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Chapter 3

Effects of Genetic Variations on Lipoprotein Metabolism in Cardiovascular Diseases

Chesa G. Chauke, Zandisiwe E. Magwebu and Jürgen V. Seier

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http://dx.doi.org/10.5772/67138

Abstract

Advances in molecular techniques have shown that genetic factors predispose individuals to cardiovascular diseases (CVD). These techniques have made it possible to identify disease-causing genes, prediction to disease susceptibility and responsiveness to drug interventions. For the purpose of this review, therapeutic intervention (niacin) was conducted in a nonhuman primate model to assess the impact of six coincident single nucleotide polymorphisms (cSNP) identified in prioritised reverse cholesterol transport (RCT) and high-density lipoprotein (HDL) metabolism genes. Gene expression findings confirmed that these genetic variants may have a direct impact on the RCT pathway and drug intervention (niacin) response.

Keywords: cardiovascular disease (CDV), candidate genes, HDL-C metabolism, sequence variants, reverse cholesterol transport (RCT)

1. Introduction

Cardiac and vascular complications are complex multifactorial pathologies and difficult to prevent since are associated with both genetic and environmental factors [1]. Research on cardiovascular diseases (CVDs) is constantly evolving and the current focus is directed towards lipid metabolism, molecular and cellular mechanisms, as well as preventive strategies. Most research in the field of lipid metabolism is motivated by an interest to understand normal lipid transport and preventative measures for atherosclerosis abnormalities [2]. Specific genes and apolipoproteins that are involved in lipid metabolism and lipoprotein synthesis have been isolated, sequenced and mapped in the human genome [3]; however, their role in the lipid

metabolism and lipid transport can only be inferred by physiological and genetic studies. To determine their overall function, further exploration of genetic alterations must be investigated.

Since the molecular regulation of lipid metabolism and reverse cholesterol transport (RCT) pathway is complex, numerous studies in humans, animals and *in vitro* have been focusing on the protective action of high-density lipoprotein cholesterol (HDL-C), RCT and cholesterol efflux, which can also be augmented for potential therapeutic strategies of CVDs [4, 5].

2. Overview of RCT and cholesterol efflux

The RCT pathway represents an important process involving the transfer of excess cholesterol by HDL particles to the liver for excretion. The ability of HDL to remove cholesterol from cells such as macrophages is linked to the anti-inflammatory and immunosuppressive functions of this lipoprotein [6]. However, the functionality of HDL is impaired in humans with chronic inflammatory diseases and this causes a reduction in the anti-inflammatory and cholesterol transport properties. Studies have shown that apolipoprotein A-I (ApoA-1), lecithin-cholesterol acyltransferase (LCAT), ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor class B type 1 (SR-B1) serve as important cofactors for a number of RCT pathway constituents [7]. The initial step of the pathway involves ApoA-1 being produced by the liver and released into the plasma where it is involved in all stages, including the formation of nascent HDL particles, HDL remodelling by LCAT and delivery of HDL cholesterol directly to the liver via SR-BI or indirectly via CETP-mediated transfer to apoB-containing lipoproteins [8]. Through this process, cholesterol efflux is promoted from the macrophages via ABCA1 and also by the ABCG1 transporter using the action of LCAT (**Figure 1**).

