DNA markers in the hyperlipidaemias and atherosclerosis

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The main function of plasma lipoproteins is to transport lipids, primarily cholesterol esters and triglycerides, between the tissues. They constitute a polydisperse carrier system and vary greatly in size, lipid composition and peptide content, but share similar structure. In general, each particle is a complex macromolecular aggregate, whose components are bound together non-covalently. They are typically spherical in shape, consisting of a hydrophobic core of triglycerides and cholesterol esters and an amphipathic surface coat consisting of a monolayer of apolipoproteins, free cholesterol and phospholipids. The protein and lipid composition of the circulating lipoprotein is not static, but in a dynamic flux.

The apolipoproteins

Apolipoproteins are important structural components of lipoprotein particles and have been shown to participate in lipoprotein synthesis, secretion, processing and catabolism [1]. At least eight of the major apolipoproteins have been characterised, and their properties and function are illustrated in Table 1. The catabolism of the various lipoprotein particles are mainly governed by the activity of endothelial-bound enzymes such as lipoprotein lipase (LPL) and lecithin-cholesterol acyl transferase (LCAT), and by the avidity of specific cell-surface receptors for their ligands.

The genes encoding the eight main apolipoproteins have been isolated and characterised [2,3]. In view of the central role of the apolipoproteins in lipid metabolism, these genes may be considered excellent 'candidate' genes for the study of the genetic components of diseases such as the hyperlipidaemias and atherosclerosis, which result from complex interactions between genotype and environment. Mutations of the apolipoproteins have been reported at both the DNA and amino acid level, and some of these have a profound effect on lipid metabolism. This review, however, will concentrate on DNA polymorphisms at the apolipoprotein gene loci and their possible association with the hyperlipidaemias and atherosclerosis.

Clinical studies using apolipoprotein genes

The chromosomal mapping of the eight apolipoprotein genes and the LDL-receptor gene are shown in Table 2.

Table 2. Chromosome location of apolipoprotein genes.

| Apolipoprotein | Chromosome |
|----------------|------------|
| Apo A-I        | 11         |
| Apo C-III      | 11         |
| Apo A-IV       | 11         |
| Apo C-I        | 19         |
| Apo C-II       | 19         |
| Apo E          | 19         |
| LDL-receptor   | 19         |
| Apo B-100      | 2          |
| Apo A-II       | 1          |

Table 1. The properties of the human apolipoproteins.

| Apolipoprotein | Molecular weight (Daltons) | Main site of synthesis | Function | Plasma levels (mg/ml) |
|----------------|---------------------------|------------------------|----------|----------------------|
| A-I            | 28,000                    | Intestine and liver    | Activates LCAT | 90-30                |
| A-II           | 17,000                    | Intestine and liver    | ? Inhibits LCAT | 30-50               |
| B-48           | 264,000                   | Intestine              | Triglyceride transport | <5                    |
| B-100          | 549,000                   | Liver                  | —Binds LDL-receptor | 80-100              |
| C-I            | 6,500                     | Liver                  | Activates LCAT | 4-7                  |
| C-II           | 8,800                     | Liver                  | Activates LPL  | 3-8                  |
| C-III          | 8,750                     | Liver                  | Inhibits LPL  | 8-15                 |
| E              | 35-39,000                 | Liver and intestine    | Ligand to LDL-receptor | 3-6                  |
There are two main clusters of apolipoprotein genes; the apo A-I/C-III/A-IV gene cluster on chromosome 11 and the apo C-II/E/C-I cluster on chromosome 19. The LDL-receptor that binds LDL via its two main apolipoprotein ligands, apo B and apo E, has also been localised to chromosome 19. Thus the localisation of apo B to chromosome 2 demonstrates that the gene is not in synteny with either apo E or the LDL-receptor.

**The apolipoprotein A-I/C-III/A-IV gene cluster**

Apo A-I is the major protein constituent of high density lipoprotein (HDL). Much of the clinical interest in HDL-Y levels derives from its inverse relationship to the risk of developing coronary heart disease (CHD). In addition, there is a genetic influence on plasma HDL-levels as indicated by certain familial disorders of lipoprotein metabolism that are characterised by reduced levels of HDL (see Table 3). The first reports of the application of cloned human apolipoprotein gene probes to the study of lipid disorders appeared simultaneously in early 1983. Both reports described the use of apo A-I cDNA probes in the restriction enzyme analysis of human chromosomal DNA. In one, Karathanasis et al. [4] studied the DNA from two sisters who exhibited a unique combination of clinical and biochemical abnormalities, including skin and tendon xanthomas, corneal clounding and severe premature coronary atherosclerosis associated with very low HDL levels and deficiencies of two apolipoproteins, namely apo A-I and apo C-III. Restriction enzyme analysis revealed an abnormality at the apo A-I gene locus in the probands, resulting in a major DNA sequence variation (an insertion of approximately 6500 nucleotides) of the coding region of the apo A-I gene. Both probands were shown to be homozygous for this defect, whilst first degree relatives were heterozygous for the DNA insertion with intermediate levels of HDL, apo A-I and apo C-III but were clinically unaffected and asymptomatic.

