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Lessons from the trials

Niemann-Pick C1-Like 1 protein: Another target for treatment of dyslipidemia? Evidence from the Myocardial Infarction Genetic Consortium and IMPROVE-IT trials

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INTRODUCTION

Absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion contributes substantially to tight control of cholesterol homeostasis. This process is mediated by a specific transporter – Niemann-Pick C1-Like 1 (NPC1L1) protein – localized to the brush border membrane of jejunal enterocytes (Figure 1, Table 1).[1] NPC1L1 was first described by Davies and colleagues in 2000 while searching for proteins homologues of human Niemann-Pick type C1 protein (NPC1) – the primary causative protein for Niemann-Pick disease type C1 – that may be involved in subcellular cholesterol trafficking.[2,3] Human liver express also NPC1L1, however its physiological significance in hepatocytes remains to be elucidated.

Ezetimibe – a potent selective inhibitor of NPC1L1 protein activity[5] – has been shown to lower plasma levels of low density lipoprotein cholesterol (LDL-C) by 12% to 20%[6–8]. It was approved, in 2012, by the Food and Drug Administration (FDA) for the treatment of hypercholesterolemia on the basis of its LDL-C lowering alone, despite lack of data on its effects on clinical end-points such as death, myocardial infarction (MI), or stroke in clinical outcome studies.

Ezetimibe failed to slow the progression of carotid intima-media thickness, when added to background statin therapy in patients with familial hypercholesterolemia in the landmark Ezetimibe and Simvastatin in Hypercholesterolemia Enhance Atherosclerosis Regression (ENHANCE) trial, reported in January 2008[11]. Unfortunately these negative results cast a shadow over ezetimibe, although the ENHANCE study was heavily criticized by a significant and unexplained 18-month delay between completion of the study and publication of results. In the latest European Society of Cardiology (ESC) and American Heart Association (AHA) prevention guidelines, the use of ezetimibe alone or in combination is considered a class IIb recommendation.[12,13]

These results move us to more uncertainty about the benefit of this drug, and stimulated the need for conducting large genetic and clinical studies in order to test the impact of NPC1L1 inhibition on clinical outcome. Data from two large studies have been recently published and reviewed here to determine the clinical efficacy and safety of ezetimibe therapy.

NPC1L1 GENE-INACTIVATING MUTATION AND CORONARY HEART DISEASE RISK

This study was conducted using DNA samples from 16 case-control studies and cohort studies, and has been recently published in *The New England Journal of Medicine* in November 2014[14]. During the first phase of the study, the 20 protein coding regions (exons) of NPC1L1 were initially sequenced in 7,364 patients with coronary heart disease (CHD) and in 14,728 controls who were of European, African,

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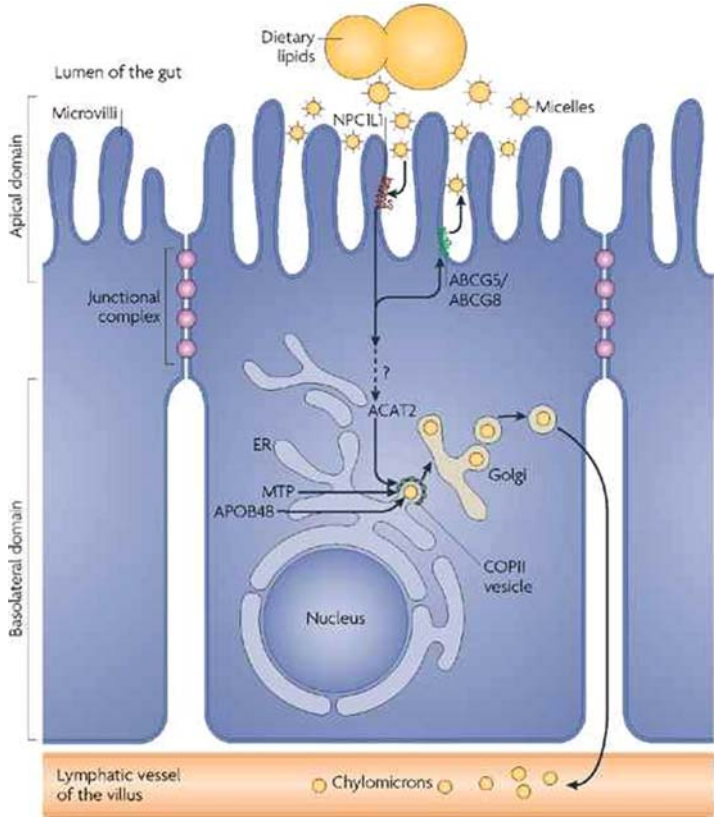


