Editorial

The present issue republishes some of the points introduced at the 2006 14th International Symposium on Atherosclerosis. Of particular interest are the American recommendations regarding the safety of statins, which were published recently (McKenney et al., American Journal of Cardiology 2006, 97, IC – 94 C).

A further clarification of the genetic forms of hypercholesterolaemia not only gives an insight into the very heterogeneous nature of the disorder but also demonstrates mutations which might explain the great variability of LDL-Ch. in the general population. This furthermore throws new light on the action mechanisms of statins and on the possibilities of new therapies involving action on the PCSK9 gene.

Finally, stroke and dementia, their relationships with cholesterol and treatment with antilipemic agents will be the subject of a meeting entitled “Stroke and Lipids”, which will be organised on 27 January 2007 in Brussels in collaboration with the Belgian Stroke Council (see below).

Prof. F.R. Heller
President
1. MYELOPEROXIDASE LEVELS ARE ASSOCIATED WITH A FUTURE RISK OF CORONARY ARTERY DISEASE IN APPARENTLY HEALTHY INDIVIDUALS

M.C. MEUWESE Medical Center – Amsterdam

Myeloperoxidase (MPO) has emerged as a mediator of atherosclerosis by inducing oxidative damage to LDL- and HDL-cholesterol as well as reducing NO availability. Elevated MPO levels are associated with an adverse outcome in patients with coronary artery disease (CAD). Data regarding the predictive value in primary prevention are lacking. The authors assessed whether MPO levels are associated with a risk of future CAD in apparently healthy individuals.

MPO was measured in baseline serum samples from a case-control study nested in the prospective EPIC-Norfolk cohort study. Cases (n=1,138) were healthy men and women, aged 45 to 79 years, who developed CAD during 6 years of follow-up. Controls (n=2,237) remained free of CAD and were matched by age, sex and enrolment time.

MPO levels were higher in cases than controls (median 608 vs 538 pM/ml, p<0.01). Logistic regression analysis showed that the future CAD risk increased in consecutive quartiles of MPO (odd ratio [OR]: 1.5) top versus bottom quartile (p for linearity <0.01). After adjustment for body mass index, smoking, diabetes, blood pressure, LDL- and HDL-cholesterol, and CRP, the OR in the top quartile remained significant at 1.3 (1.0-1.7).

Elevated MPO levels predict the future CAD risk in a primary prevention setting. This underscores the relevance of anti-inflammatory strategies targeting MPO already in the earlier phases of cardiovascular prevention.

2. ASYMMETRIC DIMETHYLARGININE (ADMA) IS ASSOCIATED WITH INCIDENCE OF CARDIOVASCULAR DISEASE IN THE GENERAL POPULATION, THE HOORN STUDY

T. TERLINCK, Medical Center - Amsterdam

Increased asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, predicts cardiovascular disease (CVD) in high-risk patient groups. T. TERLINCK prospectively studied the association between ADMA and CVD in the general population.

Increased ADMA was observed in patients who developed cardiovascular disease (CVD) during follow-up compared to controls. After adjustment for age, gender, and established risk factors (cholesterol, HDL-cholesterol, body mass index, renal function, hypertension, smoking, glucose and prevalent CVD) a high plasma concentration of ADMA (i.e. highest quintile versus lowest quintile) was associated with a hazard ratio for CVD of 1.19 (95% C.I. 1.16 to 1.90) in subjects without diabetes and 0.48 (0.24 to 0.98) in subjects with diabetes. In analyses restricted to participants without diabetes and CVD at baseline, the hazard ratio was 1.40 (1.02 to 1.91).

A high plasma concentration of ADMA independently predicts CVD in subjects without type 2 diabetes. The inverse relation between high ADMA and CVD in subjects with diabetes needs confirmation in other populations.

3. SERUM LEVELS OF OSTEOPROTEGERIN AND RANKL IN PATIENTS WITH ST ELEVATION ACUTE MYOCARDIAL INFARCTION

F. LUCA, University of Messina - Italy

OPG (osteoprotegerin) has been suggested to have an important role in atherogenesis and vascular calcification. In the present study, F. LUCA & Coll investigated serum OPG and RANKL (receptor activator of nuclear factor kappa B ligand) concentrations in patients with ST elevation AMI and established CAD (coronary artery disease).

OPG and RANKL were measured in 58 patients hospitalised in the coronary care unit with ST elevation AMI, in 52 asymptomatic male patients with an established diagnosis of CAD and in 52 healthy male controls. These last two groups were matched with the AMI patients for age and body mass index.

OPG was significantly (P<0.05) higher in patients with AMI at 1 h after AMI (8.04 +/- 4.86 pmol/l) than in both patients with established CAD (4.92 +/- 1.65 pmol/l) and healthy subjects (3.15 +/- 1.01 pmol/l). Subjects with established CAD had...
significantly (P<0.05) increased OPG levels compared with controls. RANKL levels in patients with established CAD (0.02 +/- 0.5 pmol/l) and with AMI (0.11 +/- 0.4 pmol/l) were significantly (P<0.05) lower compared with controls (0.32 +/- 0.35 pmol/l). In the AMI group, OPG decreased significantly (P<0.05) at 1 and 4 weeks after infarction (8.04 +/- 4.86 compared with 6.38 +/- 3.87 and 6.55 +/- 2.6 pmol/l respectively), but OPG levels, either at 1 h or 1-4 weeks after AMI, remained significantly (P< 0.05) higher compared with established CAD (4.92 +/- 1.65 pmol) and controls (3.15 +/- 1.01 pmol/l). These data show for the first time that OPG levels are increased in ST elevation AMI within 1 h of infarction. Whether the increase in OPG is a consequence or a causal factor of plaque destabilisation (changes in the calcification process?) deserves further investigation.