These findings, and the suggestion from previous amino acid sequence data that the genes encoding for apo A-I and apo C-III were derived by gene duplication of a common evolutionary precursor, led to the hypothesis that the two apolipoproteins were closely linked in the genome. The hypothesis was subsequently confirmed by Karathanasis et al. [5], who demonstrated that the apo C-III gene was situated 2.8 Kb downstream from the 3' end of the apo A-I gene. Furthermore, they appear to be convergently transcribed as illustrated in Figure 1.

**Table 3. Familial disorders characterised by a marked reduction of HDL.**

| Disease                          | Clinical features                                      | Lipoprotein abnormalities                        |
|----------------------------------|--------------------------------------------------------|--------------------------------------------------|
| Tangier disease (26 cases)       | Enlarged orange tonsils, relapsing neuropathy, splenomegaly, corneal opacities | Trace or absent HDL, ↓apo A-I, A-II, hypertriglyceridaemia |
| Fish eye disease (4 subjects)    | Massive corneal opacities causing impaired vision      | ↓HDL by 90%, ↓apo A-I, A-II, hypertriglyceridaemia |
| LCAT deficiency (26 subjects, 12 families) | Premature atherosclerosis, anaemia, proteinuria, uraemia, corneal opacities | ↓HDL by 70%, ↓apo A-I, A-II, hypertriglyceridaemia |
| A-I Milano (5 members of one family) | No clinical features                                   | ↓HDL by 60%, ↓apo A-I, variant protein, hypertriglyceridaemia |
| A-I Marburg (14 members of one family) | No clinical features                                   | ↓HDL levels, ↓apo A-I, variant protein, hypertriglyceridaemia |
| Apo A-I/C-III deficiency (2 sisters) | Severe premature atherosclerosis, skin and tendon xanthomas, corneal clounding | ↓HDL by 90%, absence of apo A-I and C-III |

Fig. 1. The organisation of the apo A-I/C-III gene complex in a patient with familial deficiency of apolipoprotein A-I and C-III. In the probands with familial deficiency of apolipoprotein A-I and C-III, a large DNA insert of greater than 6.5 Kb is located in the coding region of the apo A-I gene. IVS = intervening sequence (intron).
In the second study, using an apo A-I cDNA probe, Rees et al. [6,7] demonstrated an association between an Sst I restriction fragment length polymorphism (RFLP) at the apo A-I locus and hypertriglyceridaemia in Caucasian subjects [6,7]. The RFLP identifies two alleles, S1 and S2. The S2 allele is rare, being present in less than 5 per cent of normolipaemic Caucasians. However, 35-40 per cent of Caucasians with primary hypertriglyceridaemia possess this allele (p<0.001). Interestingly, this relationship does not hold for type III hyperlipidaemia as the prevalence of the S-2 allele in this group of patients approximates to that of the population at large [8]. Further characterisation of this polymorphism has shown it to result from a single base substitution in the 3'-untranslated region of the neighbouring apo C-III gene. This association between an apo C-III gene variant and hypertriglyceridaemia is consistent with the proposed physiological role of apo C-III in regulating the metabolism of triglyceride-rich lipoproteins.

Thus, these two initial reports suggest that the apolipoprotein A-I/C-III gene locus may be implicated in the susceptibility to hypertriglyceridaemia and possibly to CHD.

Subsequently, four independent clinical studies have addressed this latter possibility. Rees et al. [9], reported a significantly increased frequency (p<0.025) of the S-2 allele in subjects with severe obstructive CHD compared with subjects with minimal or absent CHD. Comparable results were reported in a similar study in 48 post-myocardial infarction patients, where a significantly increased frequency of the allele was found, compared with controls (p<0.01) [10]. A recent American study [11] reported another RFLP at this locus, demonstrated by restricting human genomic DNA with Pst I. The polymorphic restriction site is localised to the 3'-end of the apo A-I gene and identifies two alleles—a 3.3 Kb and a 2.2 Kb allele. The 3.3 Kb allele was observed in 3-4 per cent of normal controls, but in 32 per cent of 88 patients with severe CHD, (p<0.0001). In addition, the 3.3 Kb allele was found in 8 of 12 index cases of kindreds with familial hyperapolipoproteinaemia and was proposed as a further useful genetic marker for this disease and for CHD. Frossard et al. [12] reported similar findings with a Pvu II polymorphism in the apo C-III gene.

