Vol. 133, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 27, 1985 Pages 248-255

THE HUMAN APOLIPOPROTEIN B-100 GENE: A HIGHLY POLYMORPHIC GENE THAT MAPS TO THE SHORT ARM OF CHROMOSOME 2

L. Chan[1], P. VanTuinen[1], D.H. Ledbetter[1], S.P. Daiger[2], A.M. Gotto, Jr.[1], and S.H. Chen[1]

[1]Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030

[2]Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, TX 77030

Received October 14, 1985

SUMMARY: Using a cloned cDNA of apolipoprotein B-100 as hydridization probe, we have found high frequence polymorphisms in the apoB-100 gene involving sites for the restriction enzymes EcoRI, BamHI, and HindIII. The major EcoRI polymorphisms involved a 17 kb vs 15 kb variant. The incidence of the various phenotypes was estimated. In addition, other complex polymorphisms involving MspI and TaqI sites were also noted. [^{32}P]-labeled apoB-100 cDNA was used as a probe in chromosome mapping studies to detect the human apoB-100 structural gene sequence in human-Chinese hamster and human-mouse cell hybrids. Southern blot analysis of 14 hybrids localized the gene to the short arm of human chromosome 2.

Apolipoprotein (apo) B is the most abundant of the plasma apolipoproteins. It is heterogeneous, and exists primarily as 2 forms: apoB-100 and apoB-48 (1,2). ApoB-100 is found predominantly in LDL, and apoB-48 in chylomicrons. ApoB-100 is the protein determinant in LDL that recognizes the LDL receptor (3). Three-fourths to two-thirds of the LDL is metabolized by the LDL receptor pathway, mainly by the liver (4). Defects in the LDL receptor result in severe hypercholesterolemia and accelerated atherosclerosis (3).

Recently, the molecular cloning of the cDNA for apoB-100 has been reported by a number of investigators (5-7). Our laboratory has reported the cloning of a 2.8 kb long partial cDNA of human hepatic apoB-100 mRNA (7). We have used the cloned apoB-100 cDNA as a hybridization probe to search for RFLPs in the apoB-100 gene. In this communication, we report several high

Abbreviations: LDL, low density lipoproteins; kb, kilobase(s); apo, apolipoprotein; RFLPs, restriction fragment length polymorphisms.

0006-291X/85 $1.50

frequency, independent DNA polymorphisms of the apoB-100 gene. The best characterized of these polymorphisms involves an EcoRI site, although additional variation is detected with HindIII, BamHI and other restriction enzymes. Using the technique of Southern gel analysis of DNAs from a panel of human-rodent somatic cell hybrids, we have further mapped the structural gene of apoB-100 to the short arm of human chromosome 2.

EXPERIMENTAL PROCEDURES

Human ApoB-100 cDNA Probe

The apoB-100 clone, λB8, is 2.8 kilobases in length, and corresponds to the 3' end of apoB-100 mRNA (7). It contains sequences that encode an 836 amino acid length carboxyl-terminal fragment of apoB-100, as well as 303 bases in the 3' untranslated region of the mRNA, and 8 bases of the polyadenylate tail. The DNA insert was labeled with [^{32}P] by nick-translation (8), and used as hybridization probe for Southern blot analysis of genomic DNA fragments, or DNA fragments from the somatic cell hybrids.

Detection of DNA Polymorphisms in Human Leukocyte DNAs

DNA was extracted from human leukocytes by the technique of Kan et al. (9). Donors were healthy adult, Caucasian volunteers, all unrelated except for one nuclear family consisting of mother, father and two siblings. DNA was digested by various restriction enzymes under conditions recommended by the suppliers. Complete digestion was ensured by the use of excess enzyme and prolonged incubations. DNA fragments were separated on 0.7% agarose gels and transferred to nitrocellulose paper by the method of Southern (10). Prehybridization and hybridization of the nitrocellulose paper were performed as described previously (11). The filters were blotted dry and exposed to Kodak XAR-5 X-ray film with Cronex Lighting Plus intensifying screens (DuPont) at -70°C for 24-72 hr.

