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MINIREVIEW

New Apolipoprotein A-V: Comparative Genomics Meets Metabolism

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Summary

The availability of the human genome sequence and the recently completed draft sequences of two major mammalian model species, the mouse (*Mus musculus*) and the rat (*Rattus norvegicus*), allow researchers to apply novel approaches for gene identification and characterization, using methods of comparative and functional genomics. Recently, a new gene coding for apolipoprotein A-V was identified in the vicinity of APOA-I/C-III/A-IV cluster on human chromosome 11q23 by comparative sequencing method. In a relatively short time, compelling evidence accumulated for the substantial role of APOA-V in lipid metabolism. Studies in knock-out and transgenic mice revealed that its expression pattern correlates negatively with triglyceride levels. This observation was verified in human population studies in variety of ethnic and age groups. Several single nucleotide polymorphisms were described and particular SNP alleles and haplotypes in the APO A-V gene region were shown to be associated with dyslipidemia. The discovery and characterization of the APO A-V demonstrates current possibilities of the integrative approaches in biology, boosted by the available bioinformatic tools.

Key words
Apolipoprotein A-V • Comparative genomics • Triglyceride • Genetic models • SNP

Introduction

The use of animals as models for human disease is not a recent issue, particularly mice and rats have served as physiological and pharmacological models since the 19th century (recently reviewed by Jacob and Kwitek 2002). Nowadays, the availability of the draft sequence of human, mouse and rat genomes and the genome projects of other model organisms rapidly progressing to completion, it is possible to study the molecular aspects of pathological traits on the whole-genome level and even in comparison among multiple species. An emerging field, termed *comparative genomics***,** involves by definition the analysis of two or more genomes in order to identify the extent of similarity of various features, or a large-scale screening of a genome to identify sequences present in another genome. Applications range from the identification of genes and

regulatory sequences to the study of evolutionary relatedness of species (Strachan and Read 1999).

On the other hand, *functional genomics* refers to large-scale investigation of gene function and annotation of the physiological information to the respective genome. Integration of the functional and comparative approaches *via* robust bioinformatic applications allows cross-referencing various classes of genomic information among species. The ultimate goal of this process is the identification of new genes and functions that may help in deciphering the molecular basis of disease in man. Eventually, the acquired knowledge will provide fundamental information for the production of therapeutics that would causally target the underlying pathological processes. One of the areas currently capitalizing on the recent progress of genomics and concomitant development of bioinformatic tools is the research of lipid metabolism.

Plasma lipids constitute an important factor acting as a determinant of susceptibility to various common classes of disease, cardiovascular disease and atherosclerosis being on the top of the list (reviewed by Steinberg and Gotto 1999). Dyslipidemias *per se* constitute the enhanced risk for manifestation of such conditions. Furthermore, they tend to cluster with other unfavorable metabolic states, like hypertension, obesity or insulin resistance (Reaven 1995). Although there are plenty of factors contributing to individual plasma lipid levels, the apolipoproteins (apoproteins) represent a major component of lipid metabolism dynamics. At present time, the apolipoproteins A to M have been identified and the genes coding for most of them were assigned to their genomic loci. As shown in Figure 1, there are two major clusters of apolipoprotein genes on human chromosomes 11 and 19.