4. ADIPONECTIN AND THE ANTI-INFLAMMATORY EFFECTS OF FENOFRIBRATE IN PATIENTS WITH HYPERTRIGLYCERIDEMIA AND THE METABOLIC SYNDROME

R.S. ROSENSON, Northwestern Univ. Chicago - USA

Adiponectin is an adipose-secreted hormone with anti-inflammatory properties mediated by inhibition of NF-kB signalling. This study investigates whether fenofibrate alters dyslipidaemia levels in patients with hypertriglyceridaemia (HTG) and the metabolic syndrome and examines the association of dyslipidaemia with circulating inflammatory markers and cytokine production in whole blood.

The effects of fenofibrate (160 mg/d) on dyslipidaemia and other inflammatory markers were investigated in a 12-week randomised, placebo-controlled trial in 55 patients with HTG (plasma triglycerides >150 mg/dL and <600 mg/dL) and the metabolic syndrome who were not receiving lipid altering therapies.

Adiponectin levels increased by 0.34 µg/mL in the fenofibrate group and by 0.07 µg/mL in the placebo group (p=0.0003). In multivariate models including age, gender and waist circumference, correlations were demonstrated between changes in dyslipidaemia and VCAM-1 (r=0.45, p<0.001); ICAM-1 (r=0.44, p<0.001); IL-18 (r=0.39, p=0.002); MCP-1 (r=0.41, p=0.03) and MIP-1a (r=0.33, p=0.046) in fenofibrate-treated subjects. No such correlations were observed in placebo-treated subjects. There were no significant correlations between fasting dyslipidaemia and LPS-stimulated production of TNF-α; IL-6, IL-10 in either treatment group. Fenofibrate (160 mg/d) connected dyslipidaemia levels in patients with HTG and the metabolic syndrome. Changes in adiponectin could play a central role in the anti-inflammatory effects of fenofibrate.

5. SOY PROTEINS: MECHANISMS FOR CHOLESTEROL REDUCTION

M.R. LOVATI, University of Milan and T.A.B. SANDERS, King’s College – London

Soy proteins (7S soy globulin and its alpha prime subunit) have been shown to positively modulate LDL receptor activity and to decrease triglyceride synthesis both in vitro (Hep G2 cells) and in vivo (rats fed a cholesterol-rich diet).

- Cell preincubation with whole 7S soy globulin caused an increase in the mature forms of SREBP-1, both in membranes (+90%) and in the nuclei (+130%). These data suggest that 7S soy globulin is responsible for the direct up-regulation of LDL receptors via the SREBP pathway.

- Unexpectedly, the fields of PPARs and soy protein came together by the demonstration that soy isoflavones could serve as ligands for PPARs. It was demonstrated that at higher concentrations the soy isoflavones (genistein and daidzein) promote adipocyte differentiation. Subsequent studies indicated that at concentrations above 1 mM, genistein is able to activate PPARγ in a classical transactivation assay and furthermore is able to displace rosiglitazone from PPARγ, indicating direct binding. By analogy with synthetic PPARγ agonists, in vivo activation of PPARγ can be expected to result in amelioration of glucose homeostasis. Very preliminary evidence suggests that this is the case.

The consumption of intact soy protein appears to increase LDL receptors (through SREBP) and to serve as PPAR ligands. The beneficial actions of soy proteins on atherogenesis are complex but are beginning to be understood.

REPORT OF THE NATIONAL LIPID ASSOCIATION (NLA) TASK FORCE ON STATIN SAFETY

1) THE LIVER AND STATIN SAFETY

J.P.H. JONES (Houston, USA)

Asymptomatic elevations in ALT or AST liver enzymes >3 times upper limit of normal (ULN) are seen with all statins. Elevations of this magnitude are seen in <1% of patients receiving initial and intermediate doses and 2 to 3% of patients receiving 80 mg. These elevations are related to the dose of the statin but not to the LDL-cholesterol reduction. An elevation of ALT and/or AST >3 times ULN is most often transient and will resolve spontaneously in 70% of cases even if the statin and dose are continued unchanged. With a definition of ALT or AST >3 times ULN on 2 consecutive occasions, the number of patients with a significant elevation drops from 300 per 100,000 person-years to 110 per 100,000 person-years.
RECOMMENDATIONS

These data support the conclusion that there is no relationship between statin therapy and liver failure or idiosyncratic reactions resulting in liver failure. In either case, the routine monitoring of liver enzyme levels may not identify these patients and is not supported by the evidence.