Localization of ApoB-100 Structural Gene Using Somatic Cell Hybrids

Mapping of the apoB-100 structural gene to human chromosomes was performed by Southern blot analysis of DNAs isolated from a panel of human-rodent somatic cell hybries. Ten Chinese hamster-human hybrids were constructed and analyzed as previously reported (12); one additional hamster-human hybrid (1.11 HZ) was constructed in the same manner. Three mouse-human hybrids (MH-5, MH-18, and MH-7) were generated by fusions between the mouse LTMK$^-$ cell line and three human fibroblast cell lines containing balanced translocations: GM7151 [46,X,t(X;17)(q11;q11)], GM2836 [46,XY,t,(9;17)(q12;p11)], and GM0271 [46,XX,t,(17;19)(q23;p13)]. Chromosome analysis was performed by trypsin G-banding of at least 20 cells, followed by sequential G-11 staining of selected G-banded cells (12). A human chromosome was scored as present if 20% or more of the cells contained the chromosome and absent if less than 20% of the cells contained the chromosome.

For DNA preparation, cells were grown in 150 mm dishes to confluency and harvested for trypsinization. They were treated with proteinase K after washing and DNA was isolated as described (11). Digestion and agarose gel electrophoresis of cell hybrid DNA and Southern blot analysis were performed as described by Cheung et al. (11).

RESULTS

Restriction Fragment Length Polymorphism of the ApoB-100 Gene

Of 10 restriction enzymes tested on DNAs from a minimum of 6 unrelated Caucasians, 5 (AvaII, BstXI, HindII, HpaII and ScaI) detected no variation. Three (BamHI, EcoRI and HindIII) detected unequivocal RFLPs (Figure 1) and 2 (MspI and TaqI) revealed considerable inter-individual variation, but this variation was too complicated for routine analysis. The RFLPs detected with BamHI, EcoRI and HindIII are all independent of each other, i.e., individuals whose phenotypes are the same when tested with one restriction enzyme are often different from each other when tested with another enzyme. Thus, these polymorphisms probably result from the loss or gain of individual restriction sites rather than a single insertion-deletion event.

To further characterize the EcoRI RFLPs, we tested a total of 23 unrelated Caucasians and one nuclear family. In the most frequent (70%) phenotype, 3 hybridizing fragments are seen: 15, 2.1 and 1.7 kb. The next most frequent phenotype (26%) has an additional 17 kb fragment and in one individual, only the 17 kb and 1.7 kb bands are present (Figure 1). The phenotypes of the parents in the nuclear family are the first and second type, respectively, and

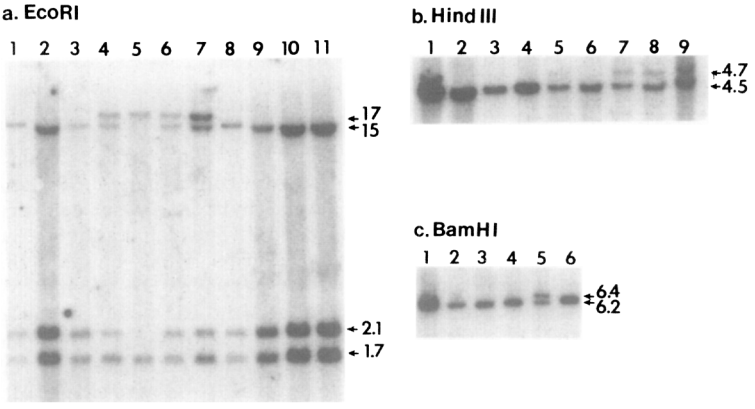

Figure 1: Restriction fragment length polymorphisms of the apoB-100 gene. Southern blots of leukocyte DNAs from unrelated Caucasians were hybridized to [^{32}P]-labeled apoB-100 cDNA clone λB8 as described in Experimental Procedures. The numbers on the right side of each panel indicate the approximate size of the individual DNA fragments in kb. a. RFLPs involving EcoRI sites. Lane 5 is the only individual out of 27 (23 unrelated individuals plus one nuclear family of 4) who showed the absence of the 15 kb band or the 2.1 kb band. These bands were present in all of the other 26 individuals studied. b. RFLPs involving HindIII sites. c. RFLPs involving BamHI sites.

the phenotypes of the sibs are likewise these two types. This is consistent with a Mendelian inheritance of the polymorphic variants.