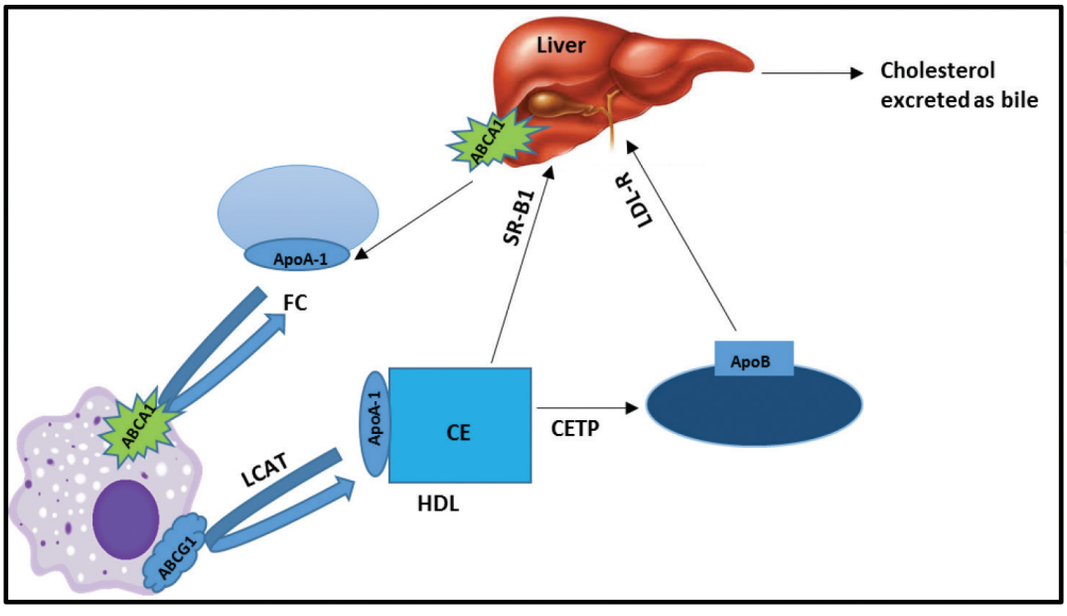

Figure 1. Reverse cholesterol transport (RCT) pathway. Major components of RCT include apolipoprotein A-I (apoA-I), high-density lipoprotein (HDL), lecithin: cholesterol acyltransferase (LCAT), ATP-binding cassette transporter (ABCA1/ABCG1) and cholesterol ester transfer protein (CETP). Free cholesterols (FC) in the HDL are delivered to the liver for excretion through scavenger receptor B1 (SR-B1). Alternatively, the cholesteryl esters (CE) could also be delivered to the liver through the low-density-lipoprotein receptor (LDLR). Figure modified from Rader et al. [4].

http://dx.doi.org/10.5772/67138

3. Vervet monkey as an animal model for CVDs

As with most areas of human biology, studies of human CVDs have been enriched and complemented by investigations of animal models. Among the nonhuman primates (NHP), the vervet monkey (*Chlorocebus aethiops*) has been validated to be an excellent research model for the study of CVDs [9]. For the purpose of this investigation, we used this NHP model to determine the protective action of HDL, its role in the reverse cholesterol transport (RCT) pathway and the expression profile of genes regulating HDL metabolism.

This study was conducted in compliance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (A5726-01) and approved by the Ethics Committee of the South African Medical Research Council (*SA*MRC) (REF 11/07). The selected subjects (25) were healthy adult female monkeys with normal plasma HDL. All individuals were kept under identical housing conditions according to the South African National Standard for the Care and Use of Animals for Scientific Purposes (The SANS 10386:2008).

3.1. Laboratory analysis

3.1.1. Candidate genes and sequence variants selection

The genetic variations were evaluated in 10 genes implicated in lipid metabolism (CETP, ABCA1, CYP7A1, apoA-1, apoB, apoE, SR-B1, LCAT, apoCI and apoCII). Twenty-two coincident single nucleotide polymorphisms (cSNPs) were selected for genotyping. These cSNPs were prioritised based on their function and location within their respective candidate gene and their association with CVD.

3.1.2. Gene expression

Blood (2 ml) was collected in EDTA-containing tubes from 25 animals using a femoral venepuncture after ketamine anaesthesia at 10 mg/kg bodyweight. DNA was extracted from whole blood using the Nucleospin Genomic Blood DNA Purification Kit (MACHEREY-NAGEL, Germany) and PAXgene Blood RNA Kit (PreAnalytiX, Qiagen) was used for RNA extraction. The extracted DNA was used for Sanger sequencing while RNA was for gene expression experiments. Turbo DNase treatment (Ambion, USA) was used for RNA purification before cDNA conversion (high-capacity cDNA kit, Applied Biosystems, USA). The effects of niacin treatment on the expression of the 10 prioritised genes were determined using quantitative real-time PCR (qRT-PCR). The gene expression data were normalised to the average of phosphoglycerate kinase 2 (PGK2: QT00219023) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH: QT01192646), which were used as housekeeping genes.

