Dyslipidemia (elevated low-density lipoprotein or elevated triglycerides and low high-density lipoprotein) is an established risk factor for the development of atherosclerosis.\(^1\) Heritability estimates for plasma lipids range from 25% to 80% and family studies suggest that within the family, genetic effects are more important than environmental.\(^2\) In a minority of cases, dyslipidemia is the result of mutation with high penetrance in a single gene, for example, familial hypercholesterolemia;\(^3\) however, in the majority of cases, dyslipidemia is a complex trait with multiple genetic and environmental factors contributing to the condition.\(^4\)

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Over the last few years, a number of genomewide association studies (GWAS) investigating the role of common genetic variants in determining plasma lipid levels have been performed. These studies culminated in the study of Teslovich et al\(^8\) who identified in a study including >100,000 individuals, 95 loci associated with plasma lipids with 59 showing a genomewide significant association with lipids for the first time. However, because these loci account for only 25% to 30% of the genetic variance in lipid levels, there has been a great deal of speculation as to what accounts for the missing heritability in GWAS.\(^5\)–\(^7\)

One factor that has been extensively discussed is the role of multiple rare variants, that is, variants with a frequency ranging from <5% to private mutations associated with single families. It has been estimated\(^8\) that only approximately 27% of new missense mutations are effectively neutral with 20% being deleterious. The remaining 53% are mildly deleterious and may have a similar effect as common variants and cumulatively play a similar role in the development of complex traits. This postulated role for rare variants in the genetics of complex traits is the basis of the common disease common variant hypothesis, which underlies the GWAS approach.\(^9\)

The chief approach to identifying the role of rare variants is the resequencing of candidate genes in probands at the extremes of lipid distribution, usually above the 95th and below the 5th percentile for age and sex. Among genes for which the importance of rare variants has been demonstrated are \(ABCA1\) and \(LIPG\) for high-density lipoprotein levels, \(PCSK9\) and low-density lipoprotein levels, and \(ANGPTL3\), \(ANGPTL4\), \(APOA5\), \(APOB\), \(GCKR\), and \(LPL\) and triglyceride levels.\(^3\) These studies provide evidence that rare variants do indeed play a role in the genetic basis of dyslipidemia.

Over the past few years, Hegele and colleagues\(^10\) have systematically investigated the genetic basis of severe hypertriglyceridemia. First, they showed that a small number of common variants could account for approximately one fourth of the variation. Next they\(^11\) directly tested the hypothesis that rare variants could account for some of the missing heritability encountered in GWAS. They resequenced 4 genes, \(APOA5\), \(GCKR\), \(LPL\), and \(APOB\), which they had previously identified in GWAS as being associated with hypertriglyceridemia, from 438 individuals with hypertriglyceridemia and 327 control subjects. Proband who had hypertriglyceridemia had almost double the frequency of rare variants compared with the healthy control subjects, 28.1% compared with 15.3%. We have been able to confirm in our laboratory the role of rare variants in the \(LPL\) and \(APOA5\) genes in the development of hypertriglyceridemia.\(^12\)–\(^13\)

In this issue of Circulation Cardiovascular Genetics, Johansen et al\(^14\) report on the extension of their resequencing studies. They have resequenced candidate genes for hypertriglyceridemia, which were not identified in GWAS, thus testing the hypothesis that there may be disease-associated genes that do not harbor common variants and so are not identified in GWAS. Three genes, \(APOC2\), \(GPIHBP1\), and \(LMF1\), were selected because patients who are homozygous for loss of function mutations have early-onset severe hypertriglyceridemia. Two transcription factor genes, \(CREB3L3\) and \(ZHX3\), which have been shown in murine studies to be involved in triglyceride metabolism, were also selected as candidates. The DNA sequence of all exons and exon-intron boundaries of 413 adult patients with hypertriglyceridemia and 324 population-based control subjects was determined. They identified 41 heterozygous rare variants (minor allele frequency [MAF] <1%), 3 heterozygous uncommon variants (MAF 1%–5%), and 3 common variants (MAF >5%). Only 3 of the rare variants were found in the dbSNP or 1000 Genomes Project databases, the rest being reported for the first time extending our knowledge on the extent of variation among rare variants of hypertriglyceridemia.

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The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Endokrinologie und Stoffwechsel, Medizinische Klinik III, Zentrum für Innere Medizin (D.E.), and Klinik und Poliklinik für Allgemeine und Interventionelle Kardiologie, Universitätsklinikum Hamburg-Eppendorf (P.D.), Martinistrasse 52, 20246 Hamburg, Germany. Correspondence to David Evans, BSc, PhD, Endokrinologie und Stoffwechsel, Medizinische Klinik III, Zentrum für Innere Medizin, Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany, Tel: 049 040 741056978, Fax: 049 040 741059036. E-mail evans@uke.uni-hamburg.de

(Circ Cardiovasc Genet. 2012;5:5-6.)

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Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org

DOI: 10.1161/CIRCGENETICS.111.962548
in these genes. The frequency of rare variants in patients with hypertriglyceridemia, 11.4% (47 of 413), was more than double that observed in control subjects, 4.9% (16 of 324). The rare variants were more frequent in genes established in hypertriglyceridemia pathophysiology.

After this proof of principle that rare variants play a significant role, we can anticipate that in the near future the application of next-generation sequencing will result in a flood of reports concerning the role of rare variants in dyslipidemia. However, a number of potential problems will need to be addressed. A consensus will be required on the design for the interpretation of such data, a point taken into account in the article under discussion in which 3 statistical models are compared.

A major difficulty will be determining the functional effect of rare variants on plasma lipids, that is, to discriminate between those variants that are genuinely associated with lipid levels and those that are incidental. To date, most studies have relied on in silico analysis using such programs as PolyPhen. Although rapid, inexpensive, and convenient, such an approach has a number of drawbacks. They are usually based on the structural stability of the protein and do not cover such factors important in discussing function such as catalytic sites and ligand binding. For example, in a resequencing study of the LIPG gene in patients with extreme high-density lipoprotein levels, a variant, A116T, was predicted in PolyPhen to be benign, but in vitro assays showed it to have reduced activity. However, the development and application of suitable in vitro assays is time-consuming and expensive and their interpretation and relevance to the in vivo situation can be problematic.

Furthermore, in cases in which genes have initially only been identified through GWAS, their function is frequently also unclear. Confirmation of the effect of a specific variant should be determined by segregation studies within families.

In conclusion, Johansen et al have demonstrated that rare variants in candidate genes identified through pathobiology and not through GWAS also contribute to the genetics of a complex trait, namely plasma triglyceride levels. This accounts in part for the missing heritability associated with GWAS.

Disclosures

None.

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Not Just Genomewide Association Studies: Rare Variants in Genes Not Identified Through Genomewide Association Studies Also Contribute to Hypertriglyceridemia

David Evans and Patrick Diemert

_Circ Cardiovasc Genet._ 2012;5:5-6
doi: 10.1161/CIRCGENETICS.111.962548

_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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