8, 11–13), Italy (14), the Netherlands (13, 15, 16), South Africa (15, 16), Norway (16), Australia (16), and in a non-Caucasian (Chinese; 17) but up to now not in Caucasians from Finland (18), Russia (13), and Israel (19) nor in non-Caucasians from Japan (19). However, a direct assessment of the prevalence in a particular population based on a unselected sample of subjects representative of the general population has not been carried out previously. Thus, the present study was performed to assess the prevalence of the point mutation in the general population of Switzerland from a sample of healthy volunteers and its confirmation by estimations from a sample of hypercholesterolemic individuals.

Abbreviations: FDB, familial defective apolipoprotein B-100; PCR, polymerase chain reaction; LDL, low density lipoprotein; FH, familial hypercholesterolemia; SE buffer, sodium chloride-EDTA buffer.

¹To whom correspondence should be addressed.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Two groups of subjects were screened for the presence of FDB. The first group consisted of 728 unrelated healthy male individuals from the German, French, and Romansh speaking parts of Switzerland recruited for military service in August 1991. The three volunteers positive for the point mutation were reexamined in August 1993 to confirm the results of the initial cholesterol determinations and to screen their families.

The second group consisted of 520 individuals from 142 unrelated families with suggested inherited forms of hypercholesterolemia which were diagnosed in the presence of LDL cholesterol levels exceeding the 95th percentile (corrected for age and gender) in the index patient and in at least three family members of two generations. Among the 142 families with hypercholesterolemia, 102 were from northern, 19 from southwestern, 5 from western, and 16 from eastern parts of Switzerland. There were 296 hypercholesterolemic (142 unrelated index patients and 154 relatives) and 224 normocholesterolemic family members.

All subjects gave their informed consent according to the Helsinki/Tokyo declaration to participate in this study. The study was approved by the ethical committee of our hospital.

Materials

Thermus aquaticus polymerase and nucleotides were purchased from Perkin Elmer Cetus Corporation (Norwalk, CT). Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer model 380 (Applied Biosystems, Foster City, CA) at the Biocenter, University of Basel, Switzerland. DNA was amplified using Cambio Intelligent Heating Blocks, model 2024 (Cambio, Cambridge, UK). Agarose was purchased from Bio-Rad (Richmond, CA) and restriction endonucleases were from Pharmacia LKB (Uppsala, Sweden). [α - 32 P] dCTP, multiprime DNA labeling systems, and Hybond NTM nylon membranes were obtained from Amersham International (Buckinghamshire, UK). The probe for analyzing the LDL receptor gene was pLDLR-2HH1, containing a 1.9 kb fragment of the full-length cDNA which hybridizes with exon 11 to exon 18 of the gene (20).

Methods

Lipoprotein analyses. Blood samples were drawn in all subjects of both groups 12 h postprandially. LDL cholesterol was determined after precipitation with heparin and HDL cholesterol after precipitation with phosphotungstic acid and magnesium ions. Total, LDL, HDL cholesterol, and triglyceride concentrations were measured in all the subjects at the same laboratory by enzymatic colorimetric methods (Boehringer Mannheim,

Germany) on a Hitachi analyzer model 737.

DNA extraction method. Total genomic DNA was extracted from white blood cells by the salting-out method (21) with the following modifications. Ten ml EDTA blood was mixed with 30 ml of an erythrocyte lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4), and left on ice for 1 h. The tubes were centrifuged (10 min, 150 g 4°C) and the cell pellet was resuspended in 30 ml of sodium chloride-EDTA (SE) buffer (75 mM NaCl, 25 mM EDTA, pH 8.0). The cells were centrifuged as described above and resuspended in 5 ml of the SE-buffer. Seventy-five μ l of proteinase K (20 mg/ml) and 15 μ l of RNase A (10 mg/ml) were added. After mixing and adding 250 μ l of 20% SDS, cells were digested overnight at 50°C. Subsequently, SE-buffer was added to a total volume of 10 ml. Proteins were salted out using 2.8 ml of 6 M NaCl and shaken vigorously for 15 sec, followed by centrifugation (15 min, 1000 g 20°C). The supernatant was transferred to another tube and precipitated with two volumes of absolute ethanol. The precipitated DNA strands were transferred to a 1.5 ml tube. DNA was once washed with 70% ethanol, twice with 100% ethanol, and dried overnight. Finally, DNA was resuspended in 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) and measured spectrophotometrically at 260 nm.

