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A Third Major Locus for Autosomal Dominant Hypercholesterolemia Maps to 1p34.1-p32

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Summary

Autosomal dominant hypercholesterolemia (ADH), one of the most frequent hereditary disorders, is characterized by an isolated elevation of LDL particles that leads to premature mortality from cardiovascular complications. It is generally assumed that mutations in the *LDLR* and *APOB* genes account for ADH. We identified one large French pedigree (HC2) and 12 additional white families with ADH in which we excluded linkage to the *LDLR* and *APOB*, implicating a new locus we named *"FH3."* A LOD score of 3.13 at a recombination fraction of 0 was obtained at markers *D1S2892* and *D1S2722*. We localized the *FH3* locus to a 9-cM interval at 1p34.1–p32. We tested four regional markers in another set of 12 ADH families. Positive LOD scores were obtained in three pedigrees, whereas linkage was excluded in the others. Heterogeneity tests indicated linkage to *FH3* in ~27% of these non-*LDLR*/non-*APOB* ADH families and implied a fourth locus. Radiation hybrid mapping located four candidate genes at 1p34.1-p32, outside the critical region, showing no identity with *FH3*. Our results show that ADH is genetically more heterogeneous than conventionally accepted.

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Introduction

Autosomal dominant hypercholesterolemia (ADH) is an inherited disorder of lipid metabolism, characterized by a selective increase of LDL particles in plasma (type IIa hyperlipoproteinemia) giving rise to tendon and skin xanthomas, arcus corneae, and premature mortality from cardiovascular complications (Goldstein and Brown 1989). Twenty-five years ago, Goldstein and Brown (1974) showed that ADH results from defects in the cell-surface receptor that removes LDL particles from plasma. They coined the disorder "familial hypercholesterolemia" (FH; MIM 143890). The LDL-particle receptor is a ubiquitous transmembrane glycoprotein of 839 amino acids that mediates the transport of LDL particles into cells via endocytosis (Goldstein and Brown 1974). The LDL-particle receptor gene (*LDLR*) was cloned by Yamamoto et al. (1984) and mapped to 19p13.1-13.3 by Lindgren (1985), and >600 mutations scattered throughout the 45-kb gene have now been reported (Varret et al. 1997*a*, 1997*b*, 1998; LDLR Mutation Database). Innerarity et al. (1987) demonstrated the genetic heterogeneity of ADH by reporting hypercholesterolemic patients with normal LDL-particle receptor activity and defective apolipoprotein B-100 that displayed low affinity for its receptor. This new molecular disorder was called "familial ligand-defective apolipoprotein B-100" (FDB; MIM 144010). The *APOB* gene spans 43 kb, is divided into 29 exons, and maps to chromosome 2p23-p24 (Law et al. 1985). To date, three mutations in the *APOB* gene associated with ADH have been reported: the R3500Q mutation is the most frequent, whereas the R3531C and the R3500W are relatively rare (Rabès et al. 1997). Thus, it convention-

ally is assumed that only two distinct genetic disorders account for the ADH phenotype.

The existence of a greater level of genetic heterogeneity has never been formulated clearly. However, careful examination of the literature shows that, by different approaches, a few families have been identified in which the ADH phenotype was not associated with defects in the *LDLR* and *APOB* genes. Lestavel-Delattre et al. (1994) studied 41 patients with the ADH phenotype (plasma LDL-cholesterol level >95th percentile, xanthomas, and/or personal or family history of coronary artery disease) and found normal LDL-particle receptor activity and normal LDL-particle binding in nine patients who did not carry the R3500Q mutation in the *APOB* gene. Further studies showed that six of these patients carried a mutation in the *LDLR* gene, but for the three other ADH subjects the genetic origin of the disease is presently unknown (Nissen et al. 1998). These results could be explained by a defect in the regulation of the expression of the *LDLR* gene or by the involvement of another locus. This latter hypothesis was indirectly confirmed by other teams (Miserez and Keller 1995; Sun et al. 1997) who reported ADH families in which they found normal LDL-particle receptor activity and a genetic exclusion of the *LDLR* and *APOB* genes. Furthermore, several cases of pseudohomozygous type IIa hypercholesterolemia have been reported by Masana et al. (1990), who showed that probands of this rare autosomal recessive hypercholesterolemia (ARH) have normal levels of LDL particles and normal LDL-particle receptor activity. In the present study we found 13 families that not only support these observations but also give evidence for the existence of a third gene involved in ADH, which we named "*FH3*." To identify *FH3*, we performed linkage analyses and exclusion mapping in a large French ADH family in which defects in *LDLR* or *APOB* have been conclusively ruled out. After positive LOD scores were obtained, confirmation of linkage in this first stage was sought in a second sample of 12 ADH families originating from France, Austria, Spain, Belgium, and New Zealand and presenting the typical ADH phenotype. Two Sardinian ARH families were also tested.

