Hum Genet (1990) 84:373–375

Human Genetics

An insertion deletion polymorphism in the signal peptide of the human apolipoprotein B gene

Sophia Visvikis[1,4], Lawrence Chan[2], Gerard Siest[1], Pierre Drouin[3], and Eric Boerwinkle[4]

[1]Center for Preventive Medicine, U.A. CNRS 597, F-54501 Vandoeuvre-les-Nancy, France
[2]Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030, USA
[3]Medical Services G, Jeanne d'Arc Hospital, F-54200 Dommartin-les-Toul, France
[4]Center for Demographic and Population Genetics, The University of Texas Health Science Center in Houston, Houston, TX 77225, USA

Received June 2, 1989

Summary. In this communication we report the genetic properties of an insertion/deletion polymorphism in the signal peptide of the human apolipoprotein B (apo b) gene. There are two alleles of the apo B signal peptide; one codes for a peptide 27 amino acids in length and the other a peptide only 24 amino acids in length. Using the polymerase chain reaction the difference of nine nucleotides between the two alleles is readily detectable after electrophoresis of the amplification products. The relative frequencies of the *Ins* and *Del* alleles are 0.655 and 0.345, respectively. The apo B signal peptide genotypes are transmitted in a manner consistent with an autosomal codominant mode of inheritance with two alleles.

Introduction

Atherosclerosis and coronary heart disease make major contributions to the morbidity and mortality of westernized populations. Epidemiological studies and clinical trials have determined that plasma lipid levels are significant risk factors for the development of disease, however information about the apolipoproteins may be better predictors of disease risk. One of these apolipoproteins, apolipoprotein B (apo B), has a central role in lipid metabolism (see Li et al. 1988 for a review). Apo B is the major apolipoprotein in postprandial chylomicrons and low-density lipoprotein particles. Elevated plasma levels of apo B are associated with increased prevalence of atherosclerosis (Brunzell et al. 1984).

The gene for human apo B has been cloned, mapped, and is well characterized (Ludwig et al. 1987). The gene is 43 kb in length with 81 bp coding for a 27 amino acid signal peptide. Although the exact physiological role of the apo B signal peptide has not been studied, other signal peptides are involved in the translocation of secretory proteins across the membrane of the endoplasmic reticulum (Chan and Bradley 1982; Benson et al. 1985; Randall and Hardy 1989). We have recently found that the signal peptide of human apo B is of variable length among individuals (Yang et al. 1989; Boerwinkle and Chan 1989). There are two apo B signal peptide alleles; one codes for a peptide 27 amino acids in length and the other a peptide only 24 amino acids in length. Amino acids -14 to -16 are absent in the shorter allele product. To our knowledge this is the first example of a polymorphism in a signal peptide of a human gene product. In this communication we characterize the genetic properties of this polymorphism reporting the allele frequencies and verifying their segregation in a large number of families. We also investigated the allele frequencies of this polymorphism in a sample of hyperlipidemic individuals and compare them to those from the population at large. This insertion/deletion mutation has potential physiological significance because it alters the structure of the signal peptide of a gene central to lipid metabolism.

Offprint requests to: E. Boerwinkle

Materials and methods

These analyses were carried out on a sample of 95 nuclear families taking part in systematic health examinations at the Center for Preventive Medicine in Nancy, France. Only the parents of these families were considered for allele frequency estimation. Children of the families were used to document the Mendelian inheritance of the apo B signal peptide insertion/deletion polymorphism. In addition to these family data, 34 type IIa hyperlipidemic subjects were selected by the hospital service in Nancy, France to estimate the allele frequencies in a clinical population.

Blood was collected into ethylenediaminetetraacetate (EDTA) vacutainers. After spinning, the buffy coat was separated and immediately frozen in liquid nitrogen until further analysis. Once in Houston, genomic DNA was purified from the buffy coat by phenol extraction. Oligonucleotide primers for the polymerase chain reaction (Saiki et al. 1988) were 22 and 23 nucleotides in length

and were synthesized on an Applied Biosystems model 380A DNA synthesizer. The 5′ oligonucleotide was 5′-CAGCTGGCGATGGACCCGCCGA-3′ end the 3′ oligonucelotide was 5′-ACCGGCCCTGGCGCCCGCCAGCA-3′. The polymerase chain reaction (PCR) was carried out in a final volume of 100 µl containing approximately 0.5 µg of genomic DNA and 0.65 µg of each oligonucleotide. The four dNTPs were present in a final concentration of 150 µ*M*. The reaction buffer used was that recommended by the manufacturer with the addition of 10% dimethylsulfoxide (DMSO). Prior to addition of 1.5 units of thermostable *Taq* polymerase (Perkin-Elmer-Cetus), the reaction mixture was boiled for 6 min and then allowed to cool briefly. Annealing, extension, and denaturation were carried out at the temperatures given below using an automated thermal cycler (Perkin-Elmer-Cetus). The denaturation step was carried out at 94°C for 1 min and annealing and extension were carried out simultaneously for 1.5 min at 64°C. Amplified DNA was subjected to electrophoresis in 8% polyacrylamide gels at 90 V for 4–5 h. PCR products were directly visualized after ethidium bromide staining of the acrylamide gels.

