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Genetic Variation in *NPC1L1* and Risk of Gallstone Disease

We read with great interest the recent paper by Ference et al. (1) using genetic variants in *NPC1L1* (target for ezetimibe) and *HMGCR* (target for statins) to predict the effect of ezetimibe and statins on low-density lipoprotein (LDL) cholesterol and risk of coronary heart disease. Ezetimibe reduces plasma levels of LDL cholesterol by inhibiting Niemann-Pick C1-like protein 1 (NPC1L1), a transporter responsible for cholesterol uptake from the intestine into enterocytes and from bile into hepatocytes in humans. In a recent study of 67,385 individuals from the general population (2), we genotyped 4 common *NPC1L1* variants, previously associated with reduced LDL cholesterol levels, and calculated a weighted genotype score. LDL cholesterol decreased stepwise up to 3.5%, and risk of ischemic vascular disease decreased up to 18% in those with the highest versus lowest genotype scores. These findings are in agreement with results from Ference et al. (1), and a recent study on rare loss-of-function variants in *NPC1L1* (3). However, in our study, genotype score also associated with a 22% increase in risk of symptomatic gallstone disease. This is biologically plausible, because in humans where NPC1L1 is expressed both in the intestine and in the liver, inhibition of hepatic NPC1L1 is likely to increase biliary cholesterol and the propensity for gallstone formation. This raises the clinically relevant question whether long-term treatment with ezetimibe might increase the risk of gallstones. According to the product insert of Zetia (Merck, Kenilworth, New Jersey), treatment of dogs (which express hepatic NPC1L1) with high doses of ezetimibe for a month increased biliary cholesterol 2- to 4-fold (4), suggesting that a long-term, on-target effect of ezetimibe monotherapy might be an increased risk of gallstones. That said, it is reassuring that 6 years of treatment with ezetimibe in combination with a statin did not seem to increase the risk of gallstones (5).

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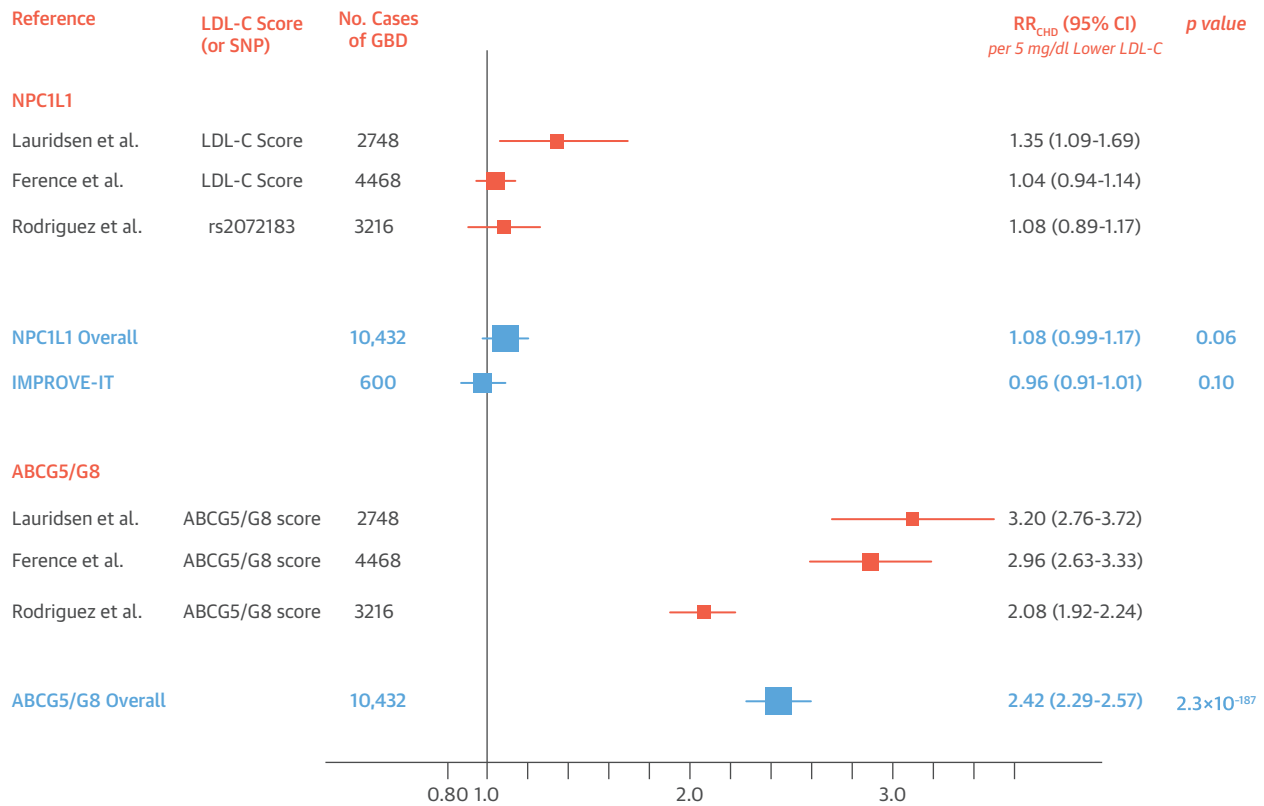
REPLY: Genetic Variation in *NPC1L1* and Risk of Gallstone Disease