Since the levels of high-density lipoprotein-cholesterol (HDL-C) are a significant determinant of a cholesterol efflux capacity, a correlation analysis was conducted to determine the relationship between the levels of HDL-C and mRNA expression of the 10 selected RCT candidate genes.

4. Effects of mutations, drug intervention and gene expression on RCT

A major area in HDL-based therapeutics is focusing on the development of pharmacological approaches to improve the activity of the RCT pathway. One of these strategies involves the combination of genetic variations and individual responsiveness to drugs [10]. Subsequently, genetic variations in gene encoding transporters contribute to individual differences in drug absorption, elimination and cellular uptake, thereby affecting drug response and toxicity [11].

Among several types of genetic variations, single-nucleotide polymorphisms (SNPs) are the most abundant throughout the genome [12]. SNPs have lately received much attention as they serve as markers of individual risk for adverse drug reactions or susceptibility to complex diseases [13]. Small-scale studies have focused on the effects of polymorphisms on physiological or biochemical factors and have provided useful information on possible mechanistic links between variation at the gene level and risk factors for CVDs [14]. For the purpose of this review, 10 'candidate' genes known to be involved in RCT and HDL metabolism were screened in the vervet model and only six cSNPs (I405V, I883M, A233S, cL96R, -62A>C and A350A) were identified in CETP, ABCA1, CYP7A1, apoCII and SR-B1, respectively (**Table 1**).

Gene	cSNP	Accession number	Chr	Exon	Nucleotide change	Amino acid change
CETP	I405V	rs5882	16	14	A/G	I/V
ABCA1	Ile883Met	rs4149313	9	18	A/G	I/M
CYP7A1	Asn233Ser	rs8192874	8	3	A/G	N/S
APOC-II	Leu96Arg	rs5167	19	3	T/G	L/R
	-62A>C	rs2288911		Promoter		
SR-B1	A350A	rs5888	12	8	C/T	A/A

Table 1. cSNPs identified in vervet monkeys.

For effective changes in lipid metabolism, niacin, as the most potent available lipid-regulating drug [7] was used as a tool to increase HDL levels (**Figure 2**). A strong inverse correlation was observed with CETP, SR-B1 and CYP7A1 concentrations (r = -0.14, -0.27, -0.30; $p < 0.001$). Concurrently, gene expression profile showed that all three genes were down-regulated when correlated with the three cSNPs (I405V, A350A and A233S) (**Figure 3**). Since I405V is known to lower plasma CETP concentration and elevate HDL-C concentration [5, 9], the presence of this variant confirmed the same effect in the vervet model. With a similar expression profile observed in SR-B1 and CYP7A1, the presence of A350A may suggest a possible influence on RCT and HDL-C synthesis and a plausible involvement of A233S in drug metabolism [6]. The remaining cSNPs (I883M, cL96R and -62A>C), however, did not influence the expression of their respective genes (ABCA1 and APOCII) despite being known to alter plasma lipid levels and influence cholesterol efflux [15, 16]. Therefore, these findings suggest that some of these identified sequence variants have significant impact on gene expression which can be correlated with biochemistry levels (HDL-C, LDL-C and triglycerides) following drug intervention.

