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Human genes involved in cholesterol metabolism: Chromosomal mapping of the loci for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase with cDNA probes

(familial hypercholesterolemia/*in situ* hybridization/somatic cell hybrids/chromosome 5/chromosome 19)

VALERIE LINDGREN*, KENNETH L. LUSKEY†, DAVID W. RUSSELL†, AND UTA FRANCKE*

*Department of Human Genetics, Yale University, New Haven, CT 06510; and †Department of Molecular Genetics, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, TX 75235

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ABSTRACT **Cellular cholesterol metabolism is regulated primarily through the coordinate expression of two proteins, the low density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). We have used cDNA probes for the human genes encoding these proteins to determine the precise chromosomal location of the two loci. By *in situ* hybridization we have regionally mapped the LDL receptor gene, *LDLR*, to the short arm of chromosome 19 in bands p13.1–p13.3. This result concurs with and extends a previous study in which *LDLR* was mapped to chromosome 19 by screening somatic cell hybrids with a species-specific monoclonal antibody. We have assigned the HMG-CoA reductase gene, *HMGCR*, to chromosome 5 by Southern blotting of DNA from a somatic cell hybrid panel and to bands 5q13.3–q14 by *in situ* hybridizations of the cDNA probe to human metaphase cells with normal and rearranged chromosomes.**

The cholesterol content of cells is regulated such that an adequate supply is available for the biosynthesis of plasma membranes, bile acids, lipoproteins, and steroid hormones, yet excessive buildup of cholesterol is prevented (1). This equilibrium is maintained by the regulated expression of two key proteins, the low density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). The number of LDL receptors on the cell surface determines the amount of extracellular cholesterol that is delivered to the interior of the cell through receptor-mediated endocytosis of LDL particles carrying cholesterol. HMG-CoA reductase, the enzyme that converts HMG-CoA to mevalonate, controls the rate of intracellular *de novo* cholesterol synthesis.

The LDL receptor is a 160-kilodalton cell surface glycoprotein that specifically binds lipoproteins containing apoprotein B or E (2). The receptor–ligand complex is internalized by endocytosis via coated pits (2). The lipoproteins are then degraded in the lysosomal compartment, and cholesterol is released. The LDL receptor protein is encoded by a gene of more than 45 kilobases (kb) that contains 18 exons (3). Distinct domains involved in ligand binding, glycosylation, membrane binding, and internalization have been identified through biochemical examination of the protein and analysis of the intron–exon structure of the gene (3–5). Regions of the LDL receptor share amino acid homology with complement component C9, the epidermal growth factor precursor, and three proteins of the blood clotting system (factor IX, factor X, and protein C) (3–6).

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Mutations in the human LDL receptor gene result in the autosomal dominant disease familial hypercholesterolemia (FH) (7). These heterozygous individuals have moderate elevation of plasma LDL–cholesterol as a consequence of having only one half the normal number of functional LDL receptors. The elevated plasma LDL–cholesterol leads to the development of premature atherosclerosis and an increased frequency of heart attacks in middle age. FH homozygotes have very high plasma LDL–cholesterol levels and signs of atherosclerosis at birth, and they generally succumb to myocardial infarction before age 20. Recent studies have identified the precise mutations in several alleles of the LDL receptor gene that encode defective receptors (8, 9).

The assignment of the LDL receptor gene (*LDLR*) to human chromosome 19 was suggested by the linkage of *FH* with the gene for the third component of complement (C3) (10), which was known to be on chromosome 19 (11). Subsequently, *LDLR* was directly mapped to chromosome 19 by the use of a monoclonal antibody directed against the human receptor to screen a panel of somatic cell hybrids (12).

The enzyme that controls intracellular cholesterol synthesis, HMG-CoA reductase, is a 97-kilodalton glycoprotein bound to the membrane of the endoplasmic reticulum (13). HMG-CoA reductase activity is regulated at the transcriptional level as well as post-transcriptionally by changes in the rate of degradation of the protein (14, 15). In hamsters, the HMG-CoA reductase gene is approximately 25 kb in length and contains 20 exons (16). The region upstream from the sites of transcription initiation contains sequences involved in the inhibition of transcription by cholesterol (17). The protein has an extensive membrane-bound domain that crosses the membrane of the endoplasmic reticulum seven times (13), and it is this domain that mediates changes in the rate of enzyme degradation in response to cholesterol (18, 19). Although mutations in the gene for HMG-CoA reductase have not yet been identified, some of the currently unexplained hypercholesterolemic conditions may be attributable to structural or regulatory defects in this gene. The HMG-CoA reductase gene (*HMGCR*) has not been assigned to a chromosome.