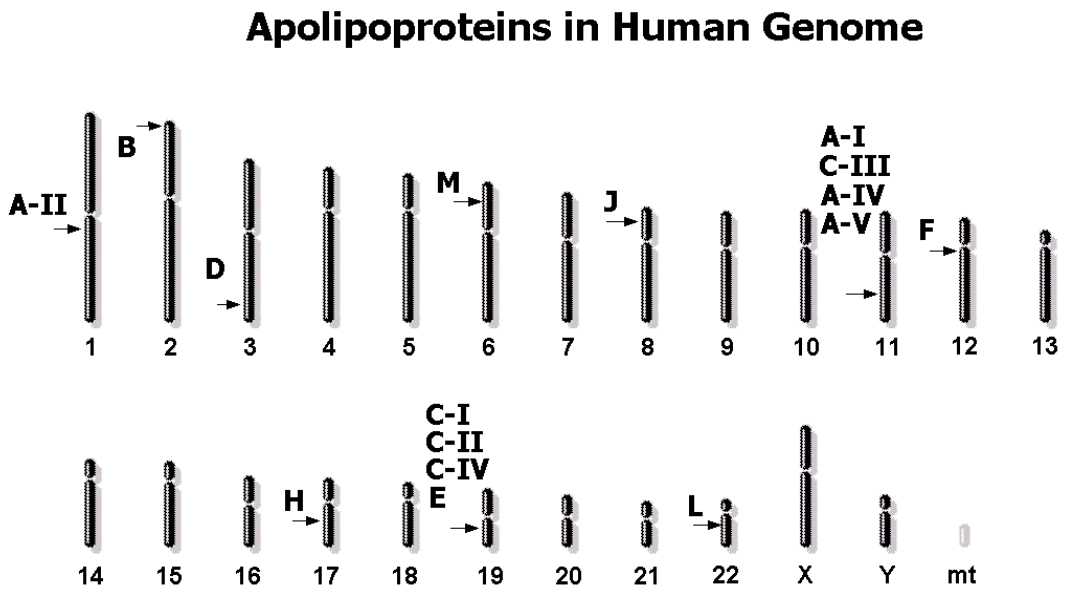

Fig. 1. *Chromosomal localization of the human apolipoprotein genes.*

The apolipoprotein gene cluster APOA-I/C-III/A-IV on human chromosome 11q23 has been thoroughly studied and has been linked to defects in lipid metabolism both in humans and model organisms (reviewed by Groenendijk *et al.* 2001). Recently, two independent research groups identified a novel apolipoprotein in the vicinity of this cluster, and designated it apolipoprotein A-V (MIM 606368). The stories of its discovery nicely depict recent possibilities of comparative genomics.

Two routes to discovery

Rat – early phase of liver regeneration

Using method of cDNA subtractive hybridization, van der Vliet *et al.* (2001) identified three novel upregulated genes in regenerating rat liver after 70 % hepatectomy, designating them regeneration associated proteins (RAP) 1, 2 and 3. After obtaining and sequencing the full-length cDNA of RAP3 from rat liver cDNA library, database

search revealed its 90 % homology with murine expressed sequence tag clones (EST) clones of the mouse liver and fetus and 80 % homology with part of the human chromosome 11q23, where the APOA-I/C-III/A-IV cluster resided. Predicted protein was compared to APOA-I and A-IV (and displayed 20-28 % homology). Using polyclonal antibody raised against recombinant RAP3, authors identified RAP3 in plasma of rats regardless of the undergone hepatectomy. Given the similarity with other apolipoproteins and its presence in the HDL lipid fraction, the novel gene was designated ApoA-V.