The frequencies of the longer (insertion, *Ins*) and shorter (deletion, *Del*) alleles were estimated by the gene counting method. Chi-square goodness-of-fit tests (Sokal and Rohlf 1981) were used to test the fit of the observed genotype frequencies to those expected assuming Hardy-Weinberg equilibrium, and to test the goodness of fit of the family data to Mendelian expectations. A contingency chi-square (Sokal and Rohlf 1981) was used to test the homogeneity of allele frequency between the normal and patient samples.

Results

The PCR products of each of the apo B signal peptide insertion/deletion genotypes are shown in Fig. 1. After polyacrylamide gel electrophoresis, the two alleles are readily distinguishable following ethidium bromide staining. The larger *(Ins)* allele is 93 bp and the smaller *(Del)* allele is 84 bp. Heterozygous individuals yield PCR products of both sizes in approximately equal molar amounts. Genotype and allele frequencies of the apo B signal peptide insertion/deletion polymorphism in both the random and type IIa hyperlipidemic samples are given in Table 1. We estimate the relative frequencies of the *Ins* and *Del* alleles in the general population to be 0.655 and 0.345, respectively. The allele frequencies between the random sample and the sample of type IIa patients do not differ significantly.

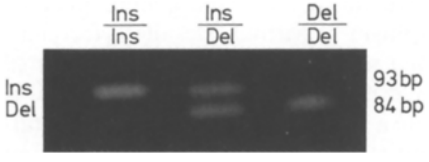

Fig. 1. Polymerase chain reaction products of each of the apo B signal peptide insertion/deletion polymorphism genotypes

Table 1. Genotype and allele frequencies for the apo B signal peptide insertion/deletion polymorphism

	Genotype frequencies[a]		
	Ins/Ins	Ins/Del	Del/Del
Sample			
Random	73 (37.6%)	108 (55.7%)	13 (6.7%)
Hyperlipidemics	13 (38.2%)	17 (50.0%)	4 (11.8%)
	Relative allele frequencies		
	Ins	Del	
Sample			
Random	65.5%	34.5%	
Hyperlipidemics	63.2%	36.8%	

[a] Counts are given and the corresponding relative frequencies are enclosed in parentheses

Table 2. Segregation of the apo B signal peptide insertion/deletion polymorphism[a]

Mating type	Number	Children			
		Ins/Ins	Ins/Del	Del/Del	Total
Ins/Ins × Ins/Ins	14	26	–	–	26
Ins/Ins × Ins/Del	32	24	41	–	65
Ins/Ins × Del/Del	7	–	14	–	14
Ins/Del × Ins/Del	35	15	33	15	63
Ins/Del × Del/Del	4	–	4	3	7
Del/Del × Del/Del	1	–	–	2	2
Total	93	65	92	20	177

[a] The observed segregation ratios do not differ from Mendelian expectations under a codominant model ($\chi^2 = 4.7$, 4 *df*)

The apo B signal peptide genotypes are transmitted in a manner consistent with an autosomal codominant mode of inheritance with two alleles. The genotypes of 177 children from 93 families whose parents were both typed for this polymorphism are given in Table 2. Data from two families with previously known genetic inconsistencies were not considered. No new inconsistencies were detected. The segregation ratios did not differ from Mendelian expectations under a model of autosomal codominant mode of inheritance ($\chi^2 = 4.7$, 4 *df*). For example, there are 32 families with both parents heterozygous *ins/del* at this locus. These families had 63 children, 15 of whom were *ins/ins*, 33 of whom were *ins/del*, and 15 of whom were *del/del*.

Discussion

We have developed a rapid and efficient method of typing an insertion/deletion polymorphism in the signal peptide region of the human apo B gene. With the polymerase chain reaction and synthetic oligonucleotides closely flanking the region of interest, alleles differing by only nine base pairs were readily distinguishable. One investigator can easily type 40 samples in a single work day. In addition to the obvious sensitivity of the PCR, its speed and simplicity make it superior to traditional Southern blot analysis for population studies. We also report the population genetic characteristics of this polymorphism including genotype and allele frequencies, and its segregation in families. Several epidemiological studies have reported significant associations between restriction fragment length polymorphisms (RFLPs) in the apo B gene and hyperlipidemia (see Humphries 1988

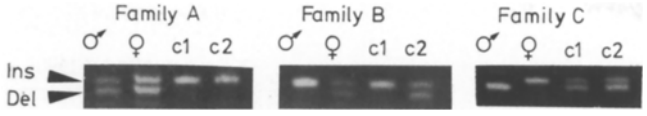

Fig. 2. Segregation of the apo B signal peptide insertion/deletion polymorphism in three families

for a review). However, several studies have also reported no significant association between apo B RFLPs and lipid levels or disease. Reasons for these discrepancies are not well understood and are likely to be complex. We found no significant difference in the frequency of this polymorphism between the population at large and a small sample of type IIa hyperlipidemic patients. The role of this signal peptide variation, if any, in lipid metabolism and cardiovascular disease risk must await further investigation.