2) MUSCLE AND STATIN SAFETY

T.A. JACOBSON (Atlanta, USA)

Muscle symptoms (i.e. pain, soreness, weakness, and/or cramps) or signs (CK elevations) are among the most prevalent and important adverse effects associated with statin therapy. The occurrence of serious muscle toxicity with currently marketed statins is fortunately rare. According to a recent meta-analysis of findings from 21 clinical trials providing 180,000 person-years of follow-up, myopathy (defined as muscle symptoms + CK > 10×ULN) occurs in 5 per 100,000 person-years and rhabdomyolysis in 1.6 per 100,000 person-years.

The most common muscle side effect of statins is myalgia (i.e. muscle pain or soreness), weakness, and/or cramps without CK elevations. These symptoms are most often tolerable, but are occasionally intolerable and debilitating and require the statin to be withdrawn. Among currently marketed statins, it appears that the risk of drug-related muscle injury is roughly the same.

The exact mechanism for muscle injury from statin therapy is not known. However, it appears to be related to the blood concentration of the statin, which is influenced by the drug’s pharmacokinetics, its potential for drug interactions, the statin dose, and the patient’s myopathic risk factors (i.e. age, renal disease, diabetes, etc.) but not by the LDL-C level achieved. Although muscle toxicity can occur in patients administered the starting dose of a statin, symptoms are much more likely to occur with higher doses. Other situations that may raise the statin’s blood levels include advanced age and frailty, small body frame, deteriorating renal function, infection, untreated hypothyroidism, and alcohol abuse.

RECOMMENDATIONS

1. It is not necessary to measure CK levels in asymptomatic patients during the course of statin therapy, since marked, clinically important CK elevations are rare and are usually related to physical exertion or other causes.
2. CK measurements should be obtained in symptomatic patients to evaluate the severity of muscle damage and facilitate a decision as to whether to continue therapy or alter doses.
3. In patients who develop rhabdomyolysis (a CK > 10,000 IU/L or a CK > 10 times ULN with an elevation in serum creatinine), statin therapy should be stopped.

INCREASING HIGH-DENSITY LIPOPROTEIN CHOLESTEROL: FROM PROMISE TO PRACTICE.

J.P. Kastelein – Amsterdam

Over the last two decades statin therapy has evolved as the mainstay of all regimens for prevention of cardiovascular disease (CVD). In fact, cardiovascular (CV) risk in all patient groups is reduced by these drugs by approximately 30%. More recent studies have emphasised the impact of robust lipid lowering by showing further reduction in CV events upon vigorous low-density lipoprotein cholesterol (LDL) reduction. Over the next few years, trial data will show whether the beneficial effect of further LDL-lowering is restricted to statin therapy, or whether it also extends to cholesterol absorption inhibitors or other novel drugs. Despite further improvements in the LDL field, current clinical practice shows that approximately two-thirds of CV events cannot be prevented (Fig 1).

Although LDL remains of the most important “actor” in atherogenesis, research has shifted beyond the LDL-particle and includes other lipoproteins that may contribute to CVD risk, such as low high-density lipoprotein-cholesterol (HDL) and elevated triglyceride (TG) levels. With the growing epidemic of obesity-induced dyslipidaemia, mainly consisting of low HDL levels and elevated TGs, in conjunction with the fact that low HDL is the most frequent lipid abnormality in patients with premature coronary heart disease, HDL-increasing strategies are beginning to emerge as an attractive tool for CV protection. Large-scale population studies have already demonstrated the impact of HDL on future CVD.

Unfortunately, statins only have minor effects on HDL levels and lifestyle modification has proven difficult to adhere to during long-term follow-up, whilst the beneficial effects on CV risk factors are, at best, modest. On the other hand, fibrates and niacin provide a safe and effective way of increasing HDL, the latter being the most potent. Whilst for both, particularly for fibrates, intervention data have emerged to show beneficial effects on CV outcome, their ability to enhance CV event reduction when added to statin monotherapy has been...
shown in small trials. In view of the consistent and strong data indicating that HDL has potent anti-atherogenic effects, it is safe to assume that future CV risk management will include both lifestyle modifications, intensive LDL-lowering therapy combined with potent HDL-increasing agents such as niacin, CETP-inhibitors or apoA-I peptides (Table 1).

Particularly, the final verdict on apoA-I peptides with regard to plaque stabilisation, regression and better survival after an acute coronary syndrome awaits further confirmation. If validated, novel interventions in patients with acute coronary syndromes and patients at very high risk of CV complications may include acute infusion of HDL mimetic peptides/reconstituted HDL, followed by long-term strategies to establish LDL-lowering with concomitant HDL-increase (Table 1).

