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CPT1A: the future of heart disease detection and personalized medicine?

M. Ryan Irvin[1], **Stella Aslibekyan**[1], **Bertha Hidalgo**[2], and **Donna Arnett**[1]

[1]Department of Epidemiology, University of Alabama at Birmingham

[2]Department of Biostatistics, Section on Statistical Genetics, University of Alabama at Birmingham

1. Introduction: CPT1A function in fatty acid metabolism and lipid transport

Fatty acid oxidation in the mitochondria is essential for energy homeostasis in the absence of a consistent energy supply, such as in prolonged fasting or exercise. Long-chain fatty acids, which make up the major dietary fraction of fatty acids, cannot enter the mitochondria by simple diffusion.[1] The carnitine palmitoyltransferase (CPT) system is a mitochondrial enzymatic complex which acts to transport long chain fatty acids across the outer and inner mitochondrial membrane.[2] The system is made up of two distinct proteins corresponding to the outer and inner membrane forms: carnitine palmitoyltransferase 1 (CPT1) and 2 (CPT2), respectively. CPT2 is ubiquitously expressed, while CPT1 exists in three tissue-specific forms, namely CPT1A in the liver, CPT1B in the muscle, and CPT1C in the brain.[3] The function of CPT1 is best understood in the liver, where CPT1A controls the fatty acid flux through the esterification and oxidative pathways with its sensitivity to malonyl-CoA, a potent CPT1 inhibitor which acts as an important intermediate in fatty acid biosynthesis. During fasting, malonyl-CoA inhibition of CPT1A is halted so that long chain fatty acid oxidation and subsequently ketogenesis become enhanced. In the postprandial state, the concentration of malonyl-CoA increases, CPT1A inhibition ensues, and long chain fatty acids are directed toward esterification. The regulation of CPT1 in extra-hepatic tissues is less clear, however, it is known to be an important constituent of energy homeostasis and maintenance in heart and skeletal muscle as well.[1]

2. Genetic studies of CPT1A and lipid metabolism

CPT1A deficiency is a very rare autosomal recessive disorder of mitochondrial fatty acid oxidation.[1] As explained above, mitochondrial fatty acid oxidation provides an alternative source of energy when carbohydrate stores are depleted by increased energy demand. Therefore, clinical symptoms of CPT1A deficiency are caused by the reduced ability to turn to fat into fuel. Patients can present with acute symptoms including hepatomegaly and hypoketotic hypoglycaemia, which can cause seizures and coma.[4] Prevention of acute symptoms is facilitated by a low-fat, high-carbohydrate diet, enriched for medium-chain fatty acids.[3] Because prevention of hypoglycaemia reduces the risk of neurologic damage, early diagnosis is essential. CPT1A deficiency has been included in newborn screening programs world-wide.[5] The gene for CPT1A is located on chromosome 11q13.1–q13.5 and, to date, fewer than 30 mutations have been described.[6] Cases of CPT1A deficiency have

been identified by homozygous mutations, but carriers of functional mutations may be at risk for lipid disorders. Several candidate gene studies have evaluated the association between *CPT1A* variants and metabolic phenotypes. In a study conducted by Rajakumar *et al.* (2009) the P479L (rs80356779) variant was very common in a Greenland Inuit population, and associated with higher levels of HDL-cholesterol (HDL-C) and ApoA1.[7] The authors concluded the variant may be protective against atherosclerosis. The same variant was also associated with obesity-related traits and fasting HDL-C in the Center of Alaska Native Health Research (CANHR) study.[8] Interestingly, the association of P479L with HDL-C was still significant after correcting for body mass index (BMI), percentage body fat (PBF), and waist circumference (WC). Their findings were consistent with those of Rajakumar *et al.* supporting the hypothesis that the L479 allele confers a selective cardioprotective advantage through increased HDL-C.[7, 8] Other studies have published associations with different functional variants. For instance, a study in a French Canadian population suggested the A275T (rs17610395) variant modulates indices of obesity by fat intake and in a separate study haplotypes of *CPT1A* were associated with left ventricular mass in essential hypertension .[9, 10] A study of 761 Alaskan Natives evaluated the association of 149 *CPT1A* SNPs with activity of delta-5 and delta-6 desaturases, which are rate limiting enzymes in the metabolism of ω3 and ω-6 fatty acids. [11] After correction for multiple testing, three independent SNPs (rs11228368, rs3019594, rs613084) were strongly associated with either red blood cell or plasma enzyme activity ($6.6*10^{-39} \leq P \leq 6.7*10^{-5}$) in addition to HDL-C level.[11] Corroborating these findings, rs11228368 and rs613084 were associated with CPT1A expression in an external Mexican American population. However, not all studies have reported a positive association of *CPT1A* variants. A study in a Japanese population found no association between *CPT1A* SNPs and obesity or fasting lipid phenotypes in individuals with T2D.[12] To the best of our knowledge, no GWAS, including a large meta-analysis (N>100,000 participants) that confirmed the association of 95 loci with fasting lipid traits, have highlighted the gene.[13] Ever improving technologies continue to make deeper interrogations of genomic variations in *CPT1A* possible.