The availability of cloned probes for the human receptor (5) and reductase genes (19) provides the opportunity for detailed mapping studies. We describe here the regional assignment of *LDLR* to the distal short arm of chromosome 19, bands p13.1–p13.3, by *in situ* hybridization. We also present

Abbreviations: bp, base pair(s); CHO, Chinese hamster ovary; FH, familial hypercholesterolemia; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; *HMGCR*, genetic locus for HMG-CoA reductase; kb, kilobase(s); LDL, low density lipoprotein; *LDLR*, genetic locus for the LDL receptor.

blotting and *in situ* hybridization data that place *HMGCR* on chromosome 5 in bands q13.3–q14.

MATERIALS AND METHODS

Somatic Cell Hybrid Formation and Characterization. Hybrid clones were produced by seven independent fusions of Chinese hamster cells (V79/380-6 or Don/a23) with human skin fibroblasts or peripheral blood leukocytes. A description of the human chromosomal content of this hybrid mapping panel of 19 clones is presented in table 1 of ref. 12; hybrid XXI-G was not included in the present study.

Human Cells. Peripheral blood lymphocytes from normal male and female donors were stimulated by phytohemagglutinin and synchronized with methotrexate. Chromosomes were harvested after thymidine release of the block (20).

Fibroblasts were obtained from a skin biopsy sample from a female donor carrying an apparently balanced chromosomal rearrangement. The abnormality consisted of the insertion of bands q13.3–q15 of chromosome 5 into band q27 of chromosome 3 [46,XX,ins(3;5) (q27;q13.3q15)] (21). Chromosomes used for *in situ* hybridization were prepared from unsynchronized cultures by standard techniques.

DNA Probes. The LDL receptor gene probe (pLDLR-2-HH1) consisted of a 1.9-kb cDNA fragment (5) inserted into the *Bam*HI site of SP64 (22). The insert contained unique sequences approximately equally divided between the 3′ end of the coding region and the 3′ untranslated region.

The human reductase gene probe (pHRed-102) used for *in situ* hybridizations was a 4.2-kb cDNA cloned in an Okayama–Berg plasmid vector (19). The insert spans the entire coding region as well as 50 base pairs (bp) of the 5′ untranslated region and 1.5 kb of the 3′ untranslated region. For Southern blotting experiments, the *Hin*dIII–*Bam*HI fragment of pHRed-102 containing 23 bp of the 5′ untranslated region and 369 bp of the coding region was used.

***In Situ* Hybridization.** The method of Harper and Saunders (23) was followed with modifications to the chromosome banding procedures as described elsewhere (24). Plasmids (pLDLR-2-HH1 and pHRed-102) were labeled by nick-translation with three tritiated nucleotides (dATP, dCTP, and dTTP) to specific activities of approximately 2×10^7 cpm/μg. Labeled probe (25 or 50 ng/ml) was hybridized overnight at 37°C to human chromosomes. For each experiment, the observed distribution of grains over each chromosome arm was compared by χ^2 analysis to a random distribution predicted on the basis of the relative length of each arm (25).

Southern Blotting. Genomic DNA, extracted from either peripheral blood leukocytes or cultured cells, was digested with *Hin*dIII and subjected to blotting as previously described (15). A uniformly ^{32}P-labeled single-stranded probe was prepared by primer extension of phage M13 subclone of pHRed-102, restriction endonuclease digestion, and purification of the labeled fragment by denaturing gel electrophoresis (45).

RESULTS

***LDLR* Regional Localization.** To determine the position of the LDL receptor gene on chromosome 19, we hybridized a human cDNA probe (pLDLR-2-HH1) to chromosomes from two normal individuals. The grains overlying all chromosomes in 156 cells were scored. Of 432 grains, 81 (18.8%) were over bands 19p13.1–p13.3, with a peak at band p13.2 (Fig. 1) ($P < 0.0001$). In the cells from one of the two experiments, more grains were observed over the short arm of chromosome 6 than expected (11 grains observed, 5.3 expected; $0.01 < P < 0.05$). However, the label was evenly distributed over the entire arm. In addition, when the data from both experiments were combined, the short arm of chromosome 6 did not appear significantly labeled ($P > 0.05$). No other chromosomal region was labeled above background (data not shown). We conclude that *LDLR* is within the region 19p13.1–p13.3 and that there are no other sites of hybridization.