<table>
<thead>
<tr>
<th>Table 1 - How to increase HDL concentrations</th>
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<tbody>
<tr>
<td>• Increasing HDL production</td>
</tr>
<tr>
<td>- Apo A1 gene therapy</td>
</tr>
<tr>
<td>- Niacin</td>
</tr>
<tr>
<td>- PPAR alpha agonists</td>
</tr>
<tr>
<td>• Improving HDL maturation</td>
</tr>
<tr>
<td>- LCAT gene therapy</td>
</tr>
<tr>
<td>- Upregulation of ABCA1</td>
</tr>
<tr>
<td>• Stimulating lipid remodelling</td>
</tr>
<tr>
<td>- CETP inhibitor (torcetrapib)</td>
</tr>
<tr>
<td>- Hepatic lipase</td>
</tr>
<tr>
<td>• Improving HDL function</td>
</tr>
<tr>
<td>- Apo A1 Milano</td>
</tr>
<tr>
<td>- Apo A1 Trimers</td>
</tr>
<tr>
<td>- Delipidated HDL</td>
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</tbody>
</table>

A meeting organized by the Belgian Lipid Club and The Belgian Stroke Council

January 27th 2007 (from 9.00 to 12.30 a.m.)
COURTYARD BY MARRIOTT
6, Avenue des Olympiades
B – 1140 (EVERE) BRUSSELS

SUBJECTS AND SPEAKERS:


FOR ADDITIONAL INFORMATION:

- Prof. Dr. HELLER, F. – e-mail: heller.cs.jolimont@skynet.be - Tél. 064/23 38 96/97
- Prof. Dr. THIJS, V. – e-mail: vincent.thijs@uz.kuleuven.ac.be - Tél. : 016/344 278
From hypercholesterolemia to hypocholesterolemia and beyond.

THE STORY OF A NEW GENE IN LIPIDOLOGY

Olivier Descamps,
Centre de Recherche Médicale de Jolimont,
Clinique de Prévention Cardiovasculaire,
Hôpital de Jolimont

A high level of low-density lipoprotein (LDL) cholesterol is a major risk factor for coronary heart disease, peripheral artery disease and stroke because it contributes to the build-up of plaque on the walls of the arteries. It has become a major cause of deaths worldwide, with figures in some industrialised countries reaching epidemic proportions. The history of the discovery of factors that modulate LDL-C levels is fascinating. The discovery of a new gene has provided a new chapter in this story.

Familial hypercholesterolemia, an heterogenous genetic disease that required a new name (ADH)

Familial hypercholesterolemia (FH) is characterized by raised plasma LDL and the depositing of cholesterol in the tendons leading to tendon xanthomas (in the homozygous form only) and, in the arteries, to premature cardiovascular events (Goldstein 2001). It is one of the most common genetic disorders (frequency 1 / 500). FH was initially attributed to mutations in the gene encoding the LDL-receptor (LDLR). More than 900 different mutations have been described throughout the world (Dedoussis 2004) and currently more than 90 mutations are known to exist in Belgium (Descamps 1997, Descamps 2000, Descamps 2001, Van Gaal 2002, Descamps 2003, Van Leuven 2001). Heterozygous (HeFH, 1 per 500) and homozygous (HoFH, 1 per 106) FH individuals have average LDL-C plasma levels of 300 mg/dL and 800 mg/dL respectively (Goldstein 2001).

Although less frequent than LDLR defects, heterozygous and homozygous mutations in the ligand-binding domain of the apolipoprotein (apo) B100, the protein moiety of LDL that interacts with the LDLR and thereby promotes LDL hepatic uptake, are also associated with the FH phenotype (sometimes called familial defective apoB or FDB) (Innerarity 1987, Soria 1989) (Table 1). The mutations in the genes encoding the LDL receptor and apolipoprotein B directly or indirectly reduce the ability of the LDL receptor to mediate the endocytosis of plasma lipoproteins containing apoB or apoE.

Recently, other diseases (phenocopies) that resemble heterozygous or homozygous forms of FH have been described (Table 2). These diseases are distinguishable however by the fact that they are inherited in a recessive manner, and thus are very rare. The rare autosomal recessive FH subtype, also called ARH or HCHOLARI, results from mutations in the ARH gene, which encodes a putative adaptor for the LDL receptor (Zuliani 1995, Garcia 2001). The clinical phenotype of ARH is milder than that of receptor-negative HoFH and resembles that observed in receptor-defective HoFH: LDL-C in ARH (553 ± 89mg/dL), in receptor-negative HoFH (830 ± 138mg/dL) and in receptor-defective HoFH (602 ± 93mg/dL). The risk of coronary artery disease is 9-times lower in ARH patients (No CHD before 20 years in ARH versus 43% in HoFH). Heterozygous

<table>
<thead>
<tr>
<th>Genes</th>
<th>LDLR</th>
<th>APOB</th>
<th>PCSK9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the disease</td>
<td>Original familial hypercholesterolemia (FH)</td>
<td>“Familial ligand-defective apo B”, (FDB)</td>
<td>No particular name</td>
</tr>
<tr>
<td>Reference MIM</td>
<td>FH or HCHOLADI (MIM 143890)</td>
<td>HCHOLAD2 (MIM 144010)</td>
<td>HCHOLAD3 (MIM 603776)</td>
</tr>
<tr>
<td>Gene product</td>
<td>LDL-R receptor</td>
<td>Apolipoprotein B</td>
<td>NARC1, a proprotein convertase</td>
</tr>
<tr>
<td>Reference MIM</td>
<td>LDLR (MIM 606945)</td>
<td>APOB (MIM 107730)</td>
<td>PCSK9 (MIM 607786),</td>
</tr>
<tr>
<td>Chromosome</td>
<td>19p13.1-p13.3</td>
<td>2p23-24</td>
<td>1p34.1-p32</td>
</tr>
<tr>
<td>Function</td>
<td>Membrane surface receptor for LDL particles</td>
<td>The ligand for the LDL receptor</td>
<td>Control on apoB100 matura-tion</td>
</tr>
<tr>
<td>Functional mutations causing ADH</td>
<td>&gt; 900 (most likely, any number can occur)</td>
<td>1 or 2</td>
<td>&gt;2 (Gain-of-function mutation)</td>
</tr>
<tr>
<td>Contribution to ADH</td>
<td>Most</td>
<td>5-10%</td>
<td>(approximately 2.3%)*</td>
</tr>
<tr>
<td>Other</td>
<td>Polymorphisms associated with LDL-C variance : is this meant to be variance?</td>
<td>Many neutral mutants; hypobetalipoproteinemia due to truncated APOB</td>
<td>Loss-of-function mutation for low cholesterol</td>
</tr>
<tr>
<td>Mutation frequency</td>
<td>1/500 (see chapter XX)</td>
<td>0.08%</td>
<td>?</td>
</tr>
</tbody>
</table>