Families and Methods

Families and Clinical Evaluation

Family HC2 is a large ADH pedigree comprising three generations (fig. 1). All affected members were characterized by levels of total cholesterol above the 97.5th percentile when compared with other French indivuduals matched by age and sex (Steinmetz 1990). The proband (II-9) is a 36-year-old woman, ascertained at age 17 years with 3.32 g/l total cholesterol, 2.36 g/l LDL cholesterol, 0.48 g/l HDL cholesterol, 0.61 g/l triglycerides, and arcus corneae. Her sister (II-7) is a 40-year-old woman ascertained at age 20 years with 4.10 g/l total cholesterol, 3.12 g/l LDL cholesterol, 0.52 g/l HDL cholesterol, 0.75 g/l triglycerides, and arcus corneae, tendon xanthomas, and xanthelasmas. Distribution of total and LDL cholesterol values in all tested HC2 members is bimodal, indicating the autosomal dominant transmission of the disease (data not shown). All lipid values were confirmed with at least a second lipid measurement. Among the 39 family members, seven (II-3, III-3, III-4, III-5, III-6, II-13, and III-15) were scored as "unknown" in linkage analyses because they presented with borderline lipid values or because only one lipid determination was available. In the second set of families, all affected subjects were scored according to their level of total or LDL cholesterol above the 95th percentile when compared with other individuals from their origin population matched by age and sex (Civeira et al. 1990; Moreda et al. 1990; Williams et al. 1993). Affected subjects ($n = 46$) had mean lipid values as follows: 3.02 ± 0.81 g/l total cholesterol, 2.25 ± 0.76 g/l LDL cholesterol, 0.52 ± 0.19 g/l HDL cholesterol, and 1.23 ± 0.51 g/l triglycerides. Unaffected subjects ($n = 54$) had mean lipid values as follows: 2.04 ± 0.27 g/l total cholesterol, 1.32 ± 0.27 g/l LDL cholesterol, 0.52 ± 0.12 g/l HDL cholesterol, and 0.88 ± 0.36 g/l triglycerides. Subjects with an unknown phenotypic status ($n = 6$) had the following mean lipid values: 2.86 ± 0.33 g/l total cholesterol, 1.94 ± 0.27 g/l LDL cholesterol, 0.52 ± 0.10 g/l HDL cholesterol, and 1.46 ± 0.83 g/l triglycerides. All these values were confirmed with at least a second lipid measurement. Finally, in the Sardinian ARH families, the four affected subjects presented an elevated level of total (>4 g/l) and LDL (>3.5 g/l) cholesterol, and normal triglyceride (<1.5 g/l) and HDL (<0.5 g/l) levels. All subjects gave informed consent.

DNA Analysis and PCR Amplifications

DNA was isolated from whole blood samples with use of a method described by Collod et al. (1994). PCR amplifications and electrophoresis were performed under conditions reported by Collod et al. (1994) or adapted from Reed et al. (1994) by use of fluorescent-labeled primers. Alleles observed in the pedigrees were numbered arbitrarily for each marker. All marker-typing data were collected blindly and independently by two investigators (M.V. and J.P.R.), and all linked 1p markers were typed twice. Nonpaternity/nonmaternity was ruled out indirectly because no incompatible phase was found with the numerous highly polymorphic markers tested.

Figure 1 Segregation of chromosome 1p34.1-p32 markers in the French family HC2. Lipid values given under each family-member symbol are (*top to bottom*): age at lipid measurement; levels of total, LDL, and HDL cholesterol (in grams per liter); levels of triglycerides (in grams per liter); and *apo*E genotype (INNO-LiPA *Apo*E, Innogenetics, Murex, Châtillon) (Harrington et al. 1994). Haplotypes (*top to bottom*) at tel-*D1S255-D1S472-D1S2892-D1S2722-D1S211-D1S197*-cen markers are shown for each family member tested. Half-blackened symbols indicate affected members, unblackened symbols indicate unaffected members, and hatched symbols indicate members with an unknown phenotypic status. The haplotype in brackets of subject I-1 was unequivocally deduced. The common region segregating with the disease phenotype in the family is boxed.