Testing for the point mutation causing FDB. A combination of allele-specific and asymmetric PCR was used (22). With the help of two primers, an upper (UOL) and a lower oligonucleotide (LOL), a DNA strand of 0.320 kb in length containing the mutation site was amplified. Provided that the mutation was present in the template, a third allele-specific oligonucleotide (ASO), complementary to the mutation, began to amplify additional DNA strands starting from the point mutation as soon as the concentration of the LOL was lower than that of the ASO. As the G-to-A substitution was 0.144 kb upstream to the 5' end of the initially amplified DNA, the additional fragment was 0.144 kb in length. In all series, DNA from patients with FDB confirmed by allele-specific oligomelting served as controls (8). All subjects with total cholesterol > 4.5 mmol/liter were tested individually in two independent runs and all reactions were carried out in duplicate. Blood samples from subjects with cholesterol levels \leq 4.5 mmol/liter were pooled (25 samples) and screened using the method of Ruzicka et al. (23). In the case of positive pools, DNA from each subject contributing to the pool was extracted and tested individually.

Major rearrangements and cosegregation analysis at the LDL receptor gene locus in families with FDB. To exclude major rearrangements at the LDL receptor locus in subjects with FDB, Southern blot investigations using the restriction enzyme *Xba* I were performed (24, 25). Five μ g genomic DNA was digested for 24 h with 10 U *Xba* I and subjected to electrophoresis. After denaturation of the gels, DNA fragments were transferred to nylon mem-

branes and fixed by UV-light radiation. The cDNA *Bam*H I-fragment of the LDL receptor gene probe was excised as described by Schuster et al. (26) and 5'-labeled using random oligonucleotide primers and [α - 32 P]dCTP (3,000 Ci/mmol). Membranes were hybridized at 65°C for 24 h, washed two times for 30 min at 65°C in 1 × SSC (150 mM NaCl, 15 mM sodium citrate) and 0.1% SDS, and exposed to Kodak X-Omat AR-5 films for 24–72 h. In all FDB families, haplotypes using eight restriction fragment length polymorphisms (RFLPs) at the LDL receptor locus (*Taq* I, *Stu* I, *Hinc* II, *Ava* II, *Apa*L I 5', *Pvu* II, *Nco* I, and *Apa*L I 3') were constructed according to the methods described elsewhere (27). Cosegregation analyses between the individuals with hypercholesterolemia and the corresponding LDL receptor haplotypes were performed.

Apolipoprotein E restriction isotyping. Restriction isotyping of human apolipoprotein E was performed by gene amplification and cleavage with *Hha* I as described by Hixson and Vernier (28).

Statistical methods. The confidence interval of the frequency of the mutation in the sample of volunteers was

calculated using methods based on a Poisson distribution since $NP < 5$, whereas N is the sample size and P is the frequency of the point mutation in the sample (29, 30). The prevalence of the mutation assessed from the sample of families with hypercholesterolemia was estimated according to the method described by Innerarity et al. (6). Data from the Lipid Research Clinics (LRC) study (48,482 individuals from North America; 31) were used to compare the present findings with a previous study (6); data from the MONICA study (3,341 individuals from Switzerland; 32) were used to estimate the prevalence of FDB in Switzerland. The prevalence of the mutation was calculated by the maximum likelihood estimation using data from both samples. Subjects from the sample of volunteers were stratified into three groups with respect to their age (19–20.9 years, 21–22.9 years, and 23–29 years). Differences of mean total cholesterol concentrations among the three age groups were calculated by the Scheffé F-test and by one-way analysis of variance (ANOVA). Fisher's exact test was used to compare frequencies of the mutation in samples from different populations. All calculations were performed on DEC and Macintosh computers.

