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The PCSK9 story

The ongoing PCSK9 adventure is an outstanding example of how human genetic studies can lead to a new therapeutic target and facilitate drug development. It started with the discovery by Abifadel *et al*. (2003) that mutations in *PCSK9* were the cause of cases of autosomal dominant familial hypercholesterolaemia (FH).[1] This seminal paper published in Nature Genetics was the first to link PCSK9 with cholesterol metabolism and its mutations to the cardiovascular complications deriving from elevated LDL-C.

This breakthrough paved the way to a plethora of studies and to a formidable mobilization of academic and pharmaceutical research teams worldwide resulting in, 13 years later, over 1155 publications on PCSK9 (*Figure 1*) and the development of a new class of lipid lowering drugs known as PCSK9 inhibitors. In the summer of 2015 Last summer, two anti-PCSK9 monoclonal antibodies (mAbs) received FDA and EMA approval: Praluent® (active ingredient: Alirocumab, by Sanofi-Aventis and Regeneron Pharmaceuticals Inc.) and Repatha® (active ingredient: Evolocumab by Amgen Inc.). These drugs were approved by the FDA both for 'use in addition to diet and maximally tolerated statin therapy in adult patients with heterozygous familial hypercholesterolemia or patients with clinical atherosclerotic cardiovascular disease such as heart attacks or strokes, who require additional lowering of LDL cholesterol' (FDA releases).[2,3]

This inspiring story of PCSK9 with its different chapters has become a reference worldwide for its exemplarity in demonstrating:

- the power of non-hypothesis driven genetic research strategies ('reverse genetics') to reveal major unknown players in human biology
- the inestimable contribution to research of networks of committed clinicians through high-quality phenotyping of patients and their extended families
- the competitive advantage of interdisciplinary collaborations
- the importance of inherited models of human diseases in providing new drug targets as well as validating efficacy and safety of medications aimed at the new target.

Next to these positive aspects should unfortunately be added. The lack of truthfulness in retracing the PCSK9 history with the downplay and non-referencing of our seminal pivotal work that launched all subsequent investigations in the field but was not given the recognition it deserves. For evidence of this, see references.[4,5]

The PCSK9 story started by the proposition of a paradigm shift: FH could be associated with mutations in other genes beyond the genes encoding the LDL receptor and its ligand apo B (*APOB*). To test this hypothesis, the French National Research for FH (chaired by C.B.) recruited numerous multigenerational families, a significant subset confirming the original hypothesis on the role of an unknown mutated gene. Genomewide search to map a new FH gene ('FH3') was than undertaken.

In 1999, our team mapped the FH3 gene on the short arm of chromosome 1 (at 1p32).[6] This was followed by rigorous and extensive molecular work. At this pre-genomic era, this represented construction of a detailed physical map of the region followed by extensive sequencing of >50 regional genes.[1] In parallel, further large families were recruited and extensively studied enabling replication and refining of the original localization.[1]

Also, a collaboration was initiated with Nabil G. Seidah, an expert in the field of proprotein convertases (PCs) at the IRCM in Canada, who had just identified in a patented database NARC-1, a new PC highly expressed in the liver,[7] encoded by a gene on chromosome 1p32. We demonstrated that the precise localization of the NARC-1 gene was in the FH3 candidate interval and identified its first two gain-of-function (GOF) mutations in three FH probands and their families.[1] The first mutation p.S127R was found in two apparently unrelated multigenerational French families, one from Nantes and the other from Dijon. In both families, high LDL-cholesterol levels were associated with overt cardiovascular disease (MIs and strokes). The second mutation, p.F216L, was identified in a third family from the Paris area and in which the proband died from myocardial infarction at the age of 49 years with an LDL-cholesterol level of 356 mg/dL. While publishing these results and in agreement with the HUGO Gene Nomenclature Committee, we baptized the gene *PCSK9*. Thus we discovered the clinical role of PCSK9, its link to cholesterol metabolism and related cardiovascular disease.[1]

The confirmation of our results occurred shortly after by the report of the third mutation of *PCSK9*, p.D374Y in the Utah kindred with which confirmation of our mapping of FH3 at 1p32 had been independently replicated.[8] Subsequently, other GOF mutations were described in Norway and different regions around the world (reviewed in Ref. 9). A decade later, new GOF mutations are very rarely identified despite systematic screening of the *PCSK9* gene for molecular diagnosis of FH.