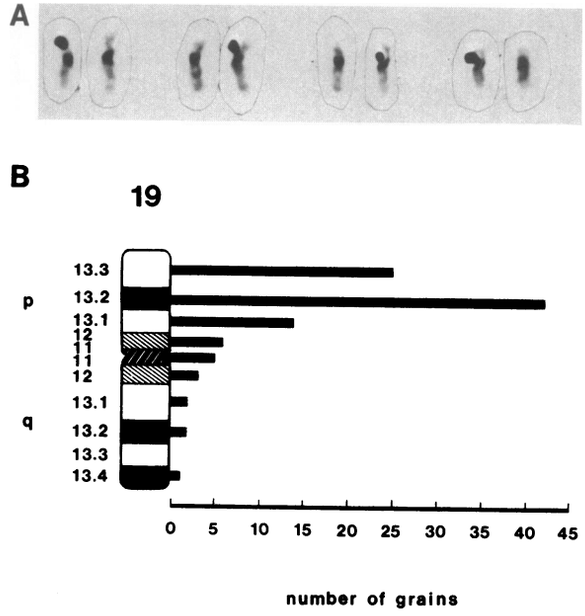

FIG. 1. *In situ* hybridization of the *LDLR* probe to normal human chromosomes. (*A*) Examples of chromosomes 19 after hybridization and autoradiography. One of each pair of homologs is labeled in the region p13.1–p13.3. (*B*) Standard idiogram (27) showing the distribution of grains over chromosome 19. Bars summarize the numbers of grains observed over each band in 156 cells from two experiments.

Mapping of *HMGCR*. A panel of 19 Chinese hamster–human somatic cell hybrids was screened with a ^{32}P-labeled probe from the 5′ end of the HMG-CoA reductase cDNA, pHRed-102 (Fig. 2). In this analysis, an 11-kb band was detected in human DNA, and a less intense 3.8-kb band was observed in Chinese hamster ovary (CHO) DNA. The 11-kb band corresponds to an 11-kb *Hin*dIII fragment found in bacteriophage λ genomic clones from the 5′ end of the human

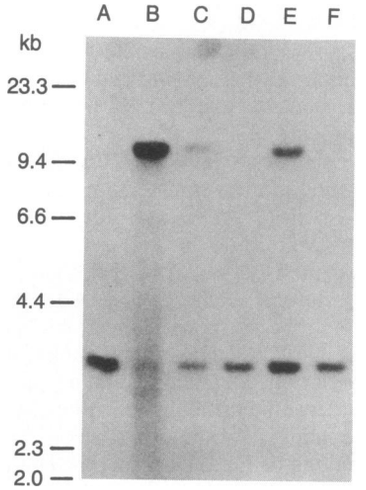

FIG. 2. Detection of human reductase-specific restriction fragment in somatic cell hybrid DNA. Cellular DNA (5 μg) from CHO cells (lane A), human peripheral leukocytes (lane B), or Chinese hamster–human somatic cell hybrid XV-A (lane C), XXV-I (lane D), XV-B (lane E), or XVII-J (lane F) was digested with *Hin*dIII and subjected to Southern blotting analysis with a 392-bp ^{32}P-labeled probe for human HMG-CoA reductase.

Table 1. Correlation of human chromosome content and presence of 11-kb human fragment specific for HMG-CoA reductase in 19 Chinese hamster–human somatic cell hybrids

Segregation (human reductase sequences*/presence of chromosome)	No. of hybrids with human chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant hybrids																							
+/+	1	4	5	3	7	5	2	5	1	3	6	5	5	6	3	4	3	4	5	5	7	7	4
−/−	9	7	6	8	12	5	11	6	11	10	5	4	7	1	6	7	9	6	3	7	3	3	2
Discordant hybrids																							
+/−	5	3	1	4	0	2	5	2	6	4	1	2	2	1	4	3	4	3	2	2	0	0	3
−/+	3	5	5	4	0	7	1	5	1	2	5	8	5	11	4	5	3	6	9	5	8	7	7
Total discordant hybrids	8	8	6	8	0	9	6	7	7	6	6	10	7	12	8	8	7	9	11	7	8	7	10
Total informative hybrids†	18	19	17	19	19	19	19	18	19	19	17	19	19	19	17	19	19	19	19	19	18	17	16
Percent discordant hybrids	44	42	35	42	0	47	32	39	37	32	35	53	37	63	47	42	37	47	58	37	44	41	62

*The 11-kb human reductase-specific fragment was detected in seven hybrid clones (XV-A, XV-B, XV-R, XVIII-M, XXI-C, XXI-E, and XXI-P). Table 1 of ref. 12 summarizes the human chromosomal content of all 19 hybrids analyzed; hybrid XXI-G was not included in the present study.
†Only intact chromosomes present in at least 10% of cells were included in the data. Therefore, the number of informative hybrids is less than 19 for some chromosomes.

reductase gene (unpublished observations). The presence or absence of human reductase sequences correlated perfectly with the presence or absence of human chromosome 5; no discordancies were detected (Table 1). There was discordant segregation for all other chromosomes in at least six hybrid clones.