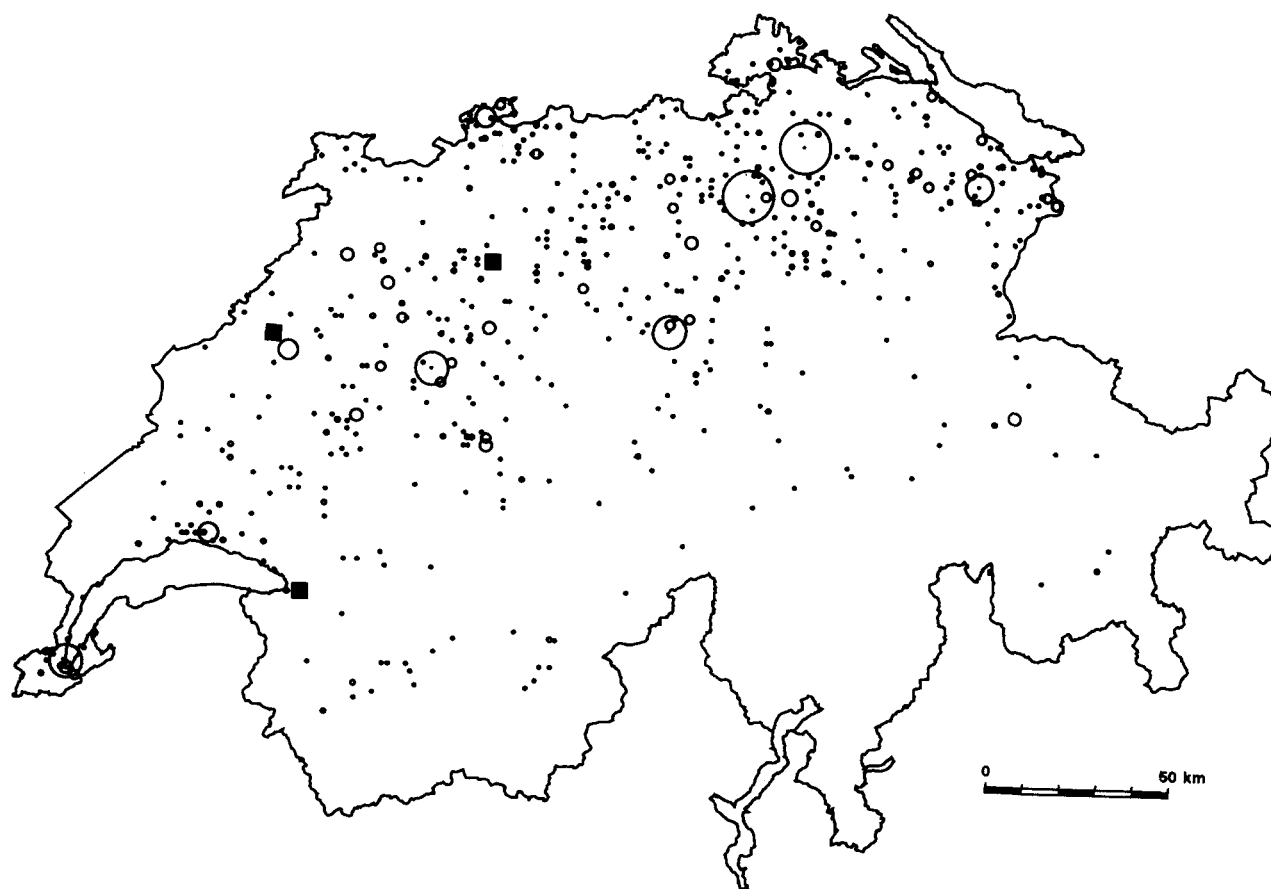


Fig. 1. The map shows the domiciles of the 728 volunteers participating in this study (closed circles represent one individual, open circles represent more than one subject whereas the areas of the circles are proportional to the number of volunteers originating from the respective city). The distribution of the domiciles of the volunteers reflects the Swiss population density at December 31 1990 (Bundesamt für Landestopografie (1992), CH-3084 Wabern, Switzerland) quite exactly. Closed squares represent the domiciles of the three individuals positive for the mutation.

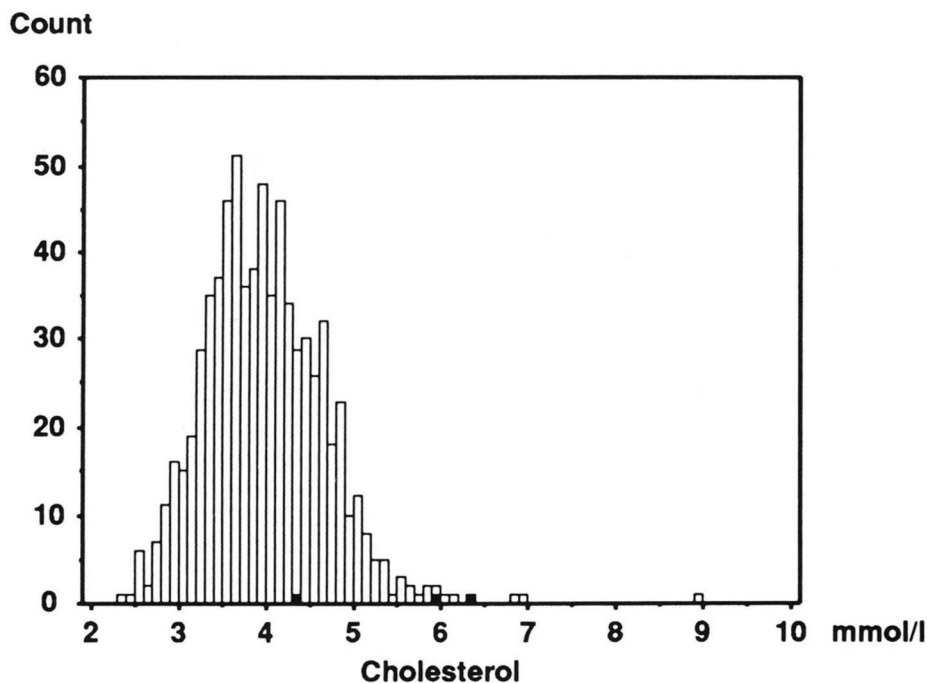


Fig. 2. Total serum cholesterol levels of 728 volunteers (closed parts of the bars: subjects with the point mutation).