(* in France, Allard 2005)
ARH carriers showed a higher level of LDL-C (+17%) than non-carrier family members (Pisciotta 2006a). Table 2 summarises what we know about the causes of autosomal recessive hypercholesterolemia.

Because of these alternative recessive forms of FH, it was decided to change the name of “familial hypercholesterolemia” to the more generic name of “Autosomal Dominant hypercholesterolemia” or ADH. Until 2003, ADH was associated with the two following genetic defects: LDLR mutations in about 65% of case reports and APOB mutations in about 10% of case reports. But the cause(s) responsible for the remaining 25% remain(s) unknown (Kastelein 2005).

**Third Locus for “Autosomal Dominant hypercholesterolemia”**

In 2003, using hypercholesterolemic families in which mutations in the LDL receptor and apoB genes had been excluded, Abifadel and co-workers mapped a region on the human chromosome 1 (1p34.1-p32) that segregated with ADH in a population of French families (Abifadel 2003). Haplotype analysis identified a critical region that contained 41 genes, including a new gene PCSK9 or proprotein convertase subtilisin kexin type 9 (initially called NARC-1). Through systematic sequencing, Abifadel et al identified mutations in the PCSK9 gene in 13% of the families with ADH. The average pre-treatment plasma LDL-C level of these patients (all adults) was 307±68 mg/dL, compared with 98±31mg/dL for the other family members. The affected individuals were found to have a G25T>A substitution in exon 2 of the PCSK9 gene, resulting in a S127R substitution at the amino acid level.

Since then, several missense PCSK9 mutations have been reported in ADH patients with normal LDLR- or apoB encoding genes, resulting in the following amino acid substitution: F216L, R218S and R357H in France (Allard 2005), and D374Y in Utah, England and Norway (Leren 2004, Sun 2005, Timms 2004). In all cases, the LDL-cholesterol level was 2 to 5-times higher than normal and was caused by an increase in LDL particles. The lipoprotein profile of patients with heterozy-

### Table 2. Causes of autosomal recessive hypercholesterolemia (ARH)

<table>
<thead>
<tr>
<th>Gene and product</th>
<th>Sitosteroneia **</th>
<th>Cholesterol 7-hydroxylase deficiency. ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td>Binds the FDNPY of the LDLR required for internalization LDLR ††</td>
<td>Control the absorption and the elimination of dietary sterols in enterocytes (also in hepatocytes)</td>
</tr>
<tr>
<td>Biology</td>
<td>Very low LDL clearance Normal LDLR function in fibroblasts</td>
<td>☒ absorption of dietary sterols (nl: &lt;5%) and ☑ secretion of sterols into the bile. sitosterol 50 x normal (from plants and shellfish).</td>
</tr>
<tr>
<td>Clinical</td>
<td>Intermediate between FH heterozygotes and FH homozygotes</td>
<td>LDL-C levels like HoFH xanthomas and atheromas †† (mainly cholesterol) Rare LDL-C normal planar xanthomas, aortic stenosis low-level hemolysis (plant sterols into red blood membranes).</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Families in Liban and Sardinian</td>
<td>Rare</td>
</tr>
<tr>
<td>Treatment</td>
<td>Statins reduced LDL-C more strongly than in homozygous FH, but most required LDL apheresis</td>
<td>extremely responsive to dietary cholesterol restriction and to bile acid resin therapy, respond poorly to statin but very well to ezetimibe</td>
</tr>
</tbody>
</table>

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igious PCSK9 mutations closely resembles that of individuals with other ADH due to LDLR or APOB heterozygous defects. No homozygous mutants of PCSK9 have yet been identified but recently two patients with particularly severe FH phenotype were found to have heterozygous mutations in the LDLR gene and in the PCSK9 gene simultaneously, suggesting an additive effect for the two defects (Pisciotta 2006b).

Proprotein convertase subtilisin kexin type 9 (PCSK9).

In the secretory pathway of the cell, many biologically inactive precursor proteins are processed by limited proteolysis to produce biologically active peptides and proteins. The enzymes that perform these cleavages are referred to as subtilisin-like proprotein convertases (SPCs). Several members of the SPC family have been identified. One of them was already known to be involved in cholesterol homeostasis (the subtilisin kexin isoenzyme-1 /site-I-protease or SK /S1P), through processing of the sterol regulatory element-binding proteins (SREBPs) (Seidah 1999, Sakai 1998).

