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Clinical Implications

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by Friedrich C. Luft

Can complex genetic diseases be solved? (and a PS on PXE)

The announcement that the human genome has in large part been sequenced and that a first draft will be made available to the interested public has aroused worldwide attention, especially in the media. Holtzman and Marteau observed that medicine has now been "shrouded in a genetic mantle" [1]. Promises have been implied, if not indeed made, by Bell, Collins, and others that understanding the genetic role in human diseases is just around the corner [1]. An impatient public expects new strategies for the prevention and treatment of disease. However, there are reasons for skepticism. The search for susceptibility-conferring genotypes for breast cancer, colon cancer, and prostate cancer have been successful, but the genotypes count for less than 3% of the cases. A large twin study involving >44,000 twin pairs from Scandinavia found that inherited genetic factors make only a minor contribution to most kinds of neoplasms [2]. However, in the area of cardiovascular disease, diabetes mellitus, and Alzheimer disease, the track record is not much better.

One explanation is that the disease risk conferred by alleles at one locus depends not only on alleles at other, independently segregating loci, which by themselves have little effect on risk, but also on environmental factors [3]. The problem of identifying susceptibility-conferring genotypes is compounded when different combinations of gene loci are implicated in a disease. The subject numbers necessary for such an analysis can be considerable. A novel approach might be to consider quantitative, rather than qualitative traits and their gene loci. Such quantitative trait loci (QTL) circumvent the discrete variable or "disease" dilemma. Examples might include arterial blood pressure or lipid concentrations. Both are important risk factors for cardiovascular disease, which when considered alone is too complex to permit genetic analyses. Furthermore, such quantitative traits are generally the result of gene families acting in concert. In this month's issue of the *Journal of Molecular Medicine,* Knoblauch et al. [4] propose such an analysis to elucidate the molecular genetics of lipid metabolism. In their analysis, they consider measured phenotypic variables, namely, chylomicrons, very-low-density lipoprotein cholesterol (VLDL), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TGL). They then rely on a defined schema involving the metabolism of these constituents previously determined by lipid biochemists. The enzymes involved in this process are known. Lipoprotein lipase, hepatic triglyceride lipase, and lecithin cholesterol acyl transferase are primarily involved. The important receptors are defined and include the LDL receptor, the remnant receptor, the scavenger receptor, and cubulin. They also consider the cholesterol ester transfer protein in their analysis. The genes for these constituents are obvious candidates to influence lipid metabolism. There are of course many others, but one has to start somewhere. Knoblauch et al. [4] suggested linking these constituents together in terms of a metabolic pathway analysis. The senior author, Jens Reich, developed a series of differential equations to define the reactions and interrelationships. The authors then tested their model by building in the known effects of lipoprotein lipase mutations. The model seems to work in that it predicts the simultaneous concentrations of the lipid constituents when gene product activity, e.g. lipoprotein lipase activity is altered. A simplified schema is provided in Fig. 1.

What is the rationale beyond this approach? Most gene products are proteins, most proteins as in this example are enzymes, and most enzymes operate as metabolic components. Therefore, genetic information that might have a bearing on how these enzymes behave and how they are regulated might be useful in predicting metabolism. Cornish-Bowden has suggested that such metabolic pathway analyses are a necessary component for understanding how metabolic systems behave. "To understand the whole, you have to look at the whole" [5]. When looking at the equation-filled schema, we can imagine how a single polymorphism in lipoprotein lipase may have only a minor effect on HDL, LDL, or TGL concentrations. However, when considered simultaneously with a polymorphism in the scavenger receptor, cubulin, or the cholesterol ester trans-

F.C. Luft (✉)
Franz-Volhard-Klinik,
Humboldt University of Berlin,
Wiltbergstrasse 50,
13125 Berlin-Buch, Germany
e-mail: luft@fvk-berlin.de