RESULTS

Characteristics of the healthy volunteers

Fig. 1 represents the geographic origin of the investigated volunteers in Switzerland spread out over the entire country except the small Italian speaking part south of the Alps. Most (91.8%) of the volunteers were between 19 and 22 years old and the median age was 20.5 years (range 19 to 29 years). Fig. 2 shows the frequency distribution of the cholesterol values in the investigated volunteers. Mean cholesterol of the 728 volunteers (\pm SEM) was 3.98 ± 0.03 mmol/liter. The mean cholesterol concentration in the 501 normocholesterolemic volunteers of the age of 19–20.9 years (group 1) was 3.90 ± 0.03 mmol/l, in the 193 volunteers of 21–22.9 years (group 2) 4.12 ± 0.06 mmol/l, and in the 26 volunteers of 23–29

years (group 3) 4.47 ± 0.18 mmol/l, respectively. Stratification of the normocholesterolemic 720 volunteers with respect to their age revealed significant differences of total cholesterol concentrations between group 1 and 2 ($\Delta = 5.6\%$, $P = 0.0011$), between 1 and 3 ($\Delta = 14.6\%$, $P = 0.0003$), and between group 2 and 3 ($\Delta = 8.5\%$, $P = 0.0437$). The overall difference among the three groups assessed by one-way ANOVA was also significant ($P = 0.0001$). In 8 of the 728 volunteers total cholesterol levels exceeded the 95th percentile (5.90 mmol/l).

Characteristics of subjects from families with primary hypercholesterolemia

The mean pretreatment level of total cholesterol of the 296 hypercholesterolemic members (\pm SEM) from the 142 families was 8.95 ± 0.14 mmol/l and mean LDL

Familial Defective Apolipoprotein B-100 G to A Substitution

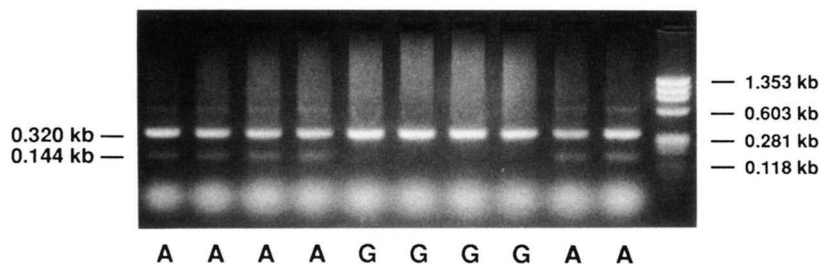


Fig. 3. Results of allele-specific asymmetric PCR. All reactions were performed in duplicate.

cholesterol was 7.09 ± 0.18 mmol/l. Mean total and LDL cholesterol of the 224 subjects not affected by primary hypercholesterolemia from these families were 5.57 ± 0.10 mmol/l and 3.61 ± 0.11 mmol/l, respectively.

Frequency of FDB in the two samples

Fig. 3 presents the result of asymmetric allele-specific PCR and agarose gel electrophoresis (all reactions carried out in duplicate) of four of the volunteers, from whom two were positive for the mutation, and one subject with proven FDB due to the G-to-A substitution which served as a control (kindly provided by H. Schuster, Munich).

From the sample of 728 unrelated, healthy volunteers, three individuals living in different regions of Switzerland

were identified as carrying the point mutation. All three were members of obviously autochthonous Swiss families. Reconstruction of the origin of the three families over four generations revealed no ancestors who had immigrated from other countries except the unaffected father (III/2) of the volunteer from the H-family (Fig. 4). Surnames and maiden names of the family members were typical for the respective geographical regions of Switzerland where the families lived. Total cholesterol concentrations initially determined in the three individuals positive for the point mutation were 4.33, 5.92, and 6.31 mmol/l, respectively. Two years after the first evaluation, the three individuals with the point mutation were reexamined. Fig. 4 presents the pedigrees of the three families of these volunteers. In the volunteer with an initially normal