Following our genetic studies, the role of PCSK9 on LDL receptor and apoB was revealed by our teams[10,11] and several others[12,13] while elucidating the impact of GOF mutations through patient-cell analysis, mutagenesis and cellular analysis, adenoviral transfection as well as animal studies. These studies showed that PCSK9 regulates the number of LDL receptors on cell surfaces. Subsequent crystal structure analyses revealed the direct interaction between PCSK9 at the level of its catalytic domain and a region of the extracellular region of the LDL receptor (the EGF-like A domain, directly below the apo B binding domain).[14,15]

After a new gene is discovered in hyper cholesterolaemia, searching for mutations associated with low LDL-cholesterol levels is the obvious next step since it is well known that missense mutations in the *APOB* gene result in hypercholesterolemia while truncating/loss-of–function (LOF) mutations result in hypocholesterolaemia.

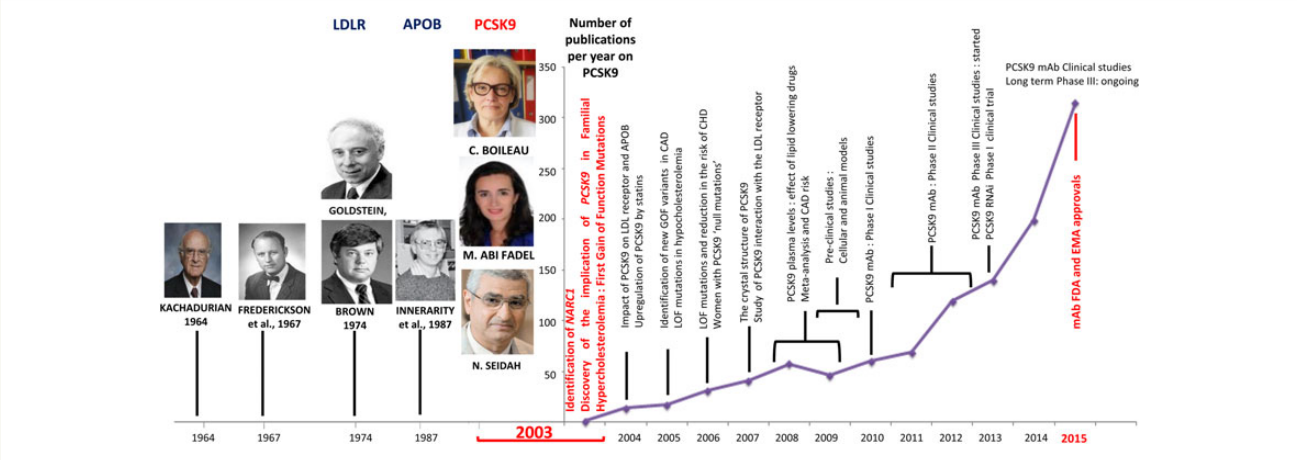

Figure 1 Autosomal dominant hypercholesterolaemia and the PCSK9 adventure.