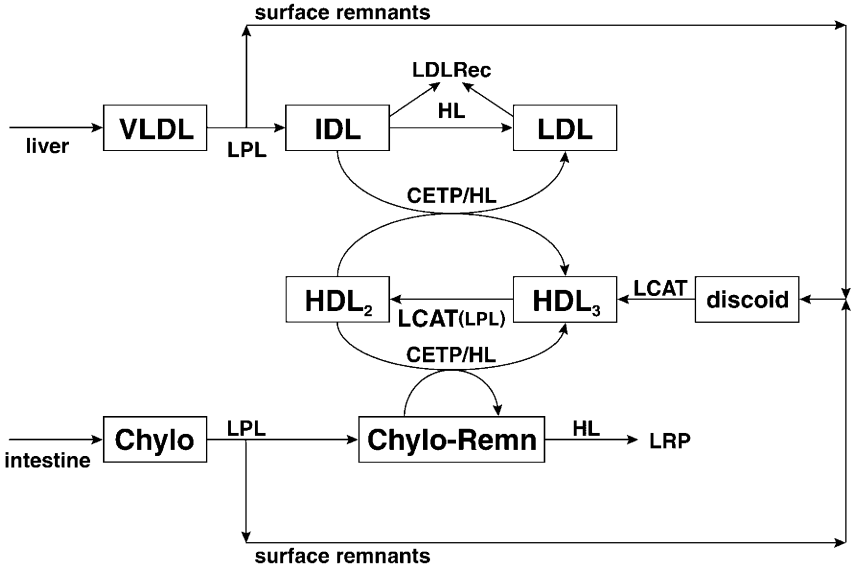

Fig. 1 Abbreviated metabolic model of lipid metabolism. *VLDL* Very low-density lipoprotein, *LDL* low-density lipoprotein, *IDL* intermediate-density lipoprotein, *HDL* high-density lipoprotein, *Chylo* chylomicrons, *LPL* lipoprotein lipase, *LRP* low-density lipoprotein receptor-related protein (the remnant receptor), *HL* hepatic triglyceride lipase, *CETP* cholesterol-ester transfer protein, *LCAT* lecithin-cholesterol-acyl transfer protein. The interconnections are defined by stoichiometric reactions (see [4])

fer protein, the cumulative effects of these polymorphisms may be much greater. This is a hypothesis the investigators have raised but have not yet tested. Large numbers of families and perhaps large numbers of single nucleotide polymorphisms (SNP) in these various genes will be required to test the model with actual genetic information.

Knoblauch et al. [4] have in essence reverted to the candidate gene approach, albeit on a larger scale. By using sibpairs and their parents from family units or from dizygotic twins, they can rely on family based association analysis, haplotype analysis, and linkage analyses. Risch [6] has indicated that by parameterizing the effect of the locus in terms of genotype relative risk, allele frequency for high relative risks, and intermediate allele frequencies, it is realistic to expect linkage analysis to provide statistical evidence for the importance of a gene locus (QTL). The investigators will necessarily rely on SNPs in their genotyping efforts. Roses has indicated that 300,000 to 500,000 SNPs will be made available to investigators by an industry sponsored SNP consortium [7]. Risch and Merikangas [8] suggested earlier that by studying coding or promoter SNP variants with potential functional significance, the greatest possible efficiency would be achieved. However, more recently Collins [9] suggested that non-coding or evenly spaced SNPs with high density could be used to track disease loci through linkage dysequilibrium. In any event, Knoblauch et al. [4] will have to find SNPs with allele frequencies high enough in the general population to be informative in their subjects.

The promise of complex genetics is strictly that – a promise and no more. Thus far, few if any novel genes have been cloned for any complex genetic diseases. The molecular approaches used so successfully to clone genes causing Mendelian syndromes have thus far failed to find the numerous genes responsible for the common causes of death. Data from the human genome project may change all that and surely the SNP consortium should prove invaluable. Knoblauch et al. [4] dare to suggest that we may already know the important genes, and by examining them in context, we may derive their individual importance. By investigating multiple genes at one time, a novel multiple candidate gene approach in the form of a metabolic control pathway analysis may be helpful. The investigators will have to test their model in several data sets and perhaps several different populations to prove its usefulness. For example, if they study 200 families with 4–6 members each and rely on more or less evenly spaced SNPs, perhaps six per gene, each job will require 40,000 or more genotypes. Let us hope that the investigators are endowed with generous funding prospects for their ambitious project.