The localization of the HMG-CoA reductase gene on chromosome 5 was independently confirmed and regionally defined by *in situ* hybridization experiments using a full-length cDNA probe, pHRed-102 (19). A total of 320 grains over the chromosomes of 119 cells from two donors were scored. Sixty-six (20.6%) of the grains were over bands 5q11.2–q14, with a peak at band q13 (Fig. 3) ($P < 0.0001$). In the cells analyzed from one of the two experiments, the long arm of chromosome 8 was labeled more often than expected by chance (10 grains observed, 5.1 expected; $0.01 < P < 0.05$). However, no peak in grain distribution was evident, and when the results of the two experiments were considered together, this region was not significantly labeled. Since no other chromosomal region was labeled above background (data not shown), these data assign the reductase gene to bands q11.2–q14 of chromosome 5.

To map the reductase gene more precisely, we employed fibroblasts from a donor carrying a balanced insertion of region q13.3–q15 of chromosome 5 into band q27 of chromosome 3 (Fig. 4) (21). The inserted region contains about one-third of the region to which the reductase gene was assigned by hybridization to normal chromosomes. Eighty-

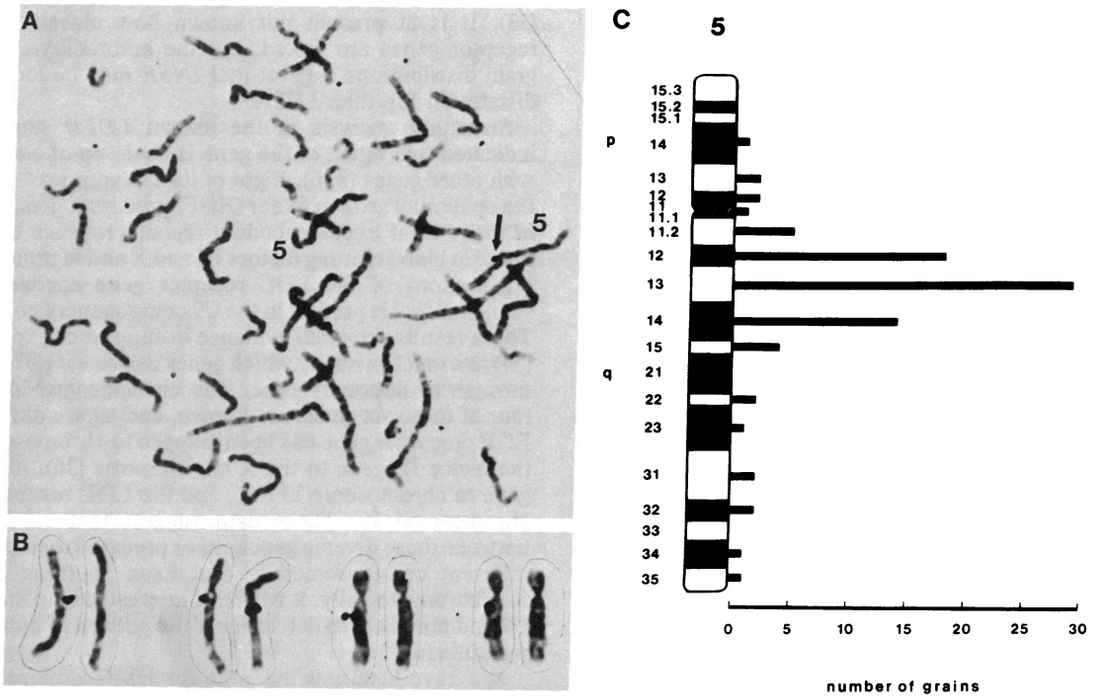

FIG. 3. *In situ* hybridization of the human HMG-CoA reductase gene probe to metaphase chromosomes from normal individuals. (*A*) Early metaphase cell with a grain (arrow) over one of the chromosomes 5. (*B*) Representative pairs of chromosomes 5, illustrating label over the proximal long arm. (*C*) Grain distribution for chromosome 5. Bars adjacent to the bands indicate the numbers of grains observed over the bands in a total of 119 cells from two experiments.