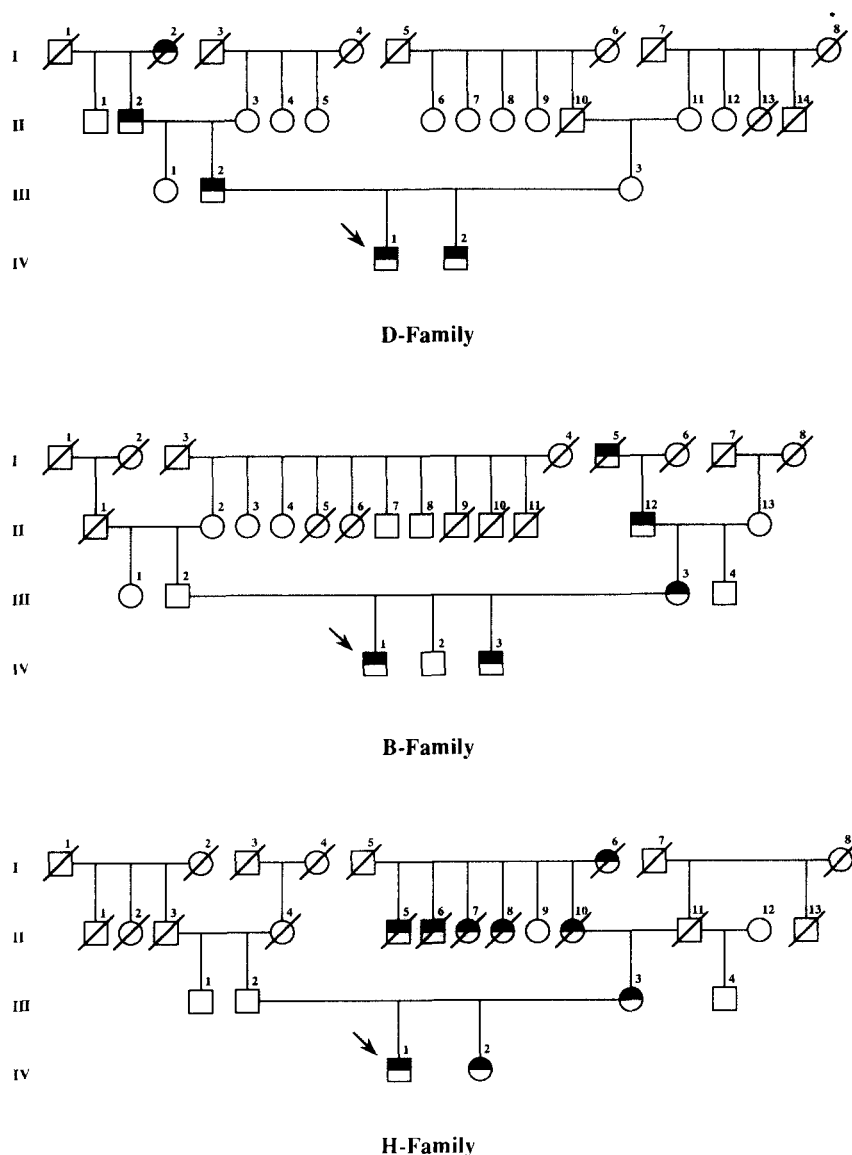


Fig. 4. Families of the three volunteers positive for the point mutation.

cholesterol concentration (4.33 mmol/l; D-family, IV/1), the determinations 2 years later revealed total cholesterol and LDL cholesterol concentrations above the 95th percentile (6.05 and 4.29 mmol/l, respectively) corresponding to an approximately 40% increase compared to the initial cholesterol value. Elevated total and LDL cholesterol concentrations had also been demonstrated in the volunteer's father (III/2): 10.60 and 8.22 mmol/l, respectively. Total cholesterol concentrations (all determined in the same laboratory) of the younger brother of the volunteer (IV/2) were strikingly inconstant between the ages 15 and 21 years, sometimes clearly elevated: 6.94 (1989), 7.59 (1992), and 6.71 mmol/l (1992), sometimes in the normal range: 5.44 mmol/l (1989), corresponding to a 40% difference. Very unstable cholesterol concentrations were also detected in the second volunteer (B-family; Fig. 4): 6.10 mmol/l (1988), 5.89 mmol/l (1991), 4.60 mmol/l (1993), 5.30 mmol/l (1993), 6.56 mmol/l (1993), corresponding to a 43% difference within one year. In the third volunteer carrying the point mutation (H-family), total cholesterol concentrations also increased clearly (from 6.31 to 7.81 mmol/l) during the 2 years corresponding to a 24% increase. The absence of hypercholesterolemia in the first volunteer (D-family) at the age of 20 years was not correlated to the apolipoprotein E3/E3 genotype as this genotype was also present in his hypercholesterolemic father (III/2) and in his hypercholesterolemic brother (IV/2). Reexamination of the three volunteers and their families confirmed the presence of the point mutations in the three probands, in the father (III/2) and the brother (IV/2) of the first volunteer (D-family), in one of the two brothers (IV/3) of the second volunteer (B-family), and in the mother (III/3) and the sister (IV/2) of the third volunteer (H-family).