Parametric Linkage Analysis

Pairwise and multipoint linkage analyses were performed with SLINK, MLINK, and LINKMAP of the LINKAGE package (Ott 1991) and the VITESSE algorithm (O'Connell and Weeks 1995), with the assumption that ADH is an autosomal dominant disease with a gene frequency of .002. Marker-allele frequencies were calculated with the data from the unrelated family members. Linkage was investigated with the assumption of equal female-to-male recombination rates. The distances between markers used in the LINKMAP analyses were estimated from family data. We assumed the penetrance for disease carriers to be .9, knowing that complete penetrance can lead to false exclusion and that reduced penetrance has been reported in FH and FDB. Penetrance for noncarriers was assumed to be zero. EXCLUDE (Edwards 1987) was used to produce the exclusion map. This program estimates the positional likelihood of the disease locus on each chromosome and the percentage of probability of the disease locus to be on any of the 22 autosomes. Heterogeneity tests were performed with HOMOG (Ott 1983), which computes likelihoods of linkage under genetic heterogeneity by estimating recombination fractions (θ) and the proportion of families showing linkage to the markers under study (α).

Nonparametric Linkage Analysis

GENEHUNTER (Kruglyak et al. 1996) performs complete multipoint analysis to infer the degree of identity-by-descent sharing among all affected family members at each map point. We used the modified version of this program, GENEHUNTER-PLUS (Kong and Cox 1997), which has been shown to be less conservative, particularly when data are less than perfectly informative. GENEHUNTER-PLUS calculates a semiparametric LOD score (ghpLS) by using a single parameter that is a measure of the inheritance vector in the pedigree and allele sharing.

Table 1

Description of the *FH3* Families Studied

Family	Origin	No. of Subjects with Status			LDL Receptor Activity[a]	LOD Score at		Mutation Screening in		Reference Population
		Affected	Unaffected	Unknown		LDLR	APOB[a]	LDLR[a]	APOB[b]	
HC2	France	6	25	7	Normal	−6.38	− 4.47	nd	Negative	Steinmetz et al. 1990
HC67	France	4	2	0	nd	−2.75	.56	nd	Negative	Steinmetz et al. 1990
A1	Austria	3	5	3	nd	−1.88	.19	Negative[c]	Negative	Williams et al. 1993
S150	Spain	3	3	0	nd	−1.18	−3.79	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
S108	Spain	3	4	0	nd	−3.53	.82	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
S113	Spain	4	4	0	nd	−1.98	−3.26	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
S517	Spain	4	1	0	nd	−.19	−1.73	Negative	Negative	Moreda et al. 1990; Civeira et al. 1990
S509	Spain	4	2	0	nd	−1.72	−1.72	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
S601	Spain	5	4	0	nd	−2.41	.30	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
S129	Spain	4	0	0	nd	−1.50	−1.61	nd	Negative	Moreda et al. 1990; Civeira et al. 1990.
S206	Spain	4	9	1	nd	−4.53	−2.74	nd	Negative	Moreda et al. 1990; Civeira et al. 1990
B1	Belgium	6	7	0	nd	−4.67	−.88	nd	Negative	Williams et al. 1993
NZ1	N. Zealand	2	3	2	Normal	−.25	−.66	Negative	Negative	Williams et al. 1993
I3	Sardinia	2	5	1	nd	−∞	nd	nd	Negative	Williams et al. 1993
I4	Sardinia	2	10	4	nd	−∞	nd	nd	Negative	Williams et al. 1993

[a] nd = not determined.
[b] R3500Q and R3531C.
[c] Promoter sequence not investigated.

Candidate Gene Mapping

We used the Radiation Hybrid Mapping Panel GENEBRIDGE 4 and performed PCR according to the protocol described by the supplier (Whitehead Institute/MIT Center for Genome Research [WI/MIT]). The primers used to test the candidate genes were reported for SCP2 (He et al. 1991), EPS15 (Genome Database; STS SHGC-14865), and FABP3 (Phelan et al. 1996). Original primers designed in the 3′ region of the *APOER2* gene were forward, 5′-TTCTTGGCTTTGGCGAAGGTC-3′ and reverse, 5′-TTGGGCTGATCTGGAAACGTC-3′.

Results

We have identified a large French family (HC2) in which linkage to either *LDLR* or *APOB* was conclusively excluded. In this family, 22 meioses are investigated. Linkage analysis with *LDLR* gene markers identified two recombinants (one affected subject and one unaffected subject). Linkage to *APOB* was excluded because seven recombinants were identified (two affected subjects and five unaffected subjects). This result was confirmed by a functional test that showed normal binding, internalization, and degradation of control LDL particles in fibroblasts from the proband (data not shown). Furthermore, no family member carried either of the two most frequent hypercholesterolemic *APOB* gene mutations (R3500Q, R3531C; table 1). Finally, to exclude the possibility of type IIb, III, or IV hyperlipoproteinemia in this family, lipid measurements were repeated and apolipoprotein E variants were determined (fig. 1).