The PCSK9 gene is mainly expressed in the liver and intestine (to a lesser extent, in the brain, skin and kidney) (Seidah 2003). The evidence that the PCSK9 gene plays a role in lipoprotein metabolism is provided by studies carried out in mice and in hepatocyte cultures.

- Downregulation of PCSK9 hepatic expression in cholesterol-fed mice (Maxwell 2003).
- Upregulation of PCSK9 in cultured hepatocytes depleted of cholesterol by a statin (Dubuc 2004).
- Upregulation of PCSK9 in mice overexpressing sterol-regulatory-element-binding protein-la (SREBPla) and SREBP2, two transcription factors activated by low levels of intracellular cholesterol (Horton 2003).
- Raised plasma LDL in mice with adenoviral-mediated overexpression of PCSK9 (no increase if the mice are also LDLR-deficient (Lalanne 2005, Maxwell 2004, Park 2004). And most importantly, the hepatic LDLR levels are reduced in mice overexpressing the PCSK9 gene. These experiments, specially the last one, give a wonderful insight into the link between the known PSCK9 mutations and hypercholesterolemia. Since the LDL receptor is the major route of uptake of LDL-C from the blood and PCSK9 overexpression is associated with hypercholesterolemia and a reduction of the number of LDL receptors, it becomes evident that the missense mutations in the PSCK9 gene that resulted in hypercholesterolemia were "gain-of-function" mutations and the PCSK9 expression was associated with hypercholesterolemia (Figure 1).

A gene in which mutations can enhance or lower LDL-C

Because gain-of-function mutations in the PCSK9 gene are associated with increased LDL-C, it was hypothesized that inactivation of the PCSK9 gene might cause an increase in LDL receptor activity, resulting in accelerated LDL clearance and a decrease in the plasma level of LDL-C. In 2005, this hypothesis was indeed confirmed by the establishment of an association between very low plasma LDL-C levels and two nonsense mutations in the PCSK9 gene (Y142X and C679X, that caused the replacement of an amino acid by a stop signal at codon 142 or 679 resulting in truncated protein) in 33 out of 1769 African Americans of the Dallas Heart Study cohort (63±23 mg/dl versus 105± 37 mg/dl, respectively, meaning a reduction in LDL-C of ~ 40 %.) (Cohen 2005) (Figure 2). Another PCSK9 gene missense (Arginine replaced by leucine or R46L) variation was also found in whites and was associated with a reduced level of LDL (reduced by about 15 %). In the individuals who were carrying the mutation, neither endogenous biosynthesis nor the intestinal absorption of cholesterol was altered, suggesting that PCSK9 loss-of-function mutations increase the functionality of LDLR (Cohen 2005) (Figure 1).

This hypothesis was definitively established by the production and characterization of PCSK9 (-/-) mice. Compared with controls, these mice presented a threefold increase in hepatic LDLR expression and LDL plasma clearance rates (Rashid 2005). The researchers deleted the PCSK9 gene in the mice. On average, mice lacking the PCSK9 gene had blood LDL-cholesterol levels of 46 milligrams per decilitre, while wild-type mice had levels around 96 mg/dl, a difference of 52 per cent. They had...
also higher levels of LDL-receptor protein expressed on the cell membrane, and were thus able to take up more LDL particles from their blood. Curiously they had no change in the level of messenger RNA, suggesting that the control of the LDL receptor by the PCSK9 gene occurs after translation into protein.

Lowering LDL – Not only the lower, the better but also the earlier, the better

These mutations made it possible to deal with the classical question of atherosclerosis prevention in a new way. How would having a lower level of circulating LDL-C for genetic reasons affect the risk of having heart disease? To answer this question, Helen Hobbs’ team (Cohen 2006) assayed the newly identified PCSK9 mutations associated with lower plasma cholesterol levels in another large population study, the Atherosclerosis Risk in Communities (ARIC) study, a 15-year study where a large proportion of the individuals (N=12,887 subjects) had multiple additional risk factors for heart disease (hypertension, diabetes, low HDL-C, smoking).

Specifically, they compared the incidence of coronary heart disease (including myocardial infarction, fatal coronary heart disease) according to the presence or absence of variants of the PCSK9 gene. Cohen et al discovered two nonsense variants in 2.6% of all black subjects. These variants were associated with an average 28% reduction in levels of LDL-cholesterol, producing a 88% decrease of CHD in the African Americans (Figure 3). In fact, only one black patient with mutant PCSK9 genes had CVD but this exception could be attributed to the multiple risk factors (smoking, obesity, diabetes, hypertension, raised lp(a)). The Caucasians with the missense mutation in the PCSK9 gene had a 15 percent reduction in LDL-C and a 46 percent reduction in events (Cohen 2006) (Figure 3).