Dr. Lauridsen and colleagues raise the biologically plausible hypothesis that inhibiting cholesterol absorption from bile during treatment with ezetimibe may increase the risk for symptomatic gallbladder disease (GBD). They report that persons with a greater number of polymorphisms in the *NPC1L1* gene (target of ezetimibe) had lower low-density lipoprotein cholesterol (LDL-C), a lower risk of coronary heart disease, and a greater risk of GBD (relative risk [RR]: 1.07; 95% confidence interval [CI]: 1.02 to 1.15; p = 0.02, comparing persons with scores above and below 5) (1). On closer inspection of these data, however, the effect of Niemann-Pick C1-like protein 1 (*NPC1L1*) polymorphisms on the risk of GBD appears to be limited to women in Copenhagen (women: RR: 1.10; 95% CI: 1.03 to 1.19; p = 0.01; men: RR: 1.00; 95% CI: 0.89 to 1.12; p = 0.83).

We therefore evaluated the effect of *NPC1L1* polymorphisms on the risk of GBD among women in our data (2). We did not find any association between *NPC1L1* polymorphisms and the risk of GBD, either alone or when combined with polymorphisms in the gene that encodes the target of statins (RR: 1.02; 95% CI: 0.97 to 1.08; p = 0.49, comparing persons with NPC1L1 scores above and below median). Our

FIGURE 1 Association of *NPC1L1* and *ABCG5/G8* Polymorphisms With GBD

Reference	LDL-C Score (or SNP)	No. Cases of GBD		RR_{CHD} (95% CI) per 5 mg/dl Lower LDL-C	p value
NPC1L1					
Lauridsen et al.	LDL-C Score	2748		1.35 (1.09-1.69)	
Ference et al.	LDL-C Score	4468		1.04 (0.94-1.14)	
Rodriguez et al.	rs2072183	3216		1.08 (0.89-1.17)	
NPC1L1 Overall		**10,432**		**1.08 (0.99-1.17)**	**0.06**
IMPROVE-IT		**600**		**0.96 (0.91-1.01)**	**0.10**
ABCG5/G8					
Lauridsen et al.	ABCG5/G8 score	2748		3.20 (2.76-3.72)	
Ference et al.	ABCG5/G8 score	4468		2.96 (2.63-3.33)	
Rodriguez et al.	ABCG5/G8 score	3216		2.08 (1.92-2.24)	
ABCG5/G8 Overall		**10,432**		**2.42 (2.29-2.57)**	2.3×10^{-187}

Boxes represent the point estimate of effect (relative risk) for the association between each polymorphism or genetic score (with all exposure alleles defined as the allele associated with lower LDL-C) and risk of GBD. **Bars** represent 95% CI. Effect estimates and standard errors are adjusted for a standard decrement of 5 mg/dl (0.13 mmol/l) lower LDL-C using the usual ratio of effect estimates method. CI = confidence interval; GBD = gallbladder disease; IMPROVE-IT = Examining Outcomes in Subjects With Acute Coronary Syndrome: Vytorin (Ezetimibe/Simvastatin) Versus Simvastatin (P04103); LDL-C = low-density lipoprotein cholesterol; RR_{CHD} = relative risk of coronary heart disease; SNP = single nucleotide polymorphism.

results agree with a recent genome-wide association study of GBD among women that reported that the *NPC1L1* rs2072183 polymorphism was not associated with GBD (p = 0.39) (3). When data from all 3 studies are combined, lower LDL-C mediated by polymorphisms in *NPC1L1* does not appear to be associated with a significantly increased risk of GBD (Figure 1). This result agrees closely with the lack of association between treatment with ezetimibe and the risk of GBD over a mean of 6-years follow-up in the IMPROVE-IT (Examining Outcomes in Subjects With Acute Coronary Syndrome: Vytorin [Ezetimibe/Simvastatin] Versus Simvastatin [P04103]) trial (4).