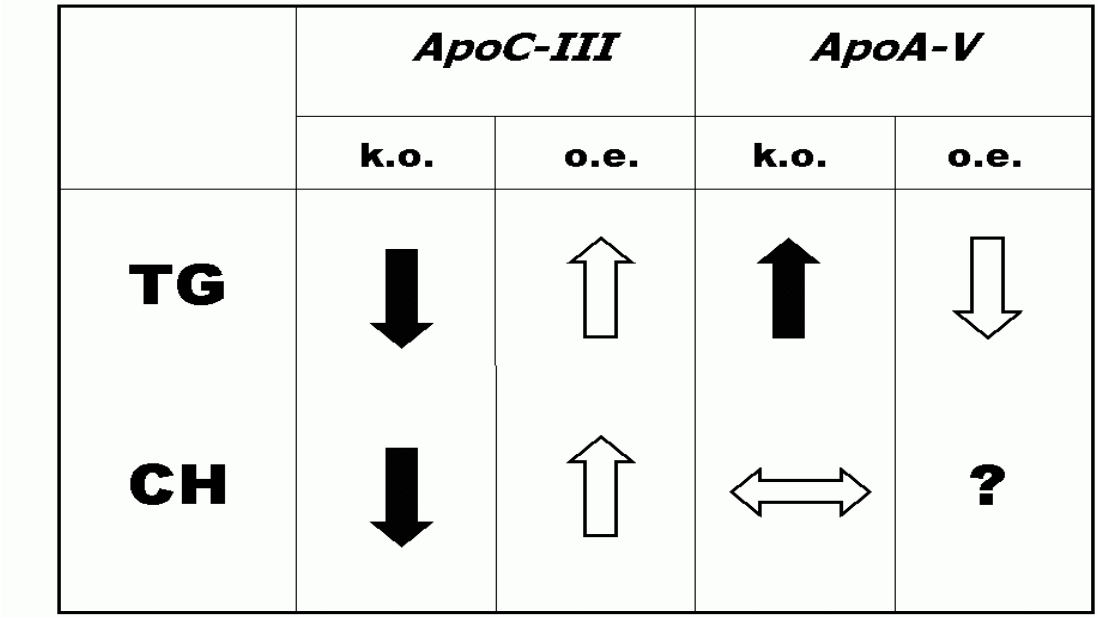

Fig. 2. *Summary of the effects of knock-out (k.o.) or overexpression (o.e.) of ApoC-III and ApoA-V genes on serum levels of triglycerides (TG) and cholesterol (CH). In case of the overexpression of ApoA-V, there are ambiguous results so far (see text).*

Mouse and human – comparative sequencing

Focusing on neighborhood of human APOA-I/C-III/A-IV cluster, Pennacchio *et al.* (2001) sequenced 200kb of orthologous mouse DNA and compared the mouse and human sequences. A region with substantial interspecies conservation of nucleotide sequence (CNS) was found, containing a putative apolipoprotein-like (APOA-V) gene (the visual representation of the alignment of the APOA-I/C-III/A-IV/A-V region among seven different species is available at http://pga.lbl.gov/cgi-bin/get_gene?id=246). Existence of matching ESTs from publicly available databases suggested the gene was transcribed. The predicted protein (sequence of 368 amino acids) showed strongest similarity to mouse ApoA-IV (24 % identity and 49 % similarity). Furthermore, the protein structure analysis revealed characteristic features of lipid-binding apolipoproteins. Expression profile in human and mouse tissues showed the gene was expressed predominantly in liver. Genetically modified mice lacking ApoA-V were derived and found to have four-fold increase in plasma triglycerides (TG), contrasting with the transgenic mice overexpressing *human* APOA-V with ~66 % decrease in plasma TG, providing direct evidence for the role of APOA-V in triglyceride metabolism. Subsequently, authors identified 4 single nucleotide polymorphisms (SNPs) surrounding the human APOA-V locus. They demonstrated in two independent cohorts that minor allele of SNPs 1 a 3 were associated with high triglyceride levels (details in sections "Human SNPs" and "Haplotype studies").

Further studies in rodents

Since ApoA-V knockout mice were shown to be hypertriglyceridemic, van der Vliet *et al.* (2002) generated mice with adenoviral overexpression of *mouse* ApoA-V. As in mice overexpressing *human* APOA-V, elevated serum ApoA-V levels were associated with a

substantial (six-fold) decrease of serum triglycerides. Unexpectedly, all lipoprotein fractions were found to have a reduced cholesterol content, resulting in reduction of total cholesterol levels by ~40 %. The discrepancy with normal cholesterol levels of *human* APOA-V overexpressing mice was attributed to a much higher expression of ApoA-V in the model used. Both studies with transgenic and knock-out mice showed consistently an effect of ApoA-V on lipid levels opposite to that observed in mice overexpressing or lacking ApoC-III, respectively (Fig. 2).