Figure 1. The Niemann–Pick C1-like-1(NPC1L1) protein (dark red) is located at the apical membrane of enterocytes and facilitates the uptake of cholesterol across the brush border membrane. In contrast, the ABCG5/G8 transporter (green) promotes the active transfer of cholesterol and plant sterols back into the intestinal lumen for excretion. Acyl CoA cholesterol acyltransferase isoform-2 (ACAT2) esterifies the absorbed cholesterol, which becomes incorporated into nascent chylomicron particles. Dietary fatty acids are used for triglyceride synthesis in the smooth ER and MTP (microsomal triglyceride transfer protein) transfers triglycerides and cholesteryl esters to APOB48. The nascent chylomicrons leave the ER in COPII-coated vesicles and are secreted through the Golgi complex to the basolateral side of the enterocyte and reach the venous circulation through lymphatic vessels[18].

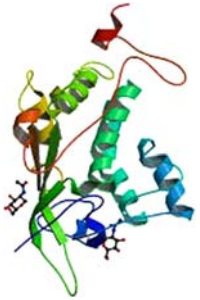
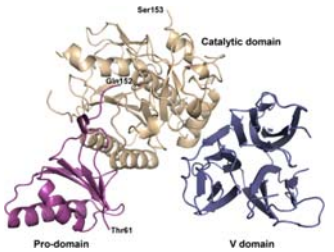
or South Asian ancestry. The most frequently observed NPC1L1 inactivating mutation during this phase was p.Arg406X. During the second phase of the study, p.Arg406X was genotyped in additional 91,002 participants.

Fifteen rare mutations (nonsense, splice-site, or frameshift mutations) that were expected to inactivate NPC1L1 protein were identified. Approximately 1 in every 650 persons was a heterozygous carrier for 1 of these mutations. No homozygotes or compound heterozygotes were identified. Carriers of any NPC1L1 inactivating mutations had a significantly lower plasma LDL-C level (mean adjusted difference, −12 mg/dL; $p = 0.04$) and a lower risk of CHD (0.04% vs. 0.09% respectively, $p = 0.008$; odds ratio for carriers, 0.47, 95% confidence interval (CI), 0.25 to 0.8) than non-carriers. No significant differences in plasma triglycerides (mean difference −12%; $p = 0.11$) or high-density lipoprotein cholesterol levels (mean difference 2 mg/dL, $p = 0.29$) were demonstrated between carriers and non-carriers.

IMPROVE-IT STUDY

The Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) was a phase III, multicenter, randomized, double-blind, placebo-controlled trial that was conducted at 1185 sites in 39 countries[15] and was presented at the annual meeting of the AHA in November 2014[16]. The study aimed to investigate the potential benefit from the addition of ezetimibe versus placebo to background simvastatin therapy in reducing cardiovascular (CV) events in patients at high risk. A total of 18,144 patients, aged 50 years or older, with recent acute coronary syndrome [ST-segment MI (STEMI) in 29%, Non ST-segment MI (NSTEMI) in 45%, and unstable angina (UA) in 24%] and plasma LDL-C 125 mg/dL

Table 1. Comparison Between NPC1L1 and PCSK9 proteins.

	Niemman-Pick type C1 L1 (NPC1L1)	Proprotein convertase subtilisin/kexin 9 (PCSK9)
Discovery	Nabil Seidah and colleagues (2003)	Davies and colleagues (2000)
Molecular structure	Domain A (residues 22-242), Domain B (residues 243 – 265)	Serine protease (prodomain, catalytic domain, and V domain)
		
Main site of production	Small intestine and liver	Liver and small intestine
Level of action in cholesterol metabolism	Promotes cholesterol absorption in small intestine	Promotes LDL receptor degradation and decreases the liver ability to clear LDL-C from blood
Prevalence of gene mutation	Inactivation mutation was detected in 1 in every 650 persons.[14]	Non-sense mutations was detected in 2% of African Americans and < 0.1% of European American.[4]
Therapeutic potential (pharmacological inhibition)	NPC1L1 inhibitors have achieved 10-20% reduction in LDL-C levels when added to background statin therapy.[6–8] NPC1L1 inhibition is associated with significant decrease in adverse CV events	PCSK9 inhibitors have achieved 60% reduction in LDL-C levels when added to background statin therapy. [9,10] The results of long term clinical outcome studies have not been yet released

(or ≤ 100 mg/dl if they were already taken statin) were randomized in a 1:1 fashion to either ezetimibe 10 mg/simvastatin 40 mg or simvastatin 40 mg.[15] Uptitration to 80 mg simvastatin occurred in 27% of the simvastatin group and 6% of the ezetimibe/simvastatin group. The target plasma LDL-C level in simvastatin arm was < 70 mg/dL. Ezetimibe was assumed to further lower LDL-C by 15 mg/dL. The primary composite endpoint was cardiovascular (CV) death, no fatal MI, no fatal stroke, readmission for UA, and coronary revascularization (≥ 30 days after randomization).