In seven of the 142 unrelated families with primary hypercholesterolemia and in 20 from 520 individuals studied, the defective apolipoprotein B-100 was identified. In all these families, there were no major rearrangements at the LDL receptor locus detectable using *Xba* I nor were there families with a cosegregation between hypercholesterolemic phenotypes and a particular LDL receptor haplotype. The mean pretreatment total and LDL cholesterol concentrations in the 20 subjects with the point mutation were 9.16 ± 0.41 mmol/l and 7.10 ± 0.30 mmol/l, respectively.

Estimation of the prevalence of FDB in the general population

The frequency of FDB in the sample of healthy volunteers was 3/728, 4.12×10^{-3} or approximately 1/240 (90% confidence interval: 1.51×10^{-3} – 1.03×10^{-2} or 1/661–1/97, respectively). The frequency of FDB in the sample of families with primary hypercholesterolemia was 7/142 (1/20) or 4.93×10^{-2} (90% confidence interval 2.53×10^{-2} – 8.82×10^{-2} or 1/39–1/12). Using data of the LRC study comprising 48,482 North American individuals (31), the prevalence of FDB would be 2.38×10^{-3} or approximately 1/420. However, mean cholesterol levels in individuals from Switzerland (32) were, depending on age and sex, 15.5%–19.7% higher than in the individuals of the LRC study. Thus, if data of the MONICA study from Switzerland were used, the prevalence in the general population was 5.20×10^{-3} or approximately 1/190 (90% confidence interval: 2.63×10^{-3} – 9.17×10^{-3} or 1/381–1/109; **Table 1**).

The estimation based on both samples (volunteers and families with primary hypercholesterolemia) was 4.79×10^{-3} or 1/209. The population of the German, French, and Romansh speaking parts of Switzerland (without the

TABLE 1. Estimation of the prevalence of familial defective apolipoprotein B-100 in Switzerland extrapolated from data of the MONICA study (Switzerland)

Age	Sex	LDL Cholesterol <i>mmol/l</i>	Number of Individuals				Frequency ^b
			This Study		MONICA Study ^a		
<i>yr</i>			Probands	Total	Group	Total	
25–34	M	> 4.97	1	9	24	3,341	0.00079816
35–44	M	> 5.45	1	18	51	3,341	0.00084805
45–54	M	> 5.64	1	18	49	3,341	0.00081479
55–64	M	> 5.47	1	15	37	3,341	0.00073830
45–54	F	> 5.27	1	17	43	3,341	0.00075708
55–64	F	4.99–5.65	1	25	52	3,341	0.00062257
55–64	F	> 5.65	1	17	35	3,341	0.00061623
Total							0.00519518

^aBurnand et al. 1993 (32).

^bFrequencies were calculated according to the formula of Innerarity et al. 1990 (6): (Probands/This Study Total) × (Group/MONICA Study Total).

Italian speaking part: 290,000 inhabitants) consisted at December 31st 1991 of 6,802,900 inhabitants (data of the Bundesamt für Statistik, CH-3003 Berne, Switzerland, 1992). Thus, assuming a prevalence of 1/209, approximately 32,500 individuals with this particular point mutation are expected in the German, French, and Romansh speaking population of Switzerland.

Table 2 presents the comparison of the present data with the estimated prevalence of FDB in other populations. The frequency of FDB in hypercholesterolemic subjects from Switzerland was significantly higher than in the sample from Finland but there was no significant difference to the FDB frequency in hypercholesterolemic subjects (FH or type IIa hyperlipidemia) from the United Kingdom or from Germany.

DISCUSSION

The sample of healthy volunteers originating from military trainees was not selected as, in Switzerland, all male individuals after the age of 19 years are drafted. The distribution of the domiciles of the volunteers was very similar to that of the general population with the exception of the small Italian speaking part south of the Alps from which no subjects were available for investigation (Fig. 1). Thus, since the disorder studied is caused by a germ line mutation located on an autosomal chromosome, the frequency of the point mutation in the present sample appears to be representative of the prevalence in the general population. As hypercholesterolemia associated with FDB

is often moderate (1), even in individuals homozygous for the mutation (33), and is rarely associated with coronary artery disease before the age of 50 years (5), the point mutation probably does not affect Darwinian fitness of these individuals. In a natural population such as the Swiss, a single gene mutation newly arisen in one parent runs a high risk of chance elimination in its immediate descendants. Probabilities for fixation or for extinction of neutral alleles arising by mutation can be calculated by equations of Kimura (34, 35). When a population is subdivided into isolate breedings of limited size (N), the ultimate probability for fixation of neutral alleles over all isolates is $P = q_0$, where q_0 is the initial frequency of a neutral allele. Assuming no selection and thus, equal Darwinian fitness of the subjects carrying the mutant allele, computer simulations of fixation and extinction of single gene mutants in a population of $N = 100$ with an initial $q_0 = 1/(2N) = 5.0 \times 10^{-3}$ result in a final $q = 4.9 \times 10^{-3}$ or 1/204 and a mean number of generations to fix the frequency of the mutant allele of $t_1 = 394$ (36). Thus, approximately 10,000 years would be necessary for fixation of a mutant in a given population. As it has been demonstrated by Innerarity et al. (6), the probability that the mutation has occurred independently more than once is very low. Furthermore, a founder gene effect has previously been assumed and it has been suggested that the mutation may have emerged in the common ancestors of a large number of Caucasian populations (6). As the G-to-A substitution at the apolipoprotein B-100 locus has not been detected among 552 hypercholesterolemic patients from the Finnish population (18) or in the St. Petersburg