Thus, 2 years after our pivotal paper linking PCSK9 to the world of cholesterol and to hypercholesterolaemia, mutations in *PCSK9* were reported in hypocholesterolaemia. Two nonsense mutations: p.Y142* in exon 3 and p.C679* in exon 12 were found in 2.6% of the African-Americans subjects of the Dallas Heart Study (a multiethnic population of Dallas County) and the Atherosclerosis Risk in Communities.[16,17] These mutations were associated with a 28% reduction in mean LDL-C level and an 88% reduction in the risk of CHD.[17] In the USA, one in every 50 African American has a nonsense mutation in *PCSK9*.[16,17] These LOF mutations were found at the same high frequency in a Nigerian population[18] as well as in 3.7% of African women from Zimbabwe and associated with a 27% reduction in LDL-C levels.[19] Thus, contrary to the very rare GOF mutations in the *PCSK9* gene, these LOF mutations are very frequent not only in populations of African ancestry but also in European Caucasians as evidenced through the identification in the latter of the p.R46L variant, associated with a 15% reduction in LDL-C levels and a 47% reduction in the risk of CHD.[17,20] These results confirmed our claim that PCSK9 was a new drug target and that its targeting would result in the lowering of circulating LDL-C levels and consequently on their associated cardiovascular complications. Finally, the safety of this possible therapy was conclusively demonstrated when human 'knock-outs' of the *PCSK9* gene were reported: a woman homozygous for the p.C679* mutation and a compound heterozygote carrying the p.Y142* mutation and an in-frame 3-bp deletion that deletes an arginine at codon 97. Both women presented very low LDL-C levels and no immunodetectable blood PCSK9. They were completely healthy and fertile thus showing that hypocholesterolaemia due to a deficiency of PCSK9 appears to be benign, with no complication.[22]

PCSK9 mutations and polymorphisms and their clinical implication have been thoroughly studied since 2003 in different populations and in different diseases especially cardiovascular diseases[20,21] (myocardial diseases, peripheral arterial diseases, etc.). The regulation of PCSK9 expression and its circulating levels are well documented.[9,23] It is also established that several antihyperlipidaemic drugs such as statins, fibrates, and ezitimibe induce an increase of PCSK9 levels.[24] This might attenuate their cholesterol-lowering effect by reducing LDL receptor abundance at the cell surface. Thus, it was suggested and proved that a combination of a statin with a PCSK9 inhibitor enhance reduction of cholesterol levels.[25,26]

Clinical trials have been launched using RNA interference or antibodies approaches to reduce PCSK9. mAbs that bind selectively to extracellular PCSK9 and prevent its interaction with the LDL receptor are the most advanced and tested approach to PCSK9 inhibition to date. Several humanized mAbs targeting PCSK9 are currently being studied in large phase III clinical trials: alirocumab[27] (Regeneron/Sanofi) and evolocumab[28] (Amgen Inc.), two fully mAbs are nearing the completion of their phase III development programme and have received FDA approval.[2,3] These antibodies given by subcutaneous injection cause a large reduction in LDL-cholesterol levels.[27,28] Long-term studies and meta-analyses that involve 20 000 patients for evolocumab and for alirocumab will provide results regarding the long-term efficacy, safety, and tolerability of these anti-PCSK9 antibodies and their cardiovascular outcomes. These are eagerly awaited.

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What have we learned so far from the biology of PCSK9?

The discovery of PCSK9 and its relation to hypercholesterolaemia via its ability to enhance the degradation of the low-density lipoprotein receptor (LDLR) has been an unexpected blessing for the treatment of cardiovascular diseases, and likely other pathologies.

PCSK9 was discovered in 2002 during our search for new secretory proteases implicated in the activation/inactivation of polypeptide hormones and protein precursors. PCSK9 turned out to be the last enzyme of a nine-membered family of mammalian secretory proteases, known as the *P*roprotein *C*onvertases, related to bacterial Subtilisin and yeast Kexin (PCSKs).

Each member of the family has specific biological functions affecting many tissues and cells in the organism, ranging from endocrine and neural functions, to differentiation and growth, as well as reproduction and metabolism. Little did we know that PCSK9 would turn out to be a major regulator of circulating LDL-cholesterol (LDLc) in a non-enzymatic fashion, likely ensuring that cholesterol is distributed to other extrahepatic tissues and at the same time restricting the influx of LDLc in hepatocytes.