Curiously, the observed genotype frequencies presented in Table 1 are significantly different from those expected assuming Hardy-Weinberg equilibrium (χ^2 = 10.4, 1 *df*). Without supporting evidence, we do not believe this deviation is due to selection. Initially, we suspected technical laboratory reasons. We have retyped over 100 individuals and corroborated their genotype designation. The observed Mendelian inheritance (Fig. 2, Table 2) also does not support this methodological hypothesis. We have typed a second sample of 93 unrelated individuals from this same population. The genotype frequencies of this second sample do not differ from Hardy-Weinberg expectations. In addition, we have typed five other polymorphic loci in the apo B gene in this same sample of families, and their genotype frequencies were each in accordance with Hardy-Weinberg expectation. We have also typed an independent sample of 194 unrelated Mexican-American individuals from Starr County, Texas, USA and these frequencies were also in Hardy-Weinberg equilibrium (data not shown). It is for these reasons that we believe that in the sample of parents from Nancy, France, the deviation of the insertion/deletion genotype frequencies from Hardy-Weinberg expectations is due to chance.

Apo B, as with most proteins, is synthesized initially in precursor form with an NH_2-terminal signal peptide sequence (Yang et al. 1989). This signal peptide is cotranslationally cleaved off during transport of the polypeptide from the site of synthesis to the endoplasmic reticulum. The role of the signal peptide sequence in the transport process can be divided into three stages: entry into the pathway, translocation across the membrane, and release on the opposite side (Randall and Hardy 1989). Information specifying the correct cellular localization often resides in the signal peptide. Any modification in the signal peptide sequence may provoke irregular protein processing (e.g., Angele et al. 1989). Signal peptides containing mutations that decrease the efficiency of export have been characterized for some export proteins (e.g., Benson et al. 1985). Such mutations may affect the three-dimensional structure or the hydrophobicity of the leader peptide, which has a crucial role in membrane translocation. To our knowledge, this apo B signal peptide polymorphism is the only such mutation described in humans. Further studies on the role of this variation on apo B synthesis and metabolism, and its effect on lipid levels will shed light on the role of signal peptides in protein function and metabolism.

Acknowledgements. The authors are grateful to Dr. Craig Hanis for making available the sample of unrelated Mexican-Americans collected in part by support from NIH AM 34666. This work was supported in part by NIH HL-40613 to E.B., NIH HL-27341 to L.C., the March of Dimes Birth Defects Foundation, the Institut National de la Santé et de la Recherche Medicale (C.R.E. 888010), the Caisse Nationale d'Assurance Maladie des Travailleurs Salaries, and by the Centre National de la Recherche Scientifique.

References

Angele C, Wellman T, Thioudellet C, Guellaen G, Siest G (1989) Expression of rat renal gamma-glutamyltransferase cDNA in *Escherichia coli*. Biochem Biophys Res Commun 160:1040–1046

Benson SA, Hall MN, Silhavy TJ (1985) Genetic analysis of protein export in *Eschericia coli* K12. Annu Rev Biochem 54: 101–134

Boerwinkle E, Chan L (1989) A three codon insertion/deletion polymorphism in the signal peptide region of the human apolipoprotein B (APOB) gene directly typed by the polymerase chain reaction. Nucleic Acids Res 17:4003

Brunzell JD, Sniderman AD, Albers JJ, Kwiterovich PO (1984) Apolipoprotein B and A-I and coronary artery disease in humans. Arteriosclerosis 4:79–83

Chan L, Bradley WA (1982) Signal peptides: properties and interactions. In: Conn PM (ed) Cellular regulation of secretion and release. Academic Press, New York London, pp 301–321

Humphries SE (1988) DNA polymorphisms of the apolipoprotein genes – their use in the investigation of the genetic component of hyperlipidaemia and atherosclerosis. Atherosclerosis 72: 89–108

Li WH, Tanimura M, Luo CC, Datta S, Chan L (1988) The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. J Lipid Res 29: 245–271

Ludwig EH, Blackhart BD, Pierotti VR, Caiati L, Fortier C, Knott T, Scott J, Mahley RW, Levy-Wilson B, McCarthy BJ (1987) DNA sequence of the human apolipoprotein B gene. DNA 4:363–372

Randall LL, Hardy SJS (1989) Unity in function in the absence of consensus in sequence: role of leader peptides in export. Science 243:1156–1159

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi RG, Horn TT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487–491

Sokal R, Rohlf FJ (1981) Biometry. Freeman, San Francisco New York

Yang C, Gu ZW, Weng S, Kim TW, Chen SH, Pownall HJ, Sharp PM, Liu SW, Li WH, Gotto AM, Chan L (1989) Structure of apolipoprotein B-100 of human low density lipoproteins. Arteriosclerosis 9:96–108