Five reports have now suggested a possible association between certain apo A-I/C-III alleles and CHD. The consistency of these observations strongly suggests that some RFLPs at this locus may be in linkage disequilibrium with genetic variants predisposing to alterations of lipid metabolism and to CHD.

Apolipoprotein B

Apo B-100 is the ligand recognised by the LDL-receptor [1], and thus plays a central role in lipid metabolism. The gene encoding for apo B has been mapped to the short arm of chromosome 2 [13]. Numerous RFLPs have been reported [3,13,14], and one detects a protein polymorphism which results in lysine-glutamic acid substitution [3]. Recently, an Xba1 RFLP has been associated with altered plasma lipids, but its physiological significance remains to be elucidated [15]. As only a small proportion of subjects with hypercholesterolaemia have been found to have a defective or absent LDL-receptor [16,17], it may be that variations in ligand structure may exist in some patients with normal receptor function but with sporadic hypercholesterolaemia and/or premature coronary heart disease.

**LDL-receptor**

A Pvu II RFLP has been reported which unambiguously cosegregates with familial hypercholesterolaemia (FH) in two index families [18]. In one FH index case, a 2 Kb deletion has been reported at the 3'-end of the gene and thought to be directly responsible for the FH phenotype [19]. It is likely that a wide spectrum of genetic abnormalities at this locus is responsible for the FH phenotype, and it is speculated that some of these alleles may be implicated in atherogenesis.

**Apolipoprotein A-II**

Apo A-II is one of the two major protein components of plasma HDL. In one recent study, plasma levels of apo A-II were found to be superior to those of apo A-I, in distinguishing control subjects from survivors of myocardial infarction [20]. The apo A-II gene has been localised to chromosome 1, and an Msp 1 RFLP close to this locus has been reported which appears to alter apo A-II composition of HDL [21]. The molecular basis for this effect and its relevance to human disease remains to be established.

**Apolipoproteins C-I, C-II and E**

The genes encoding for apolipoproteins C-I, C-II and E are clustered on chromosome 19, with the apo C-II and apo E genes within 2 centimorgans of each other. A Taq I RFLP has been reported at the apo C-II locus but no definitive disease-association has been found [22]. Apo E exists as many isoforms, due to both common genetic variation and post-translational modification due to sialylation [1]. Apo E is recognised by high affinity receptors and thus mediates the binding, internalisation and catabolism of lipoprotein particles. It can serve as a ligand for the LDL receptor present on both hepatic and extrahepatic tissues. Six common apo E phenotypes are recognised, resulting from a combination of three apo E alleles. These are designated E-2, E-3 and E-4. Amino acid sequence analyses have established that the common variants of apo E, E-4 and E-2 differ from E-3 at residue 112 because of an arginine for cysteine substitution and E-2 differs at residue 158 because of a cysteine for arginine substitution. Type III hyperlipidaemia is characterised by elevated cholesterol and triglyceride levels, delayed chylo- micron remnant clearance, xanthomas and premature CHD. In 90 per cent of cases an E2/2 phenotype is observed [1,2]. However, the disease frequency is such that only 1-2 per cent of people with the E-2/2 actually express the disease. Other genetic factors may well contribute to the aetiology, but these are not yet known.
Apo C-I is a constituent of VLDL and HDL but the precise physiological role(s) of the peptide is unknown. Human apo C-I cDNA clones have been isolated, but no studies in human disease have been reported.

Conclusions

The recent developments in recombinant DNA technology are radically changing the direction and impetus of clinical research. This is particularly evident in the study of the multifactorial, polygenic diseases such as diabetes mellitus, the hyperlipidaemias and atherosclerosis. It is now possible for almost any gene of interest to be isolated and its structure studied, even when the primary amino acid structure of the expressed protein is unknown. In addition, a substantial proportion of the genome is not expressed and may be implicated in gene regulation or other functions. It is predicted that RFLPs occur every few hundred bases throughout the genome, thus the established approach of investigating for linkage between certain ‘markers’ and a disease, is considerably enhanced by studying RFLPs rather than expressed protein polymorphisms.

The potential implications of these techniques are numerous. It is hoped that a beginning has been made to unravelling the complexities underlying the interaction of genotype and environment, leading to hyperlipaemia and atherosclerosis. In addition, it may be possible to identify certain DNA markers, the possession of which confer an increased disease-susceptibility. A fuller understanding of the aetiology of such diseases should facilitate the development of rational treatment and preventive measures, and help to allocate health-care resources more efficiently.

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