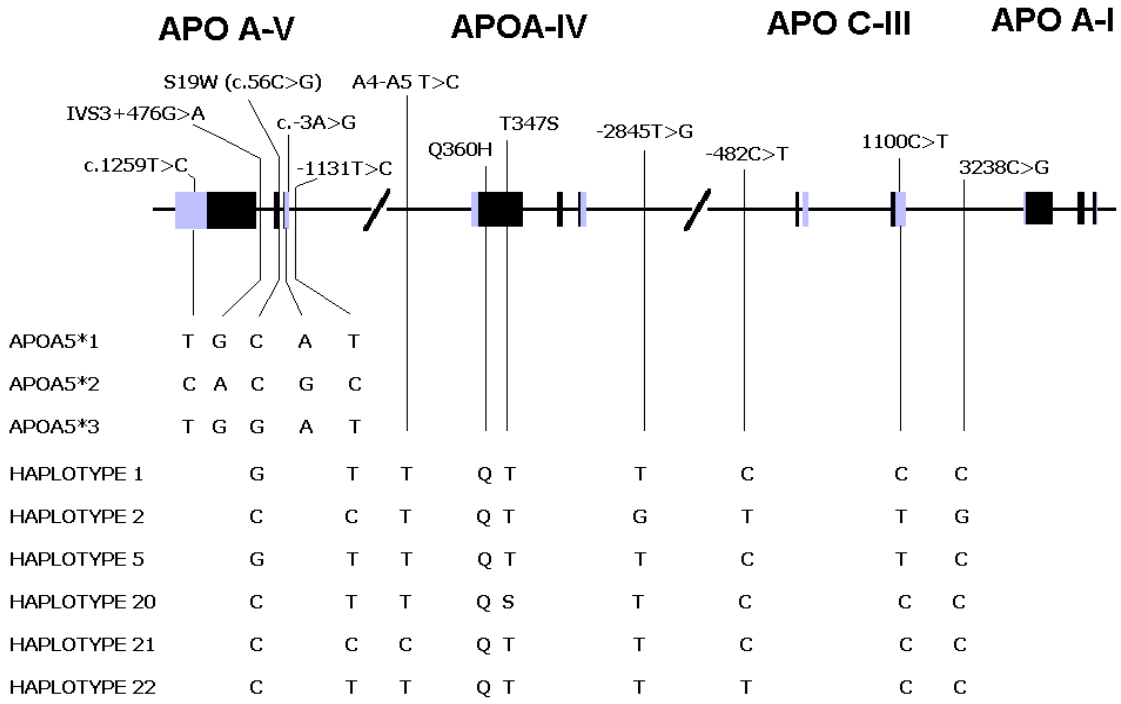

Fig. 3. *Summary of haplotype studies in the apolipoprotein cluster. APOA5*1 -3 are haplotypes designated by Penacchio et al. (2002), selected haplotypes from the total of 22 are shown according to Talmud et al. (2002) (haplotypes 1,2 and 5 represent group with highest TG levels, conversely, the carriers of the haplotypes 20, 21 and 22 displayed the lowest TG levels). Dark boxes represent exons, light boxes represent untranslated regions of the genes.*

Human SNPs

In the original paper, Pennacchio *et al.* (2002) described four SNPs within the APOA-V gene region or its close vicinity, designating them SNP 1-4. The first three of them were shown to be in a strong linkage disequilibrium, suggesting the existence of common haplotype in APOA-V region influencing plasma TG levels. To date, 11 SNPs are annotated in publicly available databases (e.g. http://www.ncbi.nlm.nih.gov, NCBI). So far, the SNP3 (-1131T>C) and S19W (serine/tryptophan) polymorphisms received most attention of several research groups.

The discovery of APOA-V and the fact that familial combined hyperlipidemia (FCHL) has been repeatedly associated with APOA-I/C-III/A-IV cluster prompted a study in which Ribalta *et al.* (2002) used SNP3 in the APOA-V promoter region as the genetic marker to search for associations between the APOA-V and TG metabolism in group of 16 FCHL families (n=103), contrasting them with normolipidemic Dutch group (n=89) and population-based Spanish control group (n=408). APOA-V seemed to be associated with TG-related variables only in FCHL group without adjustment for confounding factors (age, gender, body mass index) and the TG levels even in carriers of the minor -1131C allele were relatively low (1.82±1.33 mmol/l, 1.49±1.06 mmol/l and 0.90±0.42 mmol/l in FCHL and the two control groups, respectively) However, when the association was evaluated in normolipidemic and

hyperlipidemic individuals within the 16 FCHL families separately, carriers of the rare allele C/C displayed significantly increased plasma TG concentrations. Moreover, this APOA-V variant was present more often in FCHL patients and their relatives. Authors therefore concluded that APOA-V acts as modulator of TG concentrations only when there is altered genetic or metabolic background and can be considered a predisposing factor for FCHL. Of interest is the reported significant interaction between APOA-V and APOC-III, confirming the previously shown negative linkage disequilibrium between the two markers.