If the 17 and 15 kb EcoRI fragments are alleles of each other, then the respective gene frequencies are 61% and 39%. If the two alleles are simply the presence or absence of the 17 kb band (and the absence of the 15 kb band [and the 2.1 kb band] in one individual is the result of an unrelated mutation), then the respective gene frequencies are 83% and 17%. Our present data do not allow us to distinguish between these two models. Comparable polymorphic variation is also detected with BamHI and HindIII, and the allelic relationships within these phenotypes are equally complex. In any case, there are clearly several, independent, high frequency RFLPs at this locus.

Chromosomal Mapping of the ApoB-100 Structural Gene

When the nick-translated apoB-100 cDNA probe, λB8, was hydridized to human DNA digested with the restriction enzyme SacI, a single 7 kb DNA band was noted (Figure 2). Under the conditions of hybridization, no cross-hybridizing Chinese hamster or mouse DNA fragments were detected. We have used Southern blots of hybrid cell DNA digested with SacI for hybridization, and correlated the presence of the human specific DNA bands with the presence of specific human chromosomes in the various somatic cell hybrids.

Table I shows the results of Southern blot analysis with [^{32}P]-labeled λB8 hybridized to the DNAs from 14 cell hybrids. Synteny analysis of the various hybrid clones indicates concordance between the apoB-100 gene and human chromosome 2. Furthermore, a hybridization signal was evident in one cell hybrid, 6.1, which contained only the short arm of chromosome 2 translo-

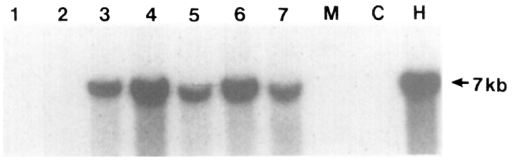

Figure 2: Hybridization of apoB-100 cDNA, λB8, to SacI-digested DNA from human-rodent somatic cell hybrids. Lanes 1-7, DNA from different somatic hybrids. Lane M, mouse DNA; lane C, Chinese hamster DNA; lane H, human DNA. Lanes 3-7 are all positive for the human specific 7 kb DNA band. They all contain human chromosome 2. Lanes 1 and 2 are negative, and do not contain human chromosome 1. For complete tabulation of results, see Table I.

Table I. Synteny Analysis of the Human apoB-100 Gene in 14 Human-Rodent Somatic Cell Hybrids by Molecular Hybridization Using a cDNA Probe λB8

Hybrids[x]	Human Chromosomes																							ApoB[@]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Y	X	
C_1IA			+	+	+	+					+	+		+	+	+	+	+			+	+		+	
1.2	+		+	+	+	+		+	+		+								+	+	+			+	
1.4			+	+												+			+		+			+	
6.1	+	*	+	+		+		+		+	+	+	+	+					+	+	+	+	+	+	+
8.2		+	+	+			+	+		+	+	+		+	+	+				+				+	+
16.1		+	+	+	+	+					+					+		+	+		+		+	+	+
MR2.2		**	+			+	+			+	+		+							+		+		+	+
MR5.11	+	+	+				+		+	+	+		+			+		+			+	+		+	+
MR6.1		+					+		+		+		+	+	+									+	+
MR7.11											+		+					+				+		+	
1.11 H2						+		+			+													+	
MH-5		+	+		+							+					x		+		+			+	+
MH-18			+		+	+	+	+	§		+	+	+	+	+		¶			+	+				
MH-7		+	+														+	+							+
Number of Concordant Hybrids	7	14	9	6	5	5	9	5	6	10	7	7	8	7	6	7	6	5	7	7	6	7	8	8	
Percent of Concordance	50	100	64	43	36	36	64	36	43	71	50	50	57	50	43	50	43	36	50	50	43	50	57	57	