TABLE 2. Comparison of the prevalence of FDB due to the G to A mutation in different population samples

City/Country	Reference	Method ^a	Sample	Affected Individuals	P^b	Prevalence
Montreal	Innerarity et al. 1990 (6)	(A, C)	343	1	(0.0012)	
San Francisco	Innerarity et al. 1990 (6)	(A, C)	388	4	(0.0103)	
Dallas	Innerarity et al. 1990 (6)	(A, C)	133	4	(0.1824)	
Salzburg	Innerarity et al. 1990 (6)	(B, C)	236	2	(0.0162)	
Total	Innerarity et al. 1990 (6)	(D)	1100	11	(0.0024)	1/500
United Kingdom	Tybjærg-Hansen et al. 1990 (7)	(A)	264	9	0.1571	1/600
Denmark	Tybjærg-Hansen et al. 1990 (7)	(B)	137	1	(0.0374)	
Sweden	Tybjærg-Hansen et al. 1990 (7)	(B)	113	0	(0.0001)	
Germany	Schuster et al. 1990 (8)	(A)	243	8	0.1555	1/700
Finland	Hämäläinen et al. 1990 (18)	(A)	552	0	0.0001	-
Switzerland	This study	(E)	142	7		1/190
	This study	(F)	728	3		1/240
Total (combined prevalence)	This study	(E, F)				1/209

^aA: Estimated from hypercholesterolemic subjects; B: Estimated from subjects with atherosclerosis; C: Estimated from normocholesterolemic subjects; D: Pooled prevalence estimated from normo- and hypercholesterolemic subjects corrected using data of the LRC study (North America; 48,482 individuals; 31); E: estimated from hypercholesterolemic subjects (type IIa hyperlipoproteinemia) corrected using data of the MONICA study (Burnand et al. 1993; 32); F: Estimated from a sample of healthy male Swiss volunteers originating from the entire country.

^b P values (Fisher's exact test): significance levels of differences between the frequency of FDB in Swiss hypercholesterolemic subjects (7/142) and the frequencies in subjects from other populations. P values in parentheses: samples are not directly comparable.

area (13), it may be hypothesized that this base exchange may have arisen after the divergence of the Finnish and the other Caucasoid populations. From genetic distances between the Finnish and the other European populations (37), this divergence is estimated to have occurred approximately 10,000 to 20,000 years ago, in any case, clearly after the divergence of Caucasoid populations and Mongoloids which was approximately 41,000 years ago (37). On the other hand, the finding of a similar prevalence of the point mutation in several European and American Caucasian populations and in a man of Chinese origin (17) as well as the finding of a prevalence which was (particularly in the Swiss) close to the theoretically expected frequency of a fixed gene (1/204), both suggest fixation of the mutant alleles in these populations. This supports the hypothesis that the mutation may be at least 10,000 years old and, if the mutation in this Chinese individual did not occur independently, even more than 41,000 years old.

However, it is a striking observation that only one mutation causing a defect of the binding site of the apolipoprotein B-100 has been described in detail since 1986 up to now. This defect impairs the interaction between the ligand, the apolipoprotein B-100, and the LDL receptor. At the LDL receptor locus itself, more than 150 different mutations have been detected causing similar pathophysiologic changes resulting in elevated LDL cholesterol concentrations (38). Thus, it may be hypothesized that, in contrast to the situation at the LDL receptor locus (27), there is a very low mutation rate at the apolipoprotein B-100 locus, or mutations other than the base exchange at position 10,708 may be lethal during embryonic development.

Total cholesterol values of the 728 healthy volunteers investigated in this trial are well characterized by a Gaussian distribution (Fig. 2). Cholesterol concentrations in two of the three subjects identified as carrying the mutation were clearly increased compared with levels of individuals at equal ages. However, the levels corrected for age and sex were lower than in FH patients reported in the literature (39) and only moderately elevated compared with three of the other six hypercholesterolemic individuals from this sample. Furthermore, we did not anticipate detecting an individual with the point mutation but with a normal cholesterol concentration. Two years after the first evaluation, the three volunteers identified as being affected were reexamined in order to confirm the results of the initial lipid determinations, to study the cosegregation between the point mutation and the clinical features within the families, and to confirm the autosomal-dominant trait of the mutation.