These data confirmed the importance of LDL levels in the development of heart disease and that a moderate lifelong reduction in the plasma level of LDL-cholesterol is associated with a substantial reduction in the incidence of coronary events, even in populations with a high prevalence of non-lipid-related cardiovascular risk factors. But the conclusion goes further. The magnitude of the reduction in heart disease found in association with these PCSK9 sequence variations was much greater than one could have anticipated on the basis of clinical trials using cholesterol-lowering drugs such as statins, where the 5-year reduction in events is comparable to the reduction in LDL-C level (1% LDL-C reduction = 1% CVD reduction). These data suggest that, if we were to initiate cholesterol-lowering therapy earlier in life, it might greatly increase the benefits of treatment.

Some lucky guys in circulation? (Don’t know what is meant. Perhaps “some people are luckier than others”? Girls are not guys, they’re dolls!)

We should notice that the different loss-of-function mutations found in Cohen et al’s paper were quite frequent in the population (about 1%). More common sequence variations (called polymorphisms) in the PCSK9 gene have also been found in the population. (Not clear here whether the two “populations” are study populations or the general population. The use of tenses would suggest the first is a study population, the second the general population) Amongst the 93 non-coding single-nucleotide polymorphisms (SNPs) at the PCSK9 locus (Kotowski 2006), some have already been studied for their effect on the cholesterol level in the general population.

In Japan, two variants in intron 1 [C(-161)T] and exon 9(I474V) of PCSK9 have been linked to plasma LDL-C (Shioj 2004) and in the US, one variant in exon 12 (E670G) has been correlated with LDL-C and the severity of atherosclerosis (Chen 2005). Thus, a spectrum of sequence variations, ranging in frequency from 0.2% to 34% and in magnitude of effect from a 3% increase to a 49% decrease, contributes to inter-individual differences in LDL-C levels, suggesting that PCSK9 activity is a major determinant of plasma levels of LDL-C in the human population.

Although no homozygous hypercholesterolemic PCSK9 mutant has yet been identified, the first compound heterozygote for two inactivating mutations in the PCSK9 gene was discovered a few weeks ago (Zhao September 2006) in a healthy, fertile college graduate, with a strikingly low plasma level of LDL-C (14 mg/dL). The very low plasma level of LDL-C and apparent good health of this individual demonstrate that the PCSK9 gene plays a major role in determining the plasma levels of LDL-C and provides an attractive target for LDL-lowering therapy.
Statins and PCSK9

Each time that we prescribe a statin, the hepatic cells of our patients increase their LDLR expression (via an increase in LDLR mRNA levels), which causes an increase in the plasma LDL clearance rate in humans. What is common in humans does not occur in mice. In normal mice, statin administration sharply increases LDLR mRNA levels but does not increase cellular LDLR protein levels or LDL clearance. To make the reaction of mice resemble that of humans, we must render the mice defective in the PCSK9 gene by gene knockout (PCSK9 (-/-) mice). They then become hypersensitive to statins and there is a strong increase in LDL clearance (Rashid 2005).

What happens is that statins, inhibitors of cholesterol synthesis, not only upregulate the expression of the LDL receptor but also PCSK9 expression (Figure 4). These findings imply that statins might simultaneously reduce the quantity of the LDLR protein and this was discovered to be so 10 year ago (Ness 1996). Now we know that it occurs through the PCSK9 induction (Dubuc 2004). PCSK9 induction is caused through the statins stimulating the activity of a protein called SREBP-2, the same protein that activates the creation of more LDL receptors. SREBP-2 would appear therefore to be a booster of LDL receptor activity and of the activity of the PCSK9 protein, which degrades these receptors (Figure 4).

This observation in mice may have a potential application in patients “resistant to statins”, perhaps because associated with statin there is excessively high PCSK9 activity or an excessively great increase in PCSK9 activity or because LDL goals are simply not reached with statins alone. A pharmacological inhibitor for the PCSK9 gene, administered in combination with statins, might be of great value for these patients. Whereas statins inhibit the biosynthesis of cholesterol, which subsequently increases the transcription of the LDLR gene to LDLR mRNA, selective PCSK9 inhibitors would directly enhance the LDLR protein levels (Figure 5).

Still much mystery about PCSK9

Genetics establishes a relationship between a gene and a phenotype, but does not necessarily provide information about mechanisms. Many enigmas remain regarding the PCSK9 gene.

The first is how the PCSK9 gene affects the quantity of LDL receptors. It decreases the amount of the LDL receptor protein, but not its mRNA. The PCSK9 gene is a proprotein convertase but at present the only known substrate for PCSK9 is PCSK9 itself and the LDL receptor does not appear to be a substrate for the protease.

The second is whether all of the lipoprotein phenotypes seen in patients with mutant PCSK9 can be explained by the deficiency in the quantity and/or activity of LDL receptors. Compared with “traditional” FH patients carrying severe heterozygous mutations on their LDLR, hypercholesterolemia in 13 patients with the D374Y substitution on the PCSK9 gene was more pronounced and associated with borderline high-plasma triglycerides (Naoumova 2005). Whether or not there is an increase in VLDL apoB production in FH patients is more controversial (Sun 2005). Oougueurram et al recently studied lipoprotein kinetics in two subjects with the S127R mutation on the PCSK9 gene and found only a 30% reduction in the fractional clearance rate (FCR) of LDL but a 3-fold increase in the production rate of VLDL apoB, along with a 2-fold increase in the production rate of LDL apoB (Oougueurram 2004).