By contrast, increased cholesterol secretion into bile mediated polymorphisms in the *ABCG5/G8* gene was robustly associated with an increased risk of GBD in all 3 studies (Figure 1). As compared to polymorphisms in *NPC1L1*, lower LDL-C-mediated polymorphisms in *ABCG5/G8* were associated with a 10-fold greater

increased risk of GBD per unit change in LDL-C and several orders of magnitude greater statistical evidence for a causal effect (p = 3.1×10^{-187} vs. p = 0.06). It would appear therefore that the culprit for increased risk of GBD is increased cholesterol secretion into bile rather than decreased cholesterol absorption. As a result, even if LDL-C has a cumulative effect on GBD, as it appears to have on coronary heart disease (5), the combined genetic data and the results of IMPROVE-IT suggest that symptomatic GBD is unlikely to be a significant on-target treatment effect of ezetimibe, even if treatment is long term.

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Duration of Triple Therapy in Patients Requiring Oral Anticoagulation After Drug-Eluting Stent Implantation

We read with much interest the paper and editorial by
Fiedler et al. (1) and Bhatt et al. (2) regarding the
optimal duration of dual antiplatelet therapy (DAPT)
in patients on systemic anticoagulation in a recent
issue of the *Journal*. In this randomized, open-label
trial, 614 patients underwent drug-eluting stent
(DES) implantation and were randomized to either
6 weeks or 6 months of clopidogrel therapy. They
found no difference in the primary endpoint
(composite of death, myocardial infarction, definite
stent thrombosis, stroke, or TIMI [Thrombolysis In
Myocardial Infarction] major bleeding) between
the 2 groups (9.8% vs. 8.8%; p = 0.63). In addition,
the secondary combined ischemic endpoint of
cardiac death, myocardial infarction, definite stent
thrombosis, and ischemic stroke was no different
(4.0% vs. 4.3%; p = 0.87), and there was also no
difference in the TIMI major bleeding between the
groups.

Despite the important findings of this study,
some concerns remain unanswered that may limit the
broader clinical application of the results of this
study. The investigators used a large number of stent
types in this study including first-generation, second-
generation, and newer-generation (bioabsorbable/
degradable) DES (Table 1). In summary, ≥44% of the
stents implanted in the study included newer-
generation DES (bioabsorbable/biodegradable stents).
These could potentially affect the efficacy and safety
of this strategy when applied to a larger unrestricted
population.

There was an increased risk of stent thrombosis
with first-generation DES that led to the development
of DES with biocompatible polymers and more re-
cently biodegradable polymers and bioabsorbable
vascular scaffolds. We have shown compelling data
for safety and efficacy of second-generation DES
with biocompatible polymers in a large spectrum
of patients including those at high risk for stent
thrombosis (3). The newer DES with bioabsorbable/
biodegradable polymers and stents that allow for
complete dissolution of polymer or bioscaffold
leaving either a residual metal platform or native
vessel would, in theory, reduce the rates of stent
thrombosis. Biodegradable polymer biolimus-eluting
stents were perceived to be safer than first-
generation sirolimus-eluting stents on the basis of
the results of individual trials that were powered for
only composite endpoints of safety and efficacy.
However, in the largest mixed comparison meta-
analysis of >61 trials involving 63,242 patients, there
was a significant increase in the odds of myocardial
infarction (1.29; 95% confidence interval: 1.02 to 1.69)
with the use of biodegradable polymer DES versus
the second-generation DES at 1 year (4). In fact, the
second-generation DES was associated with the most
favorable safety profile. Additionally, another mixed
comparison meta-analysis that included 77 studies

TABLE 1 Stent Types Used in the ISAR-TRIPLE Trial		
	6 Weeks of Therapy (n = 417)	6 Months of Therapy (n = 409)
First-generation DES (SES, PES)	29 (6)	16 (4)
Second-generation DES (EES, ZES)	203 (49)	206 (50)
BA-DES, BD-DES, DES-Ab, DEB	183 (44)	186 (45)

Values are n (%).

BA-DES = bioabsorbable-drug eluting stent(s); BD-DES = biodegradable
drug-eluting stent(s); DEB = drug-eluting balloon; DES = drug-eluting stent(s);
DES-Ab = Abluminal biodegradable polymer metallic drug-eluting stent(s); EES =
everolimus-eluting stent(s); ISAR-TRIPLE = Duration of Triple Therapy in Patients
Requiring Oral Anticoagulation After Drug-Eluting Stent Implantation; PES =
paclitaxel-eluting stent(s); SES = sirolimus-eluting stent(s); ZES = zotarolimus-
eluting stent(s).