Although we originally did not identify PCSK9 only based on its function in liver, the overwhelming genetic, biochemical, epidemiological, and clinical data now clearly support the role of PCSK9 as a powerful regulator of liver LDLR, and hence of circulating LDLc. The LDLR-regulation by PCSK9 has revolutionized the field of LDLc metabolism, resulting in a paradigm shift in the treatment of hypercholesterolaemia.

This led health authorities in North America and Europe at the end of 2015 to approve the prescription of injectable monoclonal antibodies (mAbs) that inhibit the activity of PCSK9 on the LDLR (Repatha [Evolocumab] & Praluent [Alirocumab]). Subcutaneous injections of these mAbs every 2 weeks or monthly, result in an average of ~60% reduction in the levels of circulating LDLc, irrespective of the starting LDLc levels.

The present front-line indication is for patients suffering from severe hypercholesterolaemia who are statin-resistant and/or cannot reach target LDLc using available drugs. These unexpected, yet exciting PCSK9 translational applications remind us of the adage of Christian de Duve who discovered lysosomes: 'If chance offers you a clue, follow the trail. You may not discover what you were looking for, but what you discover may be more interesting'.

However, there remain important gaps in our understanding of the biology of PCSK9, its cellular trafficking and tissue-specific mechanism of action leading us to predict a significant potential for discovery of PCSK9 targets other than the LDLR, and the mechanistic details of PCSK9-enhanced degradation of specific receptors. Such future advances will likely lead to novel and safe therapeutic applications in cardiovascular diseases and inflammatory conditions.

Below we summarize selected future directions of PCSK9 research and the expected clinical benefits that could result from new biological findings that may ensue.

(1) It is amazing that 12 years after the discovery of PCSK9 no sorting mechanism(s) emerged to explain its ability to induce in a tissue-specific manner the degradation of the LDLR or other newly identified PCSK9-targets, such as the VLDLR, ApoER2 (LRP8), and the fatty acid transporter CD36 in late endosomes/lysosomes. It is believed that a putative protein(s) binds the [PCSK9 ≡ LDLR] complex at the cell surface and/or early endosomes & escorts it to lysosomes for degradation by undefined resident hydrolases, possibly implicating the Alzheimer's disease associated γ-secretase in the initial solubilization of this membrane-bound complex in acidic endosomes.

It was reported that binding of PCSK9 to the ER-chaperone GRP94 protects LDLR from precocious intracellular degradation, likely by preventing early binding of PCSK9 to LDLR within the endoplasmic reticulum (ER). Furthermore, efficient exit of PCSK9 from the ER of cells before its secretion seems to require an undefined membrane-bound transporter that binds PCSK9 in the lumen of the ER and the adaptor protein sec24a in the cytosol. Thus, the identification and validation of PCSK9-partners and modulators that regulate its ability to enhance the degradation of specific receptors is expected to unravel the so far obscure cellular mechanism(s) of action of this fascinating protein.

(2) PCSK9 expression is highest in adult liver hepatocytes, the major source of circulating PCSK9. It is dramatically reduced in the presence of excess cholesterol in the circulation. Thus, it was not surprising that PCSK9 primarily enhances the degradation of liver LDLR to decrease the influx of LDLc, and in its absence, excess LDLc is efficiently cleared by the liver LDLR. However, the role and/or targets of PCSK9 in extra-hepatic tissues that synthesize PCSK9, although at lower levels, e.g. small intestine, kidney, β-cells of the pancreas, and cerebellum, are still largely unknown.

In addition, circulating PCSK9 can regulate the levels of certain receptors in other tissues by an endocrine mechanism. For example, PCSK9 enhances the degradation of LDLR, VLDLR, and CD36 in adipose tissues, thereby regulating fat accumulation in adipocytes. Whether these and other receptors are also targets of PCSK9 in tissues that do not endogenously synthesize this protein, e.g. heart, vascular endothelial cells and macrophages, is not yet clear.