[x]Hybrids MH-5, MH-18 and MH-7 are human-mouse hybrids, the rest are human-Chinese hamster cell hybrids.
[@]Detected by the presence of the 7 kb human specific band on digestion with the enzyme SacI and hybridization to λB8.
*Indicates the presence of the short arm (2cen→pter) of chromosome 2.
**Chromosome 2 (2p region) observed by prior DNA hybridizations (14), but not by direct G-banding analysis.
§Indicates the presence of part of long arm (9q12→qter) of chromosome 9.
#Indicates the presence of part of long arm (17q11→qter) of chromosome 17.
¶Indicates the presence of a portion (17p11→qter) of chromosome 17.

cated onto a Chinese hamster chromosome (12). All other chromosomes showed significant discordance. These results allow assignment of the structural gene for human apoB-100 to chromosome 2, in the region 2cen→2pter (Table I).

DISCUSSION

ApoB-100 is the ligand recognized by the LDL receptor (3). It thus plays a pivotal role in lipoprotein metabolism. Only a small proportion of patients with hypercholesterolemia have been found to have a defective, or absent, LDL receptor (3,4). It is possible that variations in ligand structure might be present in some patients who have hypercholesterolemia but normal receptor function. Immunochemical heterogeneity of human apoB has been demonstrated repeatedly in various laboratories (13-17). A comparison of the DNA-deduced apoB-100 amino acid sequence from a cloned apoB-100 cDNA with amino acid sequences determined directly on purified apoB-100 peptides indicates that such heterogeneity extends to the amino acid and DNA levels (7).

The present study indicates that the apoB-100 gene is polymorphic with respect to the restriction enzymes EcoRI, BamHI, and HindIII. Segregation of the EcoRI variants in a nuclear family is consistent with Mendelian inheritance and the frequency of the least frequent allele is 17% or greater. The BamHI and HindIII RFLPs are comparable in frequency to the EcoRI RFLP though the 3 polymorphisms are clearly independent of each other. MspI and TaqI detect additional polymorphic variations although the complexity of fragment patterns makes analysis difficult. This is an unusually high number of RFLPs detected with a relatively small number of restriction enzymes (18), suggesting there will be considerable additional DNA variation found at this locus. These multiple, independent RFLPs should permit detection of a considerable number of polymorphic haplotypes at the apoB-100 locus, comparable to the β-globin locus (19) or the phenylalanine hydroxylase locus (20). The RFLP haplotypes may be useful in detecting associations between genetic variants of apoB-100 and diseases of lipid metabolism.

To date, the genes for a number of apolipoproteins have been assigned to specific human chromosomes. ApoA-I has been mapped to chromosome 1 (21); apo-

A-I, apoC-II, and apoC-IV have been mapped to chromosomes 11 (11,22,23); and apoE, apoC-II, and apoC-I to chromosome 19 (24-26). We have now mapped the gene for apoB-100 to chromosome 2. The observation of a high degree of polymorphism in the apoB-100 gene locus indicates that it will be an excellent genetic marker for the short arm of chromosome 2, and will contribute substantially to the goal of saturating the human genome with DNA polymorphisms (27).

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Institutes of Health HL-27341, AM-30471, and the March of Dimes Birth Defects Foundation.