PS on Pseudoxanthoma elasticum revealed

As a monthly editorialist, I have the interesting chore of familiarizing myself with topics outside my expertise in a very short timeframe. The task is frequently daunting and commonly carries the risk that only superficial and incomplete knowledge can be accrued. Thus, errors and omissions can creep in. An example of this is the recent editoral [10] I wrote on pseudoxanthoma elasticum (PXE). I discussed the paper by Cai et al. [11], who showed that a mutation in a gene encoding an ABC transporter is responsible for PXE. I indicated that Ringpheil et al. [12] recently reported similar findings. Readers have drawn my attention to the fact that I failed to discuss two important papers on the subject that appeared at about the same time. Le Saux and colleagues [13] showed that ABC-C6 is responsible for PXE. They identified six mutations in 10 of 17 PXE patients. Of the homozygous and compound-heterozygous mutations they identified, all were associated with autosomal-recessive PXE. However, they also observed a single heterozygous PXE patient, who may represent an autosomal-dominant form of PXE. Bergen et al. [14] also identified ABC-C6 as the gene respon-

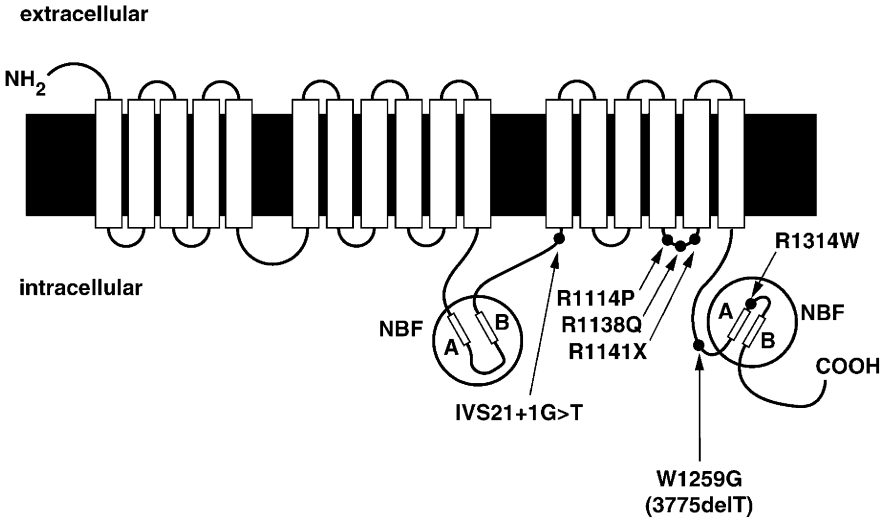

Fig. 2 Topology of the predicted APC-C6 protein [4, 5]). The molecule has 17 membrane spanning domains. *Arrows* indicate mutations observed by Le Saux et al. [4]. Circled structures are the ATP binding sites

sible for PXE. They studied both families with autosomal-recessive and autosomal-dominant PXE. Both deletions and nonsense mutations were associated with autosomal-recessive, autosomal-dominant, and sporadic PXE. They showed evidence for a patient exhibiting haplo-insufficiency at the ABC-C6 locus and concluded that de novo germline mutations probably account for most sporadic cases of PXE. A schematic diagram of the multi-drug resistance protein 6 encoded by the ABC-C6 gene is shown in Fig. 2 (adapted from [13]). Note the 17 transmembrane domains and the two ATP binding sites represented by the circles. Exactly which molecules are transported by this protein and how their failed transport results in PXE is not yet clear, but presumably the transported proteins are involved in extracellular matrix deposition or connective tissue turnover.

Finally, all the PXE papers contain large pedigrees, namely families. We should not lose sight of the fact that PXE patients and their families make the key contributions to this research by their willingness to cooperate in genetic studies. Bateson pointed out almost 100 years ago the possibilities by such unique contributions when he admonished his disciples to "treasure your exceptions" [15]. We tend to think of monogenic diseases as unusual, rare, or exotic. PXE is not particularly common. Nevertheless, for the PXE patients and their families, the incidence is 100%. There are two patient support groups to which patients can turn for support: PXE International (http://www.pxe.org). and NAPE (National Association for PXE; http://www.napxe.org).

Respectfully,
Friedrich C. Luft

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