(3) Like humans, mice lacking PCSK9 exhibit very low levels of LDLc. In these knockout (KO) mice, the fraction of the LDLR present at the cell surface of hepatocytes seems to be regulated in a sex-dependent manner. In the liver of male and female PCSK9 KO mice, a similar increase in the total amount of LDLR was observed. However, its subcellular distribution differed in a sex-dependent manner: cell surface levels of the LDLR were dramatically increased in males, but not female KO mice.

Oestrogens (17β-estradiol; E2) were shown to be responsible for the sex-dependent cell surface accumulation of the LDLR in liver and VLDLR in adipocytes. A working model is that the 'eraser' effect of PCSK9 on cell surface LDLR or VLDLR

is dominant, and that PCSK9 masks a mechanism by which E2 regulates the surface levels of these two receptors. If this E2-mediated regulation has its counterpart in humans and leads to a lower LDLR activity, the LDLc uptake by the liver may differ in hypercholesteraemic men and premenopausal women who receive therapeutic PCSK9 mAbs. An extensive analysis of available or future clinical data concerning the possible sex-dependent efficacy of PCSK9 mAbs should be informative.

(4) Sepsis is a complex disease characterized by systemic activation of inflammation and coagulation, most commonly in response to bacterial infection. Severe sepsis accompanied by dysfunction of at least one organ affects ~0.3–1% of individuals/year in the USA with mortality rates of ~30%. It has been suggested that improving microbial clearance, and thus reducing the spread of infection, minimizes the severity of sepsis.

Since LDLR KO mice exhibit increased mortality in a caecal ligation and puncture sepsis model, this suggested that LDLR plays an important role in sepsis and associated septic shock, likely by the clearance of excess toxic lipopolysaccharides (LPS) circulating in pathogen-infected individuals. This is supported by findings of lower levels of circulating LPS in bacterially infected individuals exhibiting loss-of-function (LOF) PCSK9 mutations (e.g. R46L), suggesting a protection against septic shock via more effective endotoxin clearance.

Administration of mAbs to PCSK9 may thus protect against sepsis, and familial hypercholesteraemic patients including those harbouring PCSK9 gain-of-function (GOF) mutations may be more prone to develop septic shock. Clinical trials directly testing this possibility are needed.

(5) Most of the biology of PCSK9 has been deduced from cell lines or animal models, as research on human liver is rather limited. However, recently it was shown that somatic cells isolated from the urine of normal and PCSK9 GOF or LOF mutations can be reprogrammed into human-induced pluripotent cells (hiPSCs) using episomal vectors.

Upon expression of a cocktail of differentiating factors, undifferentiated hiPSCs were efficiently differentiated into hepatocyte-like cells (HLCs). Compared with control HLCs, those derived from a PCSK9 GOF S127R individual exhibited an ~70% decrease LDLc uptake. In contrast, HLCs derived from an individual with double heterozygote PCSK9 LOF R104C/V114A displayed an ~2-fold increased LDLc uptake.

Thus, urine (or skin) samples provide an attractive and convenient source of somatic cells for reprogramming and hepatocyte differentiation. This also represents a powerful tool to further decipher the effect of mutations of human PCSK9, and likely LDLR or other receptors, during early differentiation of human hepatocytes and in adult liver cells, establishing a powerful patient-related model to further decipher PCSK9 mechanisms and functions, and to validate pharmacological inhibitors of PCSK9.

(6) There is substantial evidence supporting elevated LDLc and triglycerides as risk factors leading to coronary artery disease progression, accelerating the development of myocardial infarction (MI). The present outcome data from a limited number of patients suggest that after at least 1 year of treatment with PCSK9 mAbs an impressive ~50% reduction in major adverse cardiovascular events is observed.

If these numbers hold true when the outcome data on larger cohorts become available by 2016/2017, then targeting PCSK9 may be widely recommended for cardiac patients at risk of developing MI. Relative to statins in randomized trials, PCSK9 inhibitors have produced greater reductions in LDLc and have been at least as well tolerated.

