Editorial

From Association to Function in the Post-GWAS Era

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During the past decade, the genome-wide association studies (GWAS) have revolutionized the world of genetics by their enormous power to comprehensively detect genetic association between gene variants (e.g., SNPs) and complex phenotypes (e.g., quantitative traits or diseases). One of the most successful examples was the identification of gene loci associated with blood lipid levels in humans^{1, 2)}. The original findings obtained by a large global consortium were quite reproducible and consistent with results of other studies³⁾, implying blood lipids as highly reliable quantitative traits.

GWAS could provide a comprehensive collection of genomic variants of significant association, together with their individual chromosomal locations at the genome-wide significance level. However, GWAS themselves have only limited power to pinpoint the causative gene(s) or gene mutation(s) that underlie the complex trait (or disease) of interest. In most cases, such genomic variants with the strongest association signal (i.e., lead SNPs) are located outside the coding region of the gene; thus, providing little evidence in terms of their functional significance, whereas some of these variants are believed to affect the expression of the nearby (*cis*) or distant (*trans*) gene(s) either via direct or indirect mechanism. In general, subsequently to GWAS, several complimentary approaches have been attempted to explore and establish the causative genes, such as physical fine-mapping approach to narrow down the candidate genomic region, expression quan-

titative trait loci (eQTL) analysis to explore association between gene variants and mRNA expression levels, bioinformatics analysis in consideration with metabolic pathways and their interaction according to available databases, and functional validation approach to establish the functional significance with *in vitro* and/or *in vivo* biological experiments (**Fig. 1**). In spite of such attempts, demonstration of the causative gene/mutation has been quite difficult and remains very challenging. Although GWAS had revealed that *sortilin* (*SORT1*) was the novel target gene with the third strongest signal for association with blood low-density lipoprotein (LDL) cholesterol levels¹⁾, functional validation approaches with overexpression or knock-down experiments have produced conflicting and inconsistent results⁴⁾. Thus, the experimental strategy after GWAS has not been so straightforward, and application of an additional approach will be eagerly encouraged.

On page 455 of this issue of *Journal of Atherosclerosis and Thrombosis*, Akiyama *et al.* reports a unique complimentary approach to search for functional genes responsible for positive association signals revealed by GWAS⁵⁾. First, the authors have selected rat genes orthologous to human candidate genes revealed by the lipid GWAS mentioned above^{1, 2)} and subsequently have examined *in vivo* alterations in the mRNA expression levels of such candidate genes secondary to the intervention by feeding rats with high-fat and high-cholesterol diets (HFD) or with administration of a cholesterol-lowering agent, statin. With intervention by HFD, seven known, established candidate genes have been selected, giving authenticity to the rationality of the study design, and three novel significant and 23 additional suggestive genes have also been identified. Among them, *Stac3* appears to be an interesting candidate target of lipid metabolism because it was the only novel gene that was regulated in response to both HFD and statin treatment, with the stringent and nominal levels of statistical signifi-

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Fig. 1. Schematic overview of the experimental strategy from association to functional validation.

Gene loci identified by genome-wide association scans undergo further scrutiny by physical fine-mapping, expression quantitative trait loci (eQTL) analyses, and references to the available databases. Demonstration of the functional significance of the causative variants requires *in vitro* and/or *in vivo* functional validation approaches.

cance, respectively. Although the expression of *Stac3* is known to be most abundant in skeletal muscles and nutritionally regulated⁶, there has been no direct evidence published to suggest possible involvement of *Stac3* in lipid metabolism. On the basis of the data presented by Akiyama *et al.* in the current issue, further investigation is therefore warranted to examine such involvement.

Another impressive finding came from a comparison between the current results reported by Akiyama *et al.* and the previously published results derived from *cis*-eQTL analyses and/or from RNAi-based functional profiling^{1,7}. Despite partial overlaps observed between candidate genes that were detected by each approach, *Lipg* was the only gene commonly detectable by all three independent approaches. This finding highlights the importance of application of different experimental approaches to avoid missing crucial genes owing to reliance on a single approach alone as well as to enrich the collection of target genes of GWAS association more systematically.

Inconsistent with the brilliant results provided by GWAS, their application to clinical diagnosis or therapeutic research has been very limited. In addition, they have rarely provided mechanistic insights. In this regard, the unique experimental approach described by Akiyama *et al.* would be of potential use and might be regarded as “functional fine-mapping” because it combines the gene mapping approach with the *in vivo* functional evaluation approach performed in an animal model. Development of such diverse effective approaches will be highly encouraged to fill the great gap between association and function still left in the post-GWAS era.

Conflicts of Interest

None.

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