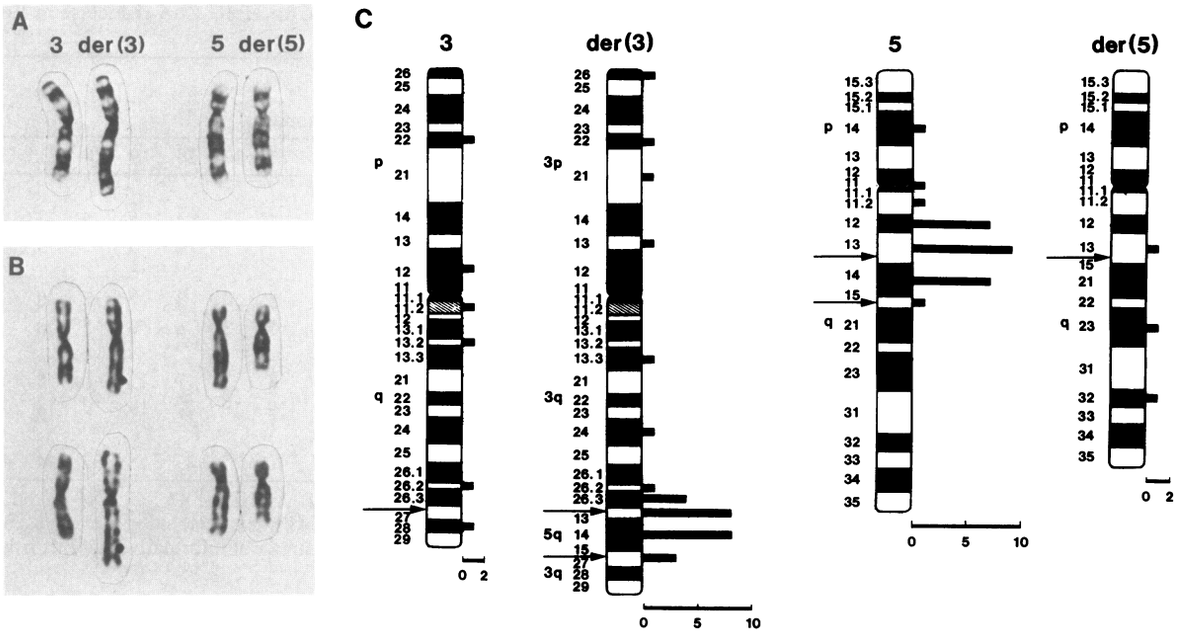

FIG. 4. *In situ* hybridization of the *HMGCR* probe to chromosomes from a donor with a balanced insertion [46,XX,ins(3;5) (q27;q13.3q15)] (21). (*A*) Trypsin–Giemsa-banded examples of the derivative chromosome 3 [der(3)], the derivative chromosome 5 [der(5)], and the normal homologs. (*B*) Partial karyotypes of two cells after hybridization. Both der(3) chromosomes have a grain over the long arm in the region where a portion of chromosome 5 is inserted. (*C*) Idiogrammatic representation of the balanced insertion with a graphic summary of the grains observed over the normal and derivative chromosomes in 84 cells. Arrows next to the normal chromosomes 3 and 5 denote the points of breakage, while arrows next to the derivative chromosomes indicate the points of rejoining. The subdivisions of band 5q13 (q13.1, q13.2, and q13.3) are not visible at the 400-band stage of mitosis and are, therefore, not shown on these diagrams (27) used to score grains. However, the positions of the arrows next to 5q13 reflect the breakpoint in q13.3 as determined by high-resolution banding studies. Scales at the bottom of each idiogram refer to the number of grains over the bands.

four cells with the rearrangement were analyzed after hybridization with pHRed-102. In a total of 218 grains over chromosomes, 24 (11.0%) grains were over bands q11.2–q14 of the normal chromosome 5 (Fig. 4). In addition, 20 (9.2%) grains were over bands 3q26.3–5q14 of the rearranged chromosome 3 [der(3)]. Only 1 (0.5%) grain was observed over the portion of band q13 remaining in the deleted chromosome 5 [der(5)]. Therefore, the segment of chromosome 5 inserted into chromosome 3 contains the HMG-CoA reductase gene, and *HMGCR* must lie within the region 5q13.3–q14.