We estimated the power of the family for linkage using SLINK (Ott 1991) assuming an autosomal dominant trait. We found a maximum LOD score (Z_{max}) of 4.13 (expected average Z_{max} in 500 replicates was 2.16, with 34% > 3.00) in family HC2, showing that this family was sufficiently informative for linkage. Two hundred four genetic markers from 22 autosomes were tested for linkage to the *FH3* locus. These $(CA)_n$ microsatellite markers spanning the human genome were chosen on the basis of two criteria: heterozygosity and spacing of ~15 cM between adjacent markers (Dib et al. 1996). Each locus was tested for linkage to the ADH phenotype by use of MLINK (Ott 1991) and VITESSE (O'Connell and Weeks 1995). The combined genotype data were also analyzed with EXCLUDE (Edwards 1987): a nonoverlapping exclusion zone of ⩾2,955 cM, correspond-

ing to 80% of the genome, was established from the cumulative exclusion intervals for each marker. The EXCLUDE analysis also indicated that the most probable position for the *FH3* locus in family HC2 was on chromosome 1, with a probability of 96%. In this region, a LOD score of 2.13 at $\theta = 0$ had been obtained at *D1S255* (table 2). Other microsatellites were tested around *D1S255,* and a Z_{max} of 3.13 ($\theta = 0$) was obtained at *D1S2892* and *D1S2722* in the HC2 family (table 1). Multipoint linkage analyses did not provide higher LOD scores (data not shown). As shown in figure 1, we found a common region (*D1S2892–D1S2722*) that segregated with the ADH phenotype, localizing *FH3* to a 9-cM interval at 1p34.1-p32 (GENATLAS).

To investigate the reproducibility of linkage, other families from different countries–with 12 presenting ADH and 2 presenting ARH–were collected through an international collaborative effort (table 1). Markers *D1S472, D1S2892, D1S2722,* and *D1S211* were tested in these families. In three families (S113, S150, and S517) originating from northeast Spain, LOD-score values near their Z_{max} were obtained for these markers. Thus, these families present a very high probability of linkage between the disease and the *FH3* locus on chromosome 1 (fig. 2, table 3). In the two Sardinian ARH families, linkage with all the markers tested was excluded (fig. 3). To investigate whether exclusion of linkage of ADH to 1p could be a result of wrong model specification, we performed model-free linkage analyses by using GENEHUNTER-PLUS (Kong and Cox 1997). Positive ghpLS scores were obtained for families HC2, S150, S113, and S601 (data not shown). This analysis confirmed that the *FH3* nonlinked families outlined by our parametric linkage analyses were not excluded because of misspecification of ADH inheritance mode. Finally, to estimate the proportion of families linked to the *FH3* locus on 1p, we performed an admixture test using HOMOG (Ott 1983), in the whole sample (family HC2 and the 12 ADH families). As shown in table 3, the test was statistically significant with markers *D1S472, D1S2892,* and *D1S2722*. Furthermore, the estimated proportion of families with linkage to the *FH3* locus was $\alpha = 27\%$ ($P = .005$) at *D1S2892* and $\alpha = 25\%$ ($P = .005$) at *D1S2722*. Multipoint HOMOG analysis gave a Z_{max} of 2.97 ($\alpha = 25\%$, $P = .0025$) between *D1S2892* and *D1S2722* at $\theta = .01$ from *D1S2892*.

The *FH3* gene maps to 1p34.1-p32, which also harbors numerous genes including those that encode a sterol carrier protein (SCP2; Ohba et al. 1994), a fatty acid–binding protein (FABP3; Phelan et al. 1996), an apolipoprotein E receptor (APOER2; Kim et al. 1997), and an epidermal growth factor receptor–pathway substrate (EPS15; Wong et al. 1994). To determine whether these positional and functional candidate genes mapped within the critical interval defined by *D1S472* and *D1S211* should be further investigated, we used the GENEBRIDGE 4 radiation hybrid panel. The results showed that all these genes did not map within the interval, excluding the possibility of identity between the *FH3* gene and one of these regional candidates (data not shown) and suggesting a novel gene for ADH on 1p34.1-p32.