REFERENCES

1. Kane, J.P., Hardman, D.A., & Paulus, H.E. (1980) Proc. Natl. Acad. Sci. USA 77, 2465-2469.
2. Kane, J.P. (1980) Ann. Rev. Physiol. 45, 637-650.
3. Goldstein, J.P., & Brown, M.S. (1977) Ann. Rev. Biochem. 46, 897-930.
4. Brown, M.S., & Goldstein, J.P. (1983) J. Clin. Invest. 72, 743-747.
5. Lusis, A.J., West, R., Mehrabian, M., Reuben, M.A., LeBoeuf, R.C., Kaptein, J.S., Johnson, D.F., Schumaker, V.N., Yuhasz, M.P., Schotz, M.C., & Elovson, J. (1985) Proc. Natl. Acad. Sci. USA 82, 4597-4601.
6. Deeb, S.S., Motulsky, A.G., & Albers, J.J. (1985) Proc. Natl. Acad. Sci. USA 82, 4983-4986.
7. Wei, C.F., Chen, S.H., Yang, C.Y., Marcel, Y., Milne, R., Li, W.H., Sparrow, J.T., Gotto, A.R, Jr., & Chan, L. (1985) Proc. Natl. Acad. Sci. USA 82, (in press).
8. Chan, L., Dugaiczyk, A., & Means, A.R. (1980) Biochemistry 19, 5631-5637.
9. Kan, Y.W., Dozy, A.M., Trecartin, R.L., & Todd, D. (1977) N. Engl. J. Med. 302, 185-192.
10. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
11. Cheung, P., Kao, F.-T., Law, M.L., Jones, C., Puck, T.T., & Chan, L. (1984) Proc. Natl. Acad. Sci. USA 81, 508-511.
12. Su, T.S., Nussbaum, R.L., Airhart, S., Ledbetter, D., Mohandas, T., O'Brien, W.E., & Beaudet, A.L. (1984) Am. J. Hum. Genet. 36, 954-964.
13. Blumberg, B.S., Dray, S., & Robinson, J.C. (1962) Nature (London) 194, 656-658.
14. Hirschfield, J. (1968) Series Haematol. 1, 38-65.
15. Butler, R., Butler, E., Scherz, R., & Pflugshaupt, R. (1978) In: Proceedings of the 25th Colloquium Protides on the Biological Fluids, (Peeters, H., ed.), pp. 255-262, Pergamon Press, New York.
16. Berg, K. (1979) In: Biochemistry in Atherosclerosis, (Scanu, A.M., Wissler, R.W., & Getz, G.S., eds.), pp. 419-480, Marcel Dekker, Inc., New York.
17. Schumaker, V.N., Robinson, M.T., Curtiss, L.K., Butler, R., & Sparkes, R.S. (1984) J. Biol. Chem. 259, 6423-6430.
18. Daiger, S.P., Hoffman, N.S., Wildin, R.S., & Su, P.-S (1984) Amer. J. Hum. Genet. 26, 736-749.
19. Antonarakis, S.E., Boehm, C.D., Giardina, P.J.V., & Kazazian, H.H. (1982) Proc. Natl. Acad. Sci. 79, 137-141.
20. Lidsky, A.S., Ledley, F.D., DiLella, A.G., Kwok, S.C.M., Daiger, S.P., Robson, K.J.H., & Woo, S.L.C. (1985) Amer. J. Human Genet. 37, 619-634.
21. Moore, M.N., Kao, F.-T., Tsao, Y.K., & Chan, L. (1984) Biochem. Biophys. Res. Commun. 123, 1-7.

22. Law, S.W., Gray, G., Brewer, H.B., Jr., Sakaguchi, A.Y., & Naylor, S.L. (1984) Biochem. Biophys. Res. Commun. 118, 934-942.
23. Karathanasis, S.K. (1985) Proc. Natl. Acad. Sci. USA (in press).
24. Lin-Lee, Y.C., Kao, F.-T., Cheung, P., & Chan, L. (1985) Biochemistry 24, 3751-3756.
25. Knott, T.J., Eddy, R.L., Robertson, M.D., Priestley, L.M., Scott, J., & Shows, T.B. (1984) Biochem. Biophys. Res. Commun. 125, 299-365.
26. Jackson, C.L., Bruns, G.A.P., & Breslow, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 2945-2949.
27. Botstein, D., White, R.L., Skolnick, M.S., & Davis, R.W. (1980) Amer. J. Hum. Genet. 32, 314-331.