DISCUSSION

Francke *et al.* (12) mapped *LDLR* to human chromosome 19 by analyzing rodent–human somatic cell hybrids for the expression of the human LDL receptor protein with a species-specific monoclonal antibody. We have confirmed this assignment by *in situ* hybridizations with a cDNA probe for *LDLR*. In addition, our experiments regionally localize the gene to 19p13.

One of the ligands of the LDL receptor, apolipoprotein E, is also encoded by a gene on chromosome 19 (28). The chromosomal location of apolipoprotein B, the other ligand of the receptor, is not yet known. Even though *LDLR* and *APOE* are syntenic, indirect evidence suggests that the two loci are separated by a considerable distance: *APOE* is very tightly linked to *APOC2*, the gene encoding apolipoprotein CII (29, 30), and *APOC2* is unlinked to *LDLR* (26). *C3* (the gene for the third component of complement), lies between *LDLR* and *APOE* and is loosely linked to both (11, 26, 28). *C3* maps to bands p13.2–p13.3 (31). Because *LDLR* and *C3* are both in the distal half of the short arm of chromosome 19, the most likely gene order is pter–*LDLR*–*C3*–*APOE*, with the centromere on either side of *APOE*. Regional mapping of *APOE* and *APOC2* (31) should resolve the position of the centromere.

Recently, the insulin receptor gene (*INSR*) has been mapped to bands p13.2–p13.3 of chromosome 19 (32), the same region to which *LDLR* maps. Although both the LDL receptor and the insulin receptor cluster in coated pits, no amino acid sequence homology is apparent between them (33). It is at present not known how closely these two receptor genes are linked, but the autoradiographic silver grain distributions suggest that *INSR* may be located more distally on 19p than *LDLR*.

Structural analysis of the human *LDLR* sequence has indicated that much of the gene is made up of exons shared with other genes (3, 6). Eight of its 18 exons are shared with the epidermal growth factor (EGF) precursor gene, and three of these eight exons encode a repeat sequence that is also found in blood clotting factors IX and X and in protein C. Five other exons of the LDL receptor gene encode a repeat sequence that is present in the C9 component of complement. These results provided evidence in support of exon shuffling (34) as a mechanism by which genes can be assembled rapidly through evolutionary time. The chromosomal locations of four of these six genes are known, and all are different. The EGF precursor gene has been mapped to chromosome 4 (35), the factor IX gene to the X chromosome (36), the factor X gene to chromosome 13 (37), and the LDL receptor gene to chromosome 19. These data suggest that exon shuffling between these diverse genes either predated their dispersal to different chromosomes or that exon shuffling can occur interchromosomally. It will be of interest to map the genes for C9 and protein C to determine if the pattern of nonsynteny is maintained.

We have localized the gene for HMG-CoA reductase to human chromosome 5 by Southern blotting of DNA from a panel of somatic cell hybrids. This result was independently confirmed by *in situ* hybridization to normal human chromosomes, which identified the region q11.2–q14 as the site of the gene. The region of assignment was further narrowed to

q13.3–q14 by hybridization to chromosomes from cells carrying a balanced chromosomal rearrangement.

Few genes have been localized to this region of chromosome 5. However, the dihydrofolate reductase locus, *DHFR*, is on chromosome 5 (38), in the proximal long arm (39, 40). In cultured Chinese hamster cells, the gene for either dihydrofolate reductase (41) or HMG-CoA reductase (15) can be amplified in the presence of competitive inhibitors of the respective enzyme. Since many genes found on chromosome 5 in man are present on chromosome 2 in the Chinese hamster (42), it is likely that both *DHFR* and *HMGCR* are present on the same Chinese hamster chromosome. The regions of the human and hamster genomes containing these two genes may be especially susceptible to gene amplification. Alternatively, the involvement of the two loci may be fortuitous; perhaps any region of the genome could be amplified under selective pressure.

The precise mapping of *LDLR* and *HMGCR*, two genes of crucial importance in regulating cholesterol homeostasis, should provide an opportunity to further address the role of genetic factors in the development of hypercholesterolemia in man. Recent studies have characterized a restriction fragment length polymorphism at the LDL receptor gene locus that cosegregates with the FH allele (43, 44). Further studies may allow us to define additional polymorphisms associated with *LDLR* and *HMGCR*. Such markers should help identify families in which abnormal expression of either the LDL receptor or HMG-CoA reductase contributes to the development of hypercholesterolemia and premature atherosclerosis.

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