Discussion

We located a third gene involved in ADH on chromosome 1p34.2-p32 and identified a large French family in which the involvement of either *LDLR* or *APOB* was excluded conclusively. This result was confirmed by a functional test that showed that fibroblasts from the proband showed normal binding, internalization, and degradation of control LDL particles. Furthermore, no family member carried either of the two most frequent hypercholesterolemic *APOB* gene mutations (R3500Q, R3531C). To exclude the possibility of type IIb, III, or IV hyperlipoproteinemia in this family, lipid measurements were repeated and apolipoprotein E variants were determined. Furthermore, linkage was also clearly excluded between the disease gene and the *LPL* locus on

Table 2

Pairwise LOD Scores for Chromosome 1 Markers and ADH in HC2

		LOD Score at θ =									
Locus	Distance[a]	.00	.001	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D1S234	...	−6.49	−4.95	−3.81	−2.60	−1.84	−.99	−.52	−.24	−.24	.40
D1S513	.15	1.05	1.06	1.09	1.16	1.17	1.03	.73	.31	1.18	.08
D1S2830	.01	−1.02	−1.01	−.94	−.70	−.49	−.22	−.08	−.01	−.01	.40
D1S255	.11	2.13	2.13	2.15	2.16	2.09	1.75	1.24	.58	2.16	.05
D1S472	.02	2.13	2.14	2.15	2.16	2.09	1.75	1.24	.58	2.17	.03
D1S2892	.08	3.13	3.13	3.11	2.98	2.77	2.22	1.54	.73	3.13	.00
D1S2722	.02	3.13	3.13	3.11	2.98	2.77	2.22	1.53	.72	3.13	.00
D1S211	.04	−4.52	.13	1.11	1.70	1.81	1.62	1.17	.57	1.81	.10
D1S197	.08	−2.97	−.86	.15	.88	1.13	1.15	.87	.43	1.19	.15

[a] Distance between two adjacent markers in θ.