Whether used as alternatives to statins or in combination with statins, PCSK9 inhibitors appear likely to substantially increase the proportion of patients at risk of cardiovascular events who achieve maximum protection through LDLc control. The potential for these agents to redefine optimal LDLc levels in patients at high risk of CVD is likely to be a focus in future clinical trials.

Conclusions

Much remains to be discovered regarding the cellular trafficking mechanism of PCSK9 together with its targeted receptors and binding to other proteins. This is definitely a very exciting period in the field of dyslipidaemia, where thanks to new PCSK9 silencing therapies LDLc levels can be lowered to unprecedented low levels. This is definitively a good outcome for hypercholesteraemic and/or cardiac patients who do not reach target levels of LDLc with the available medications. Finally, our understanding of the function and biology of PCSK9 in extra-hepatic tissues is still lacking and will require much more investigations in the future, possibly leading to new and unexpected discoveries of the multiple facets of this amazing protein.

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Nabil G. Seidah PhD

A world renowned scientist for his work on the convertase family of proteins

Nabil G. Seidah is Professor of Biochemistry, Director of the Clinical Research Institute of Montreal (IRCM) laboratory of Biochemical Neuroendocrinology and holds the Canada Research Chair in Precursor Proteolysis. He has been at the forefront of protease research.

Dr Seidah obtained his BSc in 1969 from Cairo University in Giza, Egypt, and his PhD in Biophysics in 1973 from Georgetown University, in Washington, DC. In 1974, he started studying the processing of precursor proteins at the Clinical Research Institute of Montreal (IRCM) and in 1976 he co-discovered β-endorphin and largely contributed to the biochemical characterization of proopiomelanocortin (POMC), the β-endorphin precursor and other secretory precursor proteins. Since 1983, he has been the director of the IRCM's Biochemical Neuroendocrinology Laboratory.

Dr Seidah discovered and cloned seven (PC1, PC2, PC4, PC5, PC7, SKI-1, and PCSK9) of the nine known proteases belonging to the convertase family. During this period, he also greatly contributed to demonstrating that the proteolysis by the proprotein convertases is a wide mechanism that also concerns 'non-neuropeptide' proteins such as growth factors, α-integrins, receptors, enzymes, membrane-bound transcription factors, and bacterial and viral proteins.

In 2003, he identified PCSK9 and showed that point mutations in the PCSK9 gene cause dominant familial hypercholesterolaemia, because of a gain of function related to the ability of PCSK9 to enhance the degradation of cell surface receptors, such as the low-density lipoprotein receptor. He has since worked on the elucidation of the functions and mechanisms of action of PCSK9 and other convertases both in cells, mice and human patients. He is broadly interested in the understanding of the molecular/cell biology and in the translational applications of inhibitors of the proprotein convertases.

Dr Seidah is internationally recognized as a world leader in the proprotein convertases and their physiological roles in both health and disease states. His numerous publications that tally >700 peer reviewed manuscripts have been widely recognized, and in fact he is cited as the most recognized protease expert in Canada and 6th worldwide. Indeed, Pubmed cites N.G. Seidah as the topmost in Canada and the 1st of the worldwide 20 top scientists working on 'Proprotein Convertases' since 1971.

While many of the discoveries of Dr Seidah required extensive studies over many years, it must be said that the outcomes of his discoveries were not always as planned with serendipity playing a significant role. Indeed, his constant curiosity and alertness followed the adage 'if chance offers you a clue, follow the trail. You may not discover what you were looking for, but what you discover may be much more interesting'.

