Journal of the American College of Cardiology
© 2013 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 62, No. 4, 2013
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.01.100

High Intestinal Cholesterol Absorption Is Associated With Cardiovascular Disease and Risk Alleles in *ABCG8* and *ABO*

Evidence From the LURIC and YFS Cohorts and From a Meta-Analysis

Günther Silbernagel, MD,* M. John Chapman, PhD, DSc,† Bernd Genser, PhD,‡§ Marcus E. Kleber, PhD,*‡‖ Günter Fauler, PhD,¶ Hubert Scharnagl, PhD,¶ Tanja B. Grammer, MD,‡ Bernhard O. Boehm, MD,# Kari-Matti Mäkelä, BM,** Mika Kähönen, MD,†† Rafael Carmena, MD,‡‡ Ernst R. Rietzschel, MD, PhD,§§‖‖ Eric Bruckert, MD,¶¶ John E. Deanfield, MD, PhD,## Tatu A. Miettinen, MD, PhD,*** Olli T. Raitakari, MD, PhD,†††‡‡‡ Terho Lehtimäki, MD, PhD,** Winfried März, MD‡¶§§§

Bern, Switzerland; Heidelberg, Ulm, and Mannheim, Germany; Paris, France; Salvador, Brazil; Graz, Austria; Tampere, Helsinki, and Turku, Finland; Valencia, Spain; Ghent, Belgium; and London, United Kingdom

Objectives This study sought to determine whether high intestinal cholesterol absorption represents a cardiovascular risk factor and to link *ABCG8* and *ABO* variants to cardiovascular disease (CVD).

Background Plant sterol–enriched functional foods are widely used for cholesterol lowering. Their regular intake yields a 2-fold increase in circulating plant sterol levels that equally represent markers of cholesterol absorption. Variants in *ABCG8* and *ABO* have been associated with circulating plant sterol levels and CVD, thereby suggesting atherogenic effects of plant sterols or of cholesterol uptake.

Methods The cholestanol-to-cholesterol ratio (CR) was used as an estimate of cholesterol absorption because it is independent of plant sterols. First, we investigated the associations of 6 single nucleotide polymorphisms in *ABCG8* and *ABO* with CR in the LURIC (LUdwisghafen RIsk and Cardiovascular health study) and the YFS (Young Finns Study) cohorts. Second, we conducted a systematic review and meta-analysis to investigate whether CR might be related to CVD.

Results In LURIC, the minor alleles of *rs4245791* and *rs4299376* and the major alleles of *rs41360247*, *rs6576629*, and *rs4953023* of the *ABCG8* gene and the minor allele of *rs657152* of the *ABO* gene were significantly associated with higher CR. Consistent results were obtained for *rs4245791*, *rs4299376*, *rs6576629*, and *rs4953023* in YFS. The meta-analysis, including 6 studies and 4,362 individuals, found that CR was significantly increased in individuals with CVD.

Conclusions High cholesterol absorption is associated with risk alleles in *ABCG8* and *ABO* and with CVD. Harm caused by elevated cholesterol absorption rather than by plant sterols may therefore mediate the relationships of *ABCG8* and *ABO* variants with CVD. (J Am Coll Cardiol 2013;62:291–9) © 2013 by the American College of Cardiology Foundation

From the *Department of Angiology, Swiss Cardiovascular Center, University of Bern, Bern, Switzerland; †Dyslipidemia, Inflammation and Atherosclerosis Research Unit, National Institute for Health and Medical Research, Hôpital de la Pitié-Salpetriere, Paris, France; ‡Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; §Instituto de Saúde Coletiva, Federal University of Bahia, Salvador, Brazil; ‖Division of Cardiology, Department of Internal Medicine, Ulm University, Ulm, Germany; ¶Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; #Division of Endocrinology, Department of Internal Medicine, Ulm University, Ulm, Germany; **Department of Clinical Chemistry, Fimlab Laboratories, Tampere University Hospital and University of Tampere, Tampere, Finland; ††Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland; ‡‡Division of Endocrinology and Nutrition, Department of Medicine, University of Valencia, Valencia, Spain; §§Department of Cardiovascular Diseases, Ghent University Hospital, Ghent, Belgium; ‖‖Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; ¶¶Division of Endocrinology and Metabolism, Hôpital de la Pitié-Salpétrière, Paris, France; ##Vascular Physiology