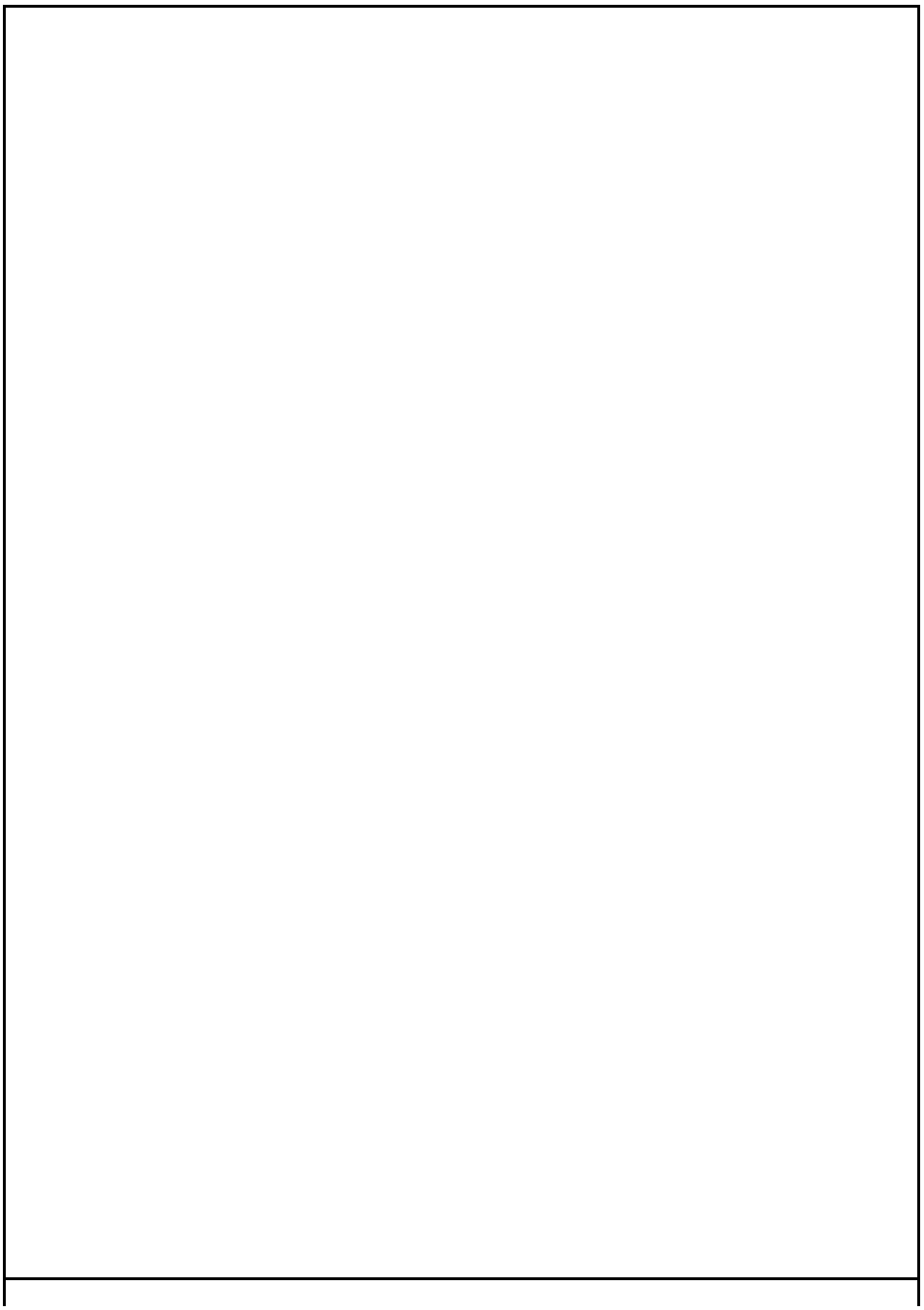

Figure 2 Segregation of chromosome 1p34.1-p32 markers in the Spanish families S113, S601, S150, and S517. Lipid values given under each family-member symbol are (*top to bottom*): age at lipid measurement levels of total, LDL, and HDL cholesterol (in grams per liter); and levels of triglycerides in (grams per liter). Haplotypes (*top to bottom*) at tel-*D1S472-D1S2892-D1S2722-D1S211*-cen markers are shown for each family member tested. Half-blackened symbols indicate affected members and unblackend symbols indicate unaffected members. The common region transmitted with the disease phenotype in each family is boxed.

chromosome 8, as well as the *APOA1-C3-A4* gene cluster on chromosome 11 and the 1q21-q23 region, both of which are associated with familial combined hyperlipidemia (Wojciechowski et al. 1991; Pajukanta et al. 1998). Because this family was clinically and biologically indistinguishable from FH or FDB families and the expected LOD score showed sufficient power in the pedigree, we used exclusion mapping to localize the disease gene. The phenotypic status of each family member was carefully and independently established by four researchers (M.D., M.K., J-P.R., and C.B.) and by other recognized French experts. To avoid spurious results from misclassification, members with borderline lipid values were scored as "unknown" in the genetic analyses (seven

Table 3

Pairwise LOD-Score Analyses for ADH and 1p Regional Markers

	Z_{max} (θ) at Marker				
Family	*D1S472*	*D1S2892*	*D1S2722*	*D1S211*	Z_{max}[a]
HC2	2.16 (.05)	3.13 (0)	3.13 (0)	1.81 (.1)	4.13
HC67	−.01 (.4)	0 (.4)	0 (.4)	0 (.4)	.86
A1	.15 (0)	−.09 (.4)	−.02 (.4)	−.09 (.4)	1.01
S150	.56 (0)	.82 (0)	.82 (0)	.82 (0)	.82
S108	nd	−.02 (.4)	−.02 (.4)	nd	.84
S113	.07 (0)	1.34 (0)	.21 (0)	.2 (0)	1.35
S517	−.03 (.4)	0 (.4)	.64 (0)	.01 (.3)	.79
S509	nd	0 (.4)	−.01 (.4)	nd	.79
S601	.09 (0)	.50 (.05)	.07 (0)	.41 (.05)	1 .61
S129	nd	−.02 (.4)	−.02 (.4)	nd	.75
S206	−.05 (.4)	.19 (.3)	−.01 (.4)	.15 (.3)	2.46
B1	−.43 (.4)	−.20 (.4)	−.2 (.4)	−.18 (.4)	1.94
NZ1	nd	−.01 (.4)	0 (.4)	nd	.50
Total Z_{max} (θ)	.17 (.3)	1.59 (.2)	1.04 (.2)	1.13 (.2)	...
	Homogeneity Admixture Text at Marker				
Total Z_{max} (θ [α])	1.43 (.1 [32%])	2.88 (0 [27%])	2.43 (0 [25%])	1.45 (.01 [46%])	...
P Value	.01	.005	.005	.11	...
	Posterior Probability of Linkage at Marker				
HC2	.985	.998	.998	.982	...
HC67	.155	.027	.004	.319	...
A1	.399	.004	.155	.154	...
S150	.625	.700	.688	.792	...
S108	.320	.014	.001	.460	...
S113	.351	.883	.351	.540	...
S517	.005	.096	.593	.344	...
S509	.320	.025	.002	.460	...
S601	.316	.533	.281	.676	...
S129	.320	.023	.009	.460	...
S206	.0004	.123	.035	.314	...
B1	.001	.001	.001	.032	...
NZ1	.320	.103	.155	.460	...