The presence of hypercholesterolemia was not dependent on the apolipoprotein E genotypes, although the initially normocholesterolemic volunteer in our study as well as the two normocholesterolemic individuals with the point mutation in the report of Gallagher and Myant (40)

were homozygous for apolipoprotein E3. One of these two normocholesterolemic subjects reported in the literature was an 8-year-old boy with total and LDL cholesterol concentrations of 3.6 and 2.7 mmol/l, respectively; the second was a 37-year-old man (5.0 and 3.4 mmol/l, respectively). The E3 phenotype is the most frequent one and thus, it may be by chance only that all the normocholesterolemic individuals with the point mutation described hitherto were homozygous for E3. Furthermore, other affected individuals of this family were also homozygous for E3 and, nevertheless, hypercholesterolemic.

Total cholesterol levels in the sample of healthy volunteers were significantly higher in subjects of 21–22.9 years than of 19–20.9 years and this phenomenon was even more pronounced in the individuals with the point mutation causing FDB. The same mechanisms leading to an increase in the LDL cholesterol concentrations during life in the normal population may also be responsible for the increase in individuals with FDB. A very unstable phase in these individuals seems to be the postpubertal period until the early adult age. During this period, cholesterol concentrations may fluctuate between normal and clearly elevated levels as demonstrated in affected members from two of the families of the volunteers. After the age of 25, compensatory mechanisms may weaken and individuals with the point mutation become hypercholesterolemic. The striking fluctuations during the postpubertal period and the distinct increase in the early twenties support the hypothesis that there may be either compensatory mechanisms to metabolize apolipoprotein B-defective LDL particles or that the gene product of the unaffected allele may be overexpressed. One of the compensatory mechanisms could be an increased uptake of the apolipoprotein E-containing IDL particles by the LDL receptor (which is capable of binding both apolipoprotein E and B) before the IDL particles are metabolized to LDL. The present data suggest that such mechanisms decompensate after the age of 15–25 years and most individuals with the mutation remain hypercholesterolemic throughout life. Nevertheless, the G-to-A mutation does not necessarily cause hypercholesterolemia and it remains to be discussed whether FDB may be defined merely by the presence of the mutation or additionally by the presence of hypercholesterolemia.

In the sample of families with hypercholesterolemia, data of the LRC study were first used to extrapolate the frequency to the general population, yielding a prevalence of approximately 1/420. However, the LRC population was not representative of the Swiss because the mean cholesterol values were 15.5%–19.7% higher in the Swiss than in the North Americans included in the LRC study (31, 32). Hence, data of the MONICA study were used to estimate the prevalence of FDB in Switzerland (Table 1). The prevalence of heterozygous FDB subjects of approximately 1/240 from a sample representative of the general

population of Switzerland and approximately 1/190 from the sample of families with primary hypercholesterolemia and thus, a prevalence of 1/209 based on the results from both samples, was very similar to the final frequency of a gene that is fixed in a population (1/204) but was higher than has been estimated in other Caucasian populations. However, the prevalence in other populations was calculated particularly from hypercholesterolemic subjects. As the presence of the point mutation, at least in young individuals, may not necessarily be associated with hypercholesterolemia, as it has been demonstrated in this study, extrapolations from hypercholesterolemic subjects may lead to an underestimation of the prevalence in the general population. Thus, the point mutation may be more frequent in other populations also. On the other hand, FDB seems to contribute to the higher frequency of hypercholesterolemic subjects in Switzerland than in North America as not only the absolute but also the relative frequency of FDB in hypercholesterolemic subjects was higher in the Swiss than in the North Americans: estimations using data of the LRC study would result in a prevalence of 1/420 in the Swiss whereas the prevalence in the North Americans using these data was 1/500 (6).

The frequency of FDB in hypercholesterolemic subjects from Switzerland was compared with those from the United Kingdom, Germany, and Finland. Samples from Montreal, San Francisco, Dallas, Salzburg, Denmark, and Sweden were not exactly comparable with the Swiss data as they included both normo- and hypercholesterolemic subjects or subjects with atherosclerosis. In contrast to FH which is estimated to occur in one of 500 subjects in the general population (39) and which is caused by more than 150 different mutations (38), the investigated base substitution causing FDB was much more prevalent than is assumed for any particular mutation (represented by a LDL receptor haplotype) causing FH in Switzerland (27). As FDB seems to be highly prevalent in Switzerland and because the disorder is possibly associated with premature coronary artery disease (5), testing for FDB using PCR-based methods may therefore become a standard procedure in the differential diagnosis of patients with familial forms of hypercholesterolemia. ■

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