JACC Vol. 62, No. 4, 2013
July 23, 2013:291–9

Abbreviations and Acronyms

CI = confidence interval

CVD = cardiovascular disease

GWA = genome-wide association

LDL = low-density lipoprotein

RR = risk ratio

SMD = standardized mean difference

SNP = single nucleotide polymorphism

The American Heart Association has recommended plant sterol–enriched foods (e.g., margarines) for cholesterol lowering (1). Moreover, the European Heart Association and the European Atherosclerosis Society have mentioned plant sterol-enriched functional foods as cholesterol lowering agents in their guidelines for the management of dyslipidemia (2). The regular intake of plant sterols reduces low-density lipoprotein (LDL) cholesterol by about 13 mg/dl (3) but also raises circulating plant sterols from about 1 mg/dl (4) by approximately 2-fold (5). Patients with sitosterolemia, a rare genetic disorder caused by mutations in the ATP-binding cassette transporters G5 and G8 (ABCG5 and ABCG8) (6), have up to 50-fold increased circulating plant sterols and may develop early onset cardiovascular disease (CVD) (7). Hence, it has been suggested that plant sterols are atherogenic (8–10). These concerns have been reinforced by the detection of plant sterols in carotid atherosclerotic plaques (11). In addition, plant sterol intake was related to increased plant sterol content in aortic valve cusps (12). Two recent studies have confirmed that consumption of plant sterols as part of a dietary portfolio and as an adjunct to treatment with ezetimibe has favorable effects on the lipid profile (13,14). Nevertheless, in continuance of the long-lasting safety discussion, these studies also garnered critical comments (15,16). The debate was further fueled by a genome-wide association (GWA) study showing that common variants in the *ABCG8* (major allele of *rs41360247* and minor allele of *rs4245791*) and *ABO* (minor allele of *rs657152*) genes increased both circulating plant sterols and cardiovascular risk (17). Correlations of variants in *ABCG8* (minor allele of *rs4299376*) and *ABO* with CVD subsequently have been replicated in other large-scale GWA studies (18–20). However, because circulating plant sterols are markers for cholesterol uptake (21), the genetic data may also indicate adverse vascular effects of high cholesterol absorption.

The current study consisted of a genetic analysis and a meta-analysis; its purpose was to investigate whether high intestinal cholesterol absorption represents a cardiovascular risk factor and to link *ABCG8* and *AB0* variants to CVD. We used the ratio of circulating cholestanol-to-cholesterol to estimate levels of intestinal cholesterol absorption independently of plant sterol concentrations (22).

Methods

Genetic analyses. STUDY DESIGN AND PARTICIPANTS. Genetic association studies were performed in the LURIC (LUdwigshafen RIsk and Cardiovascular health study) and the YFS (Young Finns Study) (23,24).

LURIC is a cross-sectional and prospective German cohort study designed to investigate biochemical and genetic cardiovascular risk factors. A total of 3,316 participants referred for coronary angiography were recruited between July 1997 and January 2000 at the Ludwigshafen Heart Center (23). Measurements of lathosterol, cholestanol, campesterol, and sitosterol were completed in 1,257 LURIC participants who did not receive statins and did not have type 1 diabetes (25). Individuals in this subgroup with available data on *ABCG8* or *ABO* single nucleotide polymorphisms (SNPs) were included in the current analyses.

YFS is a Finnish population-based, 27-year follow-up study on the evolution of cardiovascular risk factors from childhood to adulthood (24). The first cross-sectional study was conducted in 1980 at 5 centers and included 3,596 participants in the age groups of 3, 6, 9, 12, 15, and 18 years who were randomly chosen from the national population registry. In 2001, a total of 2,620 individuals, who were then aged 24 to 39 years, were studied. The sterol and lipid determinations used in the current analysis were taken from the year 2001 participants. Sterol and genetic data were available in 434 subjects.

Both studies were approved by the local ethical committees and performed according to the Declaration of Helsinki. Informed written consent was obtained from all participants (23,24). Diabetes mellitus was categorized according to the 2009 criteria of the American Diabetes Association (26).

LABORATORY ANALYSES. All laboratory measurements were performed on fasting blood samples. In LURIC, cholesterol was measured with enzymatic reagents from WAKO (Neuss, Germany) on a WAKO 30 R or Olympus AU640 analyzer (Tokyo, Japan) (23). Lipoproteins were separated by a combined ultracentrifugation precipitation method (beta-quantification).

Unit, Institute of Cardiovascular Sciences, University College London, London, United Kingdom; ***Division of Internal Medicine, Department of Medicine, University of Helsinki, Biomedicum Helsinki, Helsinki, Finland; †††Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; ‡‡‡Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland; and the §§§Synlab Academy, Mannheim, Germany. Dr. Miettinen passed away before the completion of the work. LURIC has received funding through the 6th Framework Program (integrated project Bloodomics, grant LSHM-CT-2004-503485) and 7th of Framework Program (integrated project AtheroRemo, Grant Agreement number 201668) of the European Union. YFS has been financially supported by the Academy of Finland grants 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; the Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 and 9N035) (Dr. Lehtimäki); the Juho Vainio Foundation; the Paavo Nurmi Foundation; the Finnish Foundation of Cardiovascular Research; the Finnish Cultural Foundation; the Tampere Tuberculosis Foundation; the Emil Aaltonen Foundation (Dr. Lehtimäki); and the 7th Framework Program (integrated project AtheroRemo, Grant Agreement number 201668) of the European Union. This study has been supported by grants from Danone Research. Dr. Silbernagel received a research grant from Unilever. Dr. Chapman has received research funding from Pfizer; Kowa, and Merck and has served on the advisory boards of Merck, Kowa, Amgen, Danone, and Roche. Dr. Genser has received a research grant and lecture fees from Danone Research. Dr. Bruckert has received honoraria for meetings or participation on advisory boards from Merck, Pfizer, Astra Zeneca, Unilever, Danone, Kraft, sanofi-avantis, MSD-Schering Plough, Servier, Genfit, and Aegerion. Dr. März has received lecture fees, grants, and consulting honoraria from Danone Research and a research grant from Unilever. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received December 3, 2012; accepted January 3, 2013.