Note.—nd = not determined.

[a] Z_{max} encountered in the 500 SLINK replicates.

subjects). We chose this conservative approach despite loss of power. In the absence of established genetic parameters for this autosomal dominant disease, we assumed it to be comparable to FH or FDB. Therefore, linkage analyses were performed with a gene frequency of .002 and penetrance of .9. At *D1S2892* and *D1S2722,* the Z_{max} exceeded the threshold of 3 in the family ($Z_{max} = 3.13, \theta = 0$; table 2), strongly supporting the hypothesis of an ADH locus at 1p. As shown in figure 1, family data also showed an affected subject (II-5) with a proximal recombination event, between *D1S2722* and *D1S211,* and an unaffected subject (III-1) with a distal recombination event between *D1S472* and *D1S2892*. Furthermore, all affected individuals shared the region flanked by markers *D1S2892* and *D1S2722*. Together these data place *FH3* in a region <9 cM, flanked by markers *D1S472* and *D1S211*. Interestingly, the subjects with no definite diagnosis (II-3, III-3, III-4, III-5, III-6, II-13, and III-15), did not carry the disease-associated two-marker haplotype. This observation supported our conservative approach. However, an 8-year-old boy (III-3), with a total-cholesterol level of 1.46 g/l and an LDL-cholesterol level of 0.72 g/l, also carried the disease-associated region. This observation explains why the Z_{max} was not reached at 1p.

In an attempt to replicate this positive linkage, we studied a second set of 12 ADH families from various countries (table 1). These families met the following clinical and biological criteria: (1) ADH phenotype; (2) normal LDL-particle receptor activity, and/or absence of mutations in the whole *LDLR* gene, and/or genetic exclusion of the *LDLR* gene; and (3) genetic exclusion of *APOB* and/or absence of mutations R3500Q and R3531C in *APOB*. Phenotypic status was established for each subject on the basis of the appropriate national reference population. Three families (S113, S150, and S517) gave two-point LOD scores, with *D1S2892* and *D1S2722* very close to their possible Z_{max} (table 3), sup-

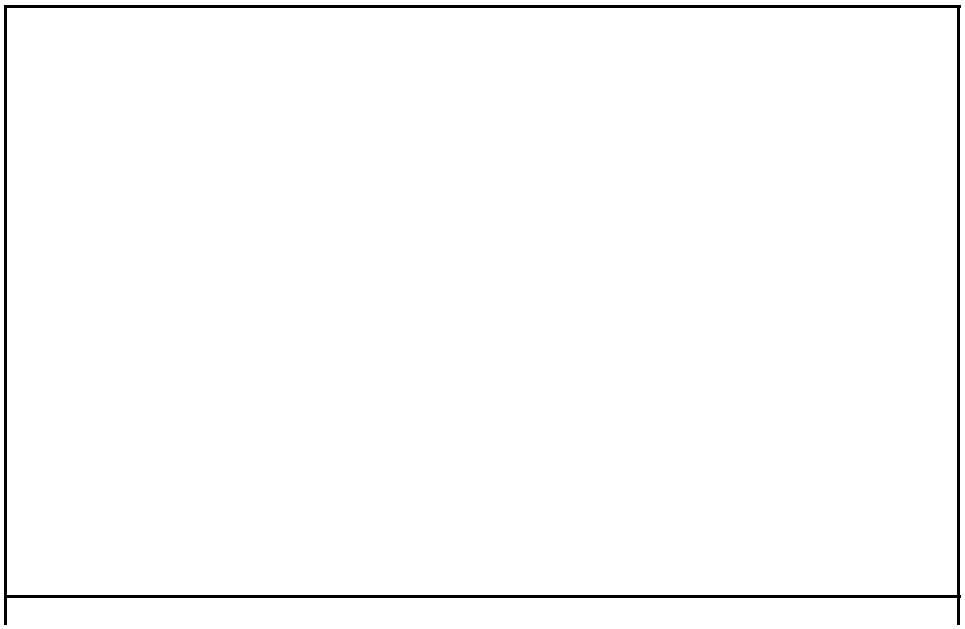

Figure 3 Segregation of chromosome 1p34.1-p32 markers in the Sardinian families It3 and It4. Lipid values given under each family-member symbol are (*top to bottom*): age at lipid measurement; levels of total, LDL, and HDL cholesterol (in grams per liter); and levels of triglycerides (in grams per liter). Haplotypes (*top to bottom*) at tel-*D1S2892-D1S2722-D1S211*-cen markers are shown for each family member tested. Blackened symbols indicate affected members, unblackened symbols indicate unaffected members, and hatched symbols indicate members with an unknown phenotypic status.