2. Expansion to epigenomic studies of CPT1A and lipid metabolism

To date, cardiovascular genetic research has almost entirely focused on heritable allelic variation, namely, genetic polymorphisms in the nucleotide sequence of DNA in populations. Over the last 20 years, intense research in the field has resulted in considerable progress. However, one of the most important lessons, especially apparent in the aftermath of the completion of the Human Genome Project, is that the genetic background of cardiovascular disease (CVD) is much more complex than originally anticipated. Known loci do not fully explain the observed variance expected to be attributed to genetic background.[14] Several possible explanations for this deemed “missing heritability” problem have been put forth, including the inability of current genetic assays to tag the causal variants, unaccounted environmental influences, and complex epigenetic factors.[14] Moreover, due to an essential role in the regulation of DNA transcription, epigenetic factors may prove crucial to filling in current knowledge gaps. Facilitating discovery, genomic technology now enables a genome-wide approach to interrogating variation in DNA methylation, an epigenetic process involving methylation of cytosine, usually at cytosine-to-

guanine (CpG) dinucleotides in the promoter region or within genes.[15] In contrast to DNA sequence variation, DNA methylation is sensitive to both inherited and environmental inputs. Therefore, processes involving DNA methylation have the potential to pass phenotypic variation through generations and/or allow a response to the environment through changes in gene expression.

Genome-wide studies of DNA methylation and CVD related traits have been largely unexplored. We recently conducted an epigenome wide association study of fasting blood lipids, diabetes-related traits, and adiponectin in 888 participants from the Genetics of Lipid Lowering Drugs and Diet (GOLDN) study.[16] DNA was isolated from CD4+ T cells harvested from stored buffy coats and methylation was quantified using the Illumina Infinium Human Methylation 450 array.[17] CD4+ T cells were selected for three reasons. First, DNA methylation patterns are often tissue specific. For instance, studies of whole blood reflect methylation variations within each blood cell type that may act to confound epigenomic association results. [18] Second, many key genes involved in lipid metabolism are expressed in lymphocytes and other immune cells (e.g. *PPARs*). [19, 20] Third, blood collection is the most viable tissue collection method among healthy individuals. Our results showed that after adjustment for gender, age, T-cell purity, and family relationship, two markers in *CPT1A* (intron 1) reached striking levels of statistical significance with multiple traits including fasting triglycerides (P=5.3*10^{-14} and 1.2*10^{-9}), very low density lipoprotein cholesterol (P=6.8*10^{-11} and 7.8*10^{-7}), adiponectin (P=5.5*10^{-9}), insulin (P=1.5*10^{-4}), and HOMA-IR (P=1.0*10^{-6} and P=2.8*10^{-5}) in a random subset of the GOLDN study (N=593). The association of the top CpG site replicated in the remaining 295 participants (P<3.0*10^{-5}). Further adjustment for other potential confounders did not materially change the results (BMI, alcohol use, smoking status). These findings were presented as late breaking news as part of the American Heart Association's Emerging Science Series in the summer of 2013 and extracted from an analysis published in the journal *Diabetes*.[21, 22]

4. The promise of *CPT1A* variation as a clinically useful biomarker

This study provides robust and internally replicated evidence of association between variable methylation in intron 1 of *CPT1A* and multiple CVD related traits in a large, healthy population of Caucasian adults. The findings implicate a potentially pleiotropic role for CpG methylation in *CPT1A* in relation to CVD related traits beyond DNA sequence variants. Therefore, *CPT1A* expression or action may prove useful as a biomarker for CVD risk or even in drug development in the future. However, we are still several steps upstream of therapeutic implications. First, the results have not yet been replicated in an external study population and, we cannot rule out systematic DNA methylation measurement errors or other laboratory, analytic, or quality control errors are responsible for the observed associations. Currently, we are actively seeking external replication of our findings. Additionally, since GOLDN is representative of healthy Caucasians, replication may expand generalizability of our results to other ethnic and clinical populations. Finally, our study design was cross-sectional and we were unable to determine if the observed associations are due to methylation effects on lipids or vice-versa, necessitating longitudinal follow-up. In sum, much work is necessary in relevant observational and/or clinical populations to

validate these findings in addition to their functional impact on *CPT1A* expression across relevant tissues before *CPT1A* can be evaluated as a clinically useful biomarker. Still given the strong the associations observed and biologic plausibility, this finding has exciting potential and warrants concerted efforts to expand association studies and pursue functional studies. In conclusion, though in early stages of discovery, variation in *CPT1A* methylation represents a promising discovery in genomics that could prove useful in CVD treatment and prevention.

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