Over a median follow up period of 7 years, the primary end-point was significantly lower in the ezetimibe/simvastatin arm compared with the simvastatin arm (32.7% vs. 34.7% respectively; hazard ratio [HR] 0.94; 95% CI 0.89-0.99; p = 0.016; number needed to treat [NNT] = 50). Ezetimibe/simvastatin therapy reduced LDL-C level to an average of 54 mg/dL, compared with 69 mg/dL for those treated with simvastatin alone. In terms of individual components of primary end-points, patients randomized to ezetimibe/simvastatin had significantly less incidence of MI (13.1% vs. 14.8% respectively, p = 0.002), stroke (3.4% vs. 4.1% respectively, p = 0.008), and CV death/MI/stroke (20.4% vs. 22.2% respectively, p = 0.003) compared to those randomized to simvastatin alone. No differences were detected in all-cause mortality (15.4% vs. 15.3%, p = 0.78), CV mortality (6.9% vs. 6.8%, p = 0.99) and need for coronary revascularization (21.8% vs. 23.4%, p = 0.11). Patients with diabetes had a greater benefit with ezetimibe/simvastatin (HR = 0.86, p = 0.023). No differences were observed in cancer incidence (10.2% vs. 10.2%, p = 0.57), myopathy (0.2% vs. 0.1%, p = 0.32), or transaminitis (2.5% vs. 2.3%, p = 0.43).

DISCUSSION

Data came from these studies have emphasized the clinical benefit of genetic or pharmacological inhibition of NPC1L1 protein in reducing the risk of CHD. Naturally occurring DNA sequence variants that affect the activity of a particular protein target can be used to estimate the potential efficacy and safety of a drug targeting such proteins.[17] Interestingly, rare protein-inactivating mutations in NPC1L1 have been shown to reduce both the plasma LDL-C concentration and the risk of CHD.

These findings were supported by the new results of the long-delayed and eagerly awaited IMPROVE-IT study that showed a modest benefit in reducing CV events when ezetimibe was added to background simvastatin therapy in high-risk patients. IMPROVE-IT study is the first outcome study to show an incremental clinical benefit for a non-statin agent when added to a statin in reducing CV events, especially MI and stroke. There were no significant differences in cancer, muscle and gall bladder related events, which confirm the safety profile of this drug. The mean levels of LDL-C in the ezetimibe/simvastatin arm of IMPROVE-IT study were less than 60 mg/dl which reaffirms "the lower is better" LDL hypothesis. However certain points need to be raised when these studies were thoroughly analyzed:

(1) The 53% relative risk reduction (RRR) in the risk CHD that was detected in carriers of NPC1L1 inactivation mutation – as compared to only 13% RRR in the incidence of MI in the IMPROVE-IT trial – is a strong stimulus for future attempts to reproduce it clinically. However, lifelong genetic inhibition is totally different from pharmacologic inhibition that is initiated in adulthood and lasts for several years. In addition, pharmacological inhibition is counterbalanced by toxic effects that would not be tested in a genetic model.

(2) The RRR in the IMPROVE-IT study is small over a long period of time. There were only 6% RRR in the primary composite end-point, primarily driven by reductions in non-fatal end points, and no mortality benefit. However NNT is only 50 to prevent one CV event. Moreover, the addition of ezetimibe was associated with 21% RRR in the incidence of stroke and 13% RRR in the incidence of MI which positively affect the quality of life of those patients.

(3) The prolonged duration of the IMRPOVE-IT trial as well as the controversy surrounding the trial leads to concern that dropout and crossover rates may significantly reduce the possible benefits of ezetimibe.

(4) The clinical benefit of further LDL-C lowering in patients with a baseline LDL-C <70 mg/dl is unknown and not established, as reported in AHA guidelines.

(5) The statin used in IMPROVE-IT study was simvastatin. Other available statins – such as atorvastatin or rosuvastatin – are more potent.

(6) The effect of ezetimibe monotherapy in patients with higher LDL-C, particularly those who are statin-intolerant, was not assessed. No clinical outcome trials have been performed to investigate the clinical efficacy of ezetimibe in these patients.

WHAT HAVE WE LEARNED?

These data are another proof for how genetic studies are very helpful in clinical practice. In addition, it reaffirms the LDL hypothesis; low is good but lower is better which need to be re-addressed in future prevention guidelines. The positive results of IMPROVE-IT study support the use of ezetimibe as a reasonable adjunct to statin therapy in treating high risk patients. Moreover, it may reassure the FDA and help early approval of the PCSK9 inhibitors by accepting LDL-C lowering as a surrogate endpoint, without waiting evidence from clinical outcomes trials. Current evidence supports the future exciting role of NPC1L1 and PCSK9 inhibition in the treatment of hypercholesterolemia.

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