porting the localization of the *FH3* gene. In families S113 and S150, a common four-marker haplotype was carried by all the affected members. Conversely, in family S517 a positive LOD score was obtained only at *D1S2722,* but careful examination of family data did not show a conserved region shared by affected members. In family S601, LOD scores were of small value; however, all affected members shared a common region. Furthermore, this region was also shared by an unaffected 24-year-old woman (II-9; 1.94 g/l total cholesterol and 1.29 g/l LDL cholesterol). This observation could be compared with that of subject III-13 of the HC2 family. It is unlikely that both these subjects are double recombinants. However, it is highly possible that, with the criteria used for the classification of family members, the penetrance of the disease gene is not complete in children and young adults.

Admixture tests were performed on a sample comprising the HC2 family and the set of 12 ADH families. The hypothesis of genetic homogeneity was rejected against the hypothesis of heterogeneity at a significance level of 1%. These results showed that the ADH phenotype is more heterogeneous than what we at first had assumed. For the remaining ADH non-1p families, another locus, named "FH4," could be involved. We also studied two Sardinian families displaying ARH, the rare pseudohomozygous type IIa hypercholesterolemia disease with an autosomal recessive transmission. Despite the different transmission modes, we thought it was possible that some mutations in the *FH3* gene could give rise to ADH and that some others could lead to ARH, as has been reported for other diseases, such as retinitis pigmentosa (MIM 268000). As shown in figure 3, no allele identity was apparent between the affected sibs (II-2 and II-3) of family It3, and identical alleles are shared by the affected sibs (II-1 and II-4) of family It4 and their unaffected sib (II-2). These results show that, in the two ARH families, the disease is not linked to the *FH3* gene. In these families, the disease gene could be identical to either the *FH4* gene or another as yet unidentified gene.

Our data provide strong evidence for an additional locus contributing to ADH and its assignment to chromosome 1p34.1-p32. Positioning the disease locus in relation to the genetic map indicates that the *FH3* gene is located within a 9-cM interval flanked by *D1S472* and *D1S211*. These microsatellite markers map to a region that contains four candidate cloned genes, as follows: *SCP2* (sterol carrier protein 2), which encodes a lipid transport basic protein believed to facilitate the movement of cholesterol and phospholipids within the cell (Ohba et al. 1994); *MDGI/FABP3* (mammary-derived growth inhibitor/fatty acid–binding protein 3-muscle and heart), which encodes a protein that transports vehicles of hydrophobic fatty acids throughout the cytoplasm and is a candidate tumor-suppressor gene for human breast cancer, although no mutation in this gene has been reported in sporadic breast tumors (Phelan et al. 1996); *APOER2* (apolipoprotein E receptor 2), which encodes a receptor that resembles LDL and very-low-density lipoprotein receptors and is most highly ex-

pressed in human brain and placenta (Kim et al. 1997); and *EPS15* (epidermal growth factor receptor pathway–substrate), which encodes a protein that is implicated in the receptor-mediated endocytosis pathway (Wong et al. 1994). To clarify the positional relationship between these regional candidate genes and the markers linked to the *FH3* gene, we mapped them relative to the WI/MIT radiation hybrid map. The results showed that all these candidate genes are not located within the *D1S472–D1S211* interval, excluding possible identity between the *FH3* gene and one of these candidates and suggesting a novel gene for ADH on 1p34.1-p32.

To conclude, the 13 families we have identified give evidence for the existence of a greater level of genetic heterogeneity than conventionally has been assumed for ADH. Our results indirectly show that *FH3* encodes a protein, as yet unknown, whose function is important in the control of cholesterol homeostasis. Defects in this new pathway could explain the resistance to drug therapy observed among some patients with hypercholesterolemia. The identification of the *FH3* gene may help to develop new intervention strategies to limit elevation of LDL particles and prevent morbidity and mortality from premature atherosclerosis.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GENATLAS, http://citi2.fr/GENATLAS (for markers used in cytogenetic localization)

Genome Database, http://gdbwww.gdb.org (for primers used)

LDLR Mutation Database, http://www.umd.necker.fr:2004/ (for *LDLR* mutations)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FH [MIM 143890], FDB [MIM 144010], and Retinitis Pigmentosa [MIM 268000])

WI/MIT, http://www.genome.wi.mit.edu/ (for marker and gene physical mapping)

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