This does not necessarily indicate that APOB production is the main mechanism in the raising of LDL-C in PCSK9 mutation. Such a large reduction (-35%) in the clearance rate of LDL is actually also found in heterozygotes (-65% in homozygotes) (Bilheimer 1979) and many studies have reported an increase in apoB production in FH due to an LDL R defect, especially in null allele or binding-defective LDL receptors (but not necessarily in those with receptors that stall in the secretory pathway) (Twisk 2000).
However, the demonstration that the PCSK9 gene modulates the overall metabolism of apoB-containing lipoprotein via direct alteration of VLDL re-uptake and/or production could mean that it also has a potential role in the metabolic syndrome, because the overproduction of VLDL is a hallmark of insulin resistance and Type 2 diabetes (Costet 2006).

Finally, PCSK9 is expressed in the brain. A gene called NARC1 was first discovered as being a gene possibly implicated in the differentiation of cortical neurons (Seidah 2003). Following the work of Abifadel et al it became evident that this gene plays its major role in lipoprotein metabolism. However, its role in neurogenic ontogeny and possible implication in pathologies have yet to be explored.

Conclusion

1. The discovery of variations in the PCSK9 gene causing hypercholesterolemia provides a new clue to the cause of certain kinds of familial hypercholesterolemia, not associated with LDLR or APOB mutations.

2. The discovery of variations in the PCSK9 gene causing reductions in plasma levels of LDL-cholesterol could lead to new strategies for the prevention of atherosclerotic cardiovascular disease. This finding is important because researchers previously thought there would be no new way of exploiting the LDL receptor pathway as a means to lowering LDL-cholesterol. On the basis of the current evidence, the PCSK9 gene is a promising therapeutic target in the treatment of hypercholesterolemia. Our present evidence suggests that new drugs targeting the PCSK9 gene may be able to act in conjunction with statin drugs to further lower LDL-cholesterol levels. New cholesterol-lowering drugs based on blocking the PCSK9 gene could also be effective on their own, so providing another option for individuals unable to take statins. If the PCSK9 gene is involved in APOB production, it may also be a potential therapeutic target for the dyslipidemias observed in metabolic diseases or diabetes.

3. The PCSK9 gene is not the first protein found to regulate the function of the LDL receptor. The protein responsible for autosomal recessive hypercholesterolemia (ARH), the “ARH adaptor protein”, was also shown to be essential for the internalization of the LDL receptor in hepatocytes (but not fibroblasts) by binding the cytoplasmic tail of the LDL receptor and promoting LDLR clustering into clathrin-coated pits on the basolateral membrane of hepatocytes (Garcia 2001, Eden 2002, Jones 2003, Garuti 2005). This protein regulates the ability of the receptor to bind to LDL via apoB, but not b-VLDL via apoE (Michaely 2004). Together with the ARH adaptor protein, the PCSK9 protein extends the picture to give a much more complex representation of the LDL receptor and its role in regulating LDL-cholesterol and intracellular cholesterol. Such a complex system usually makes it possible to keep the body’s levels of metabolites (like cholesterol) fine-tuned and to minimise big swings in the cholesterol content of normal cells, since too much or too little cholesterol can damage or kill cells.

4. The findings of reduced cardiovascular disease amongst patients with the mutant PCSK9 has public health implications. It suggests that earlier introduction of therapy to lower LDL cholesterol levels could be of great benefit. Of course, there is a difference between genetic effects on LDL cholesterol and pharmaceutical effects. Genes help keep a person’s LDL cholesterol levels low from birth while statins are usually only prescribed after cholesterol levels have become too high. There are a number of solutions, such as prescribing statins drugs earlier, combining statin therapy with other lipid-lowering drugs like ezetimibe to decrease LDL-C levels even further or, in the future, combining the therapy with drugs that target the PCSK9 gene. But the simplest solution is perhaps to concentrate our efforts on encouraging children and young adults to follow a heart-healthy diet and lifestyle that will help keep their cholesterol levels low for most of their lives.

As mentioned by Professor Helen Hobbs recently in her lecture at the ISA meeting in Rome, the practical home message suggested by all these studies is that when managing our patients for cardiovascular prevention, we are perhaps still doing “a little too little” and “a little too late”.

Thanks

I would like to take the opportunity in this paper to thank Professor Helen Hobbs for having accepted me for his post-doctoral fellowship from 1990-1993 and for helping me in the work carried out and providing advice during the following years. Helen Hobbs is currently Professor of Internal Medicine and Molecular Genetics, Director of the Eugene McDermott Center for Human Growth and Development and of the Donald W. Reynolds Cardiovascular Clinical Research Center at the University of Texas Southwestern Medical Center at Dallas. The three figures 1, 2, and 5 have been kindly provided by her and were part of her slides during her most interesting presentation at the International Atherosclerosis Society meeting in Rome in June 2006. During this meeting, she was honoured for her outstanding contribution to the understanding of the genetic causes of lipoprotein diseases. Figure 3 has been adapted from a paper by Jonathan Cohen (Cohen 2006). Figure 4 is a personal conceptual illustration. The two tables, Figure 4 and a great part of the text are taken from my PhD.

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