Very recently, two studies performed in Japanese populations assessed the association of the SNP3 allele with triglyceridemia and other aspects of lipid metabolism. These studies have found significant association of the SNP3 minor (C/C) allele with higher levels of triglycerides in both the general population (Nabika *et al.* 2002) and the cohort of 552 school children (Endo *et al.* 2002), confirming the importance of this polymorphism in a non-Caucasian population. In another recent study of Czech cohort of 1142 men and 1181 women, the SNP3 C/C allele was also found to be associated with high TG in both sexes. Although the S19W polymorphism showed significant effect only in women, carriers of minor (W19) allele had higher triglyceridemia (Hubáček *et al.* 2002). Moreover, homozygous carriers of either allele were found to be more prone to the myocardial infarction. However, since there are no data on biological significance of SNP3 (no obvious transcription factor binding sites could be identified), the association of SNP3 minor allele with high TG levels may be just a marker of its linkage disequilibrium with another functional site (e.g. APOC-III -482C>T allele within an APOC-III insulin response element, as discussed below) within this undoubtedly important region in terms of lipid metabolism.

Haplotype studies

Actually three haplotypes are described in the APOA-V gene region, designated APOA5*1, 2 and 3. Pennacchio *et al.* (2001) showed in the first study that the minor haplotype APOA5*2 defined by SNPs1, 2 and 3 was associated with plasma triglyceride levels in two independent cohorts (500 unrelated Caucausian men and another group of Caucasian men and women) with no concurrent effect of *SstI* polymorphism in APOC-III gene. In a follow-up study (Pennacchio *et al.* 2002), new haplotype APOA5*3 (Fig. 3) was found to differ from the common (APOA5*1) variant in a G/C substitution, leading to non-synonymous substitution of tryptophan for serine (S19W). The frequency of this allele was not different between Caucasians (0.06) and African Americans (0.07), but was substantially higher in Hispanics (0.15). The APOA5*3 haplotype was significantly more common among men and women with high plasma triglyceride concentrations and was shown to be systematically associated with increased triglyceridemia in men and women from three different ethnic groups (Caucasian, African-American, Hispanic), under three different dietary regimens and was independent of the effect of APOA5*2 haplotype.

Talmud *et al.* (2002) carried out an elegant study focusing on the whole APOA-I/C-III/A-IV/A-V cluster. They assessed the strength of linkage disequilibrium (LD) across this region of human chromosome 11 using nine SNPs (Fig. 3) in a set of 2808 men (NPHSII study), confirming strong LD between APOA-V and APOC-III. The homozygous carriers of APOA-V rare alleles in SNP1 and S19W were shown, in consent with previous findings, to have significantly elevated TG levels. In order to discern whether these effects are independent or reflect the strong LD with APOC-III (or vice versa), haplotype analysis was performed. Twenty-two haplotypes out of 512 theoretical combinations were present in more than 10 individuals from the cohort. Significant differences were found among TG levels and independent effect of APOA-V W19 and APOC-III -482T rare "TG raising" alleles was ascertained (they were found separately in the groups with highest TG, i.e. haplotypes 1, 2 and 3 (Fig. 3).

The above mentioned haplotype APOA5*3 corresponded to the haplotype 1, i.e. the one with the highest triglyceridemia. On the other hand, authors suggested that the effect of the APOA5*2 haplotype was probably due to its strong LD between APOA-V -1131C and the APOC-III -482T "TG raising" allele and does not represent a functional change by itself.

In summary, considerably large amount of data have accumulated over a relatively short period from the APOA-V discovery, establishing this novel gene as an important player in lipid (mostly triglyceride) metabolism. The identification of the gene itself was facilitated by employing the current methods of comparative genomics, integrating and processing the genomic information from different species with the available arsenal of bioinformatic tools. It is presumable that studies dissecting the exact function of APOA-V within the metabolic networks will shortly follow as our

knowledge in this respect is largely hypothetical. The methods of functional and comparative genomics, particularly in defined genetic models, will also prove themselves to be invaluable tools.

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