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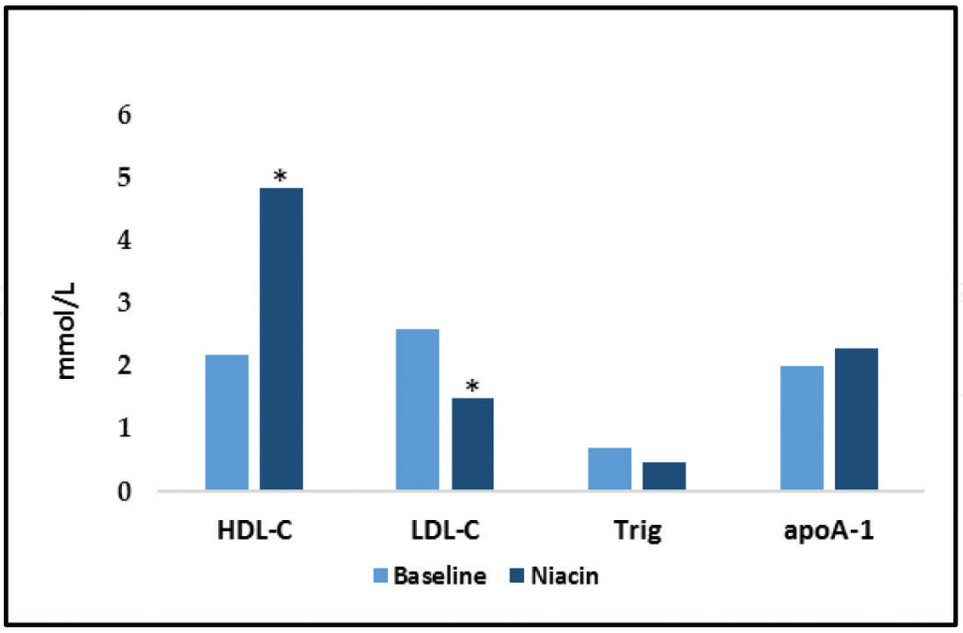

Figure 2. The effect of niacin treatment on HDL-C, LDL-C, triglycerides (Trig) and apoA-1. *Significant level ($p < 0.05$).

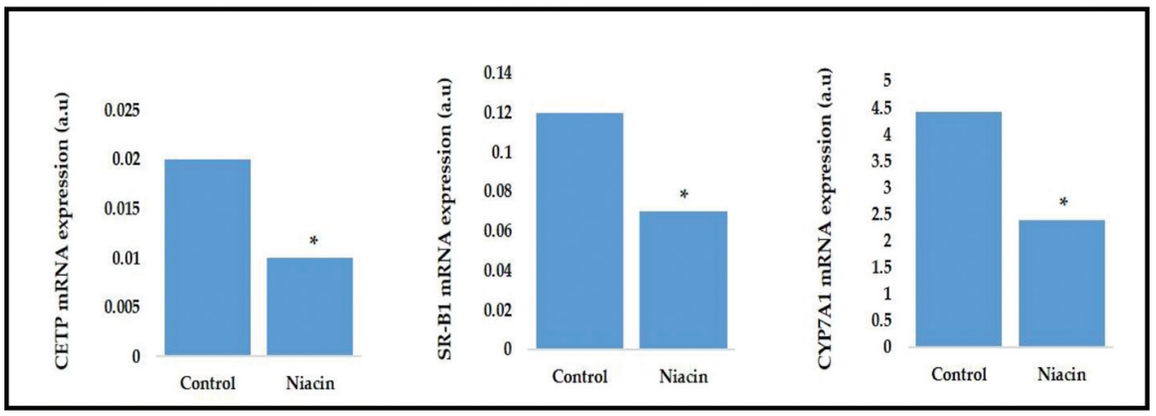

Figure 3. The effect of niacin treatment on CETP, SR-B1 and CYP7A1 mRNA expression. The data were expressed as mean ± SD and mRNA expression in a.u. (arbitrary units). *Significant level ($p < 0.05$).

5. Conclusion

It is a fact that characterisation of polymorphisms in lipid metabolism is challenging, however it remains essential for the optimal regulation and functioning of the RCT pathway. This review demonstrates that the genetic determinants of lipid transport and metabolism may provide additional significant benefit in pharmacological therapy for CVDs. Genetic approaches have shown that sequence variants can be correlated with biochemistry levels such as HDL-C, LDL-C and triglycerides following drug intervention. Although cholesterol lowering alone may explain the anti-atherosclerotic effect of niacin on HDL-C, in this review, gene expression data has shed some light in supporting the hypothesis that genetic variants may influence the expression of genes involved in RCT, which may also play a role in the anti-atherosclerotic effect of niacin.

It is also noteworthy that this is the first report to provide data of a controlled pharmacological intervention linked to genetic determinants of lipid metabolism in vervet monkeys.

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