A. Tofield

The biology of PCSK9 inhibition: some unanswered questions

Cell biology of PCSK9

The serine protease PCSK9 is a secreted inhibitor of the LDL receptor (LDLR), mainly expressed by the liver. The PCSK9 precursor undergoes intra-molecular autocatalytic processing in the endoplasmic reticulum (ER). The resulting heterodimer is then routed towards the secretory pathway. Following secretion, PCSK9 binds to the EGF-A domain of the LDLR at the cell surface and is internalized together with the receptor by endocytosis. The affinity between LDLR and PCSK9 increases as a result of acidic conditions in endosomes.[1]

The interaction between PCSK9 and the receptor locks the LDLR in an open conformation, which precludes normal recycling of the LDLR to the plasma membrane and targets it for endo-lysosomal degradation[2] (*Figure 1*). GRP94 an ER-resident protein expressed in hepatocytes binds to PCSK9 and thereby prevents an early degradation of the LDLR within the secretory pathway.[3] Thus, PCSK9 is major circulating inhibitor of LDLR expression and function. As such, it has become a prime therapeutic target for lowering LDL cholesterol (LDL-C).[4]

Its unique mode of action has prompted the development of circulating PCSK9 inhibitors such as the two fully human anti-PCSK9 monoclonal antibodies Praluent® and Repatha®, both recently approved for clinical use to treat severe or statin-resistant hypercholesterolaemia.

Heterogeneity of circulating PCSK9: does it matter?

A significant proportion of PCSK9 circulating in the plasma is bound to apoB-containing lipoproteins.[5–8] Up to 40% of total PCSK9 in human plasma can be found associated with LDL, with a K_d of 160–320 nM.[5] The interaction of PCSK9 to LDL is a common event for PCSK9, however, the stoichiometry of the interaction suggests that it is a rare event for LDL, with only one in 500–1000 LDL particles carrying one PCSK9 molecule.[9]

Interestingly, although PCSK9 can bind apoB within hepatocytes,[10] it is not found associated with very low-density lipoproteins (VLDLs),[11] suggesting that the association between PCSK9 and

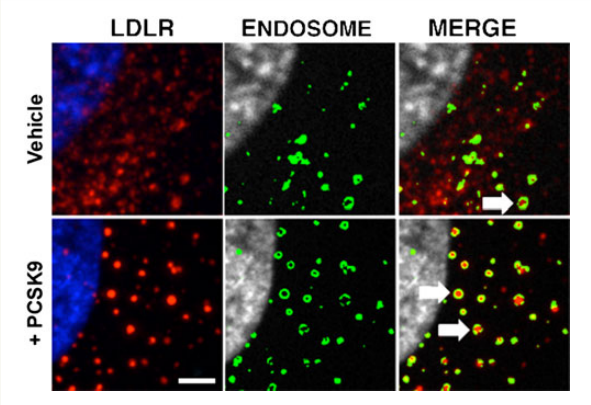

Figure 1 PCSK9 targets the low-density lipoprotein receptor toward endo-lysosomal degradation. Human dermal fibroblasts were grown in serum depleted culture conditions in the presence of 10 μg/mL mevastatin to maximally upregulate low-density lipoprotein receptor expression. Recombinant PCSK9 (300 ng/mL) or vehicle control was added to the culture medium for 10 min. Fibroblasts were then fixed, permeabilized, and visualized by confocal microscopy using fluorescent antibodies for the low-density lipoprotein receptor (clone C7 at 4 μg/mL [in red]), and Rab5 (PA3-915 at 1 μg/mL [in green]) a specific marker of endolysosomes. Nuclei were counterstained with DAPI (in blue). Arrows indicate the colocalization of low-density lipoprotein receptor within endo-lysosomes. Scale 1 μm.

apoB-containing lipoproteins occurs in the plasma and requires VLDL lipolysis to LDL. PCSK9 does not bind to chylomicrons or remnant lipoproteins (Tavori *et al.*, unpublished observation). It remains to be seen whether PCSK9 associates with lipoprotein (a) [Lp(a)].

The *in vivo* relevance of PCSK9 association with LDL was first shown in patients undergoing lipoprotein apheresis (LA), a dialysis procedure that removes apoB-containing lipoproteins from plasma in a matter of few hours. In addition to the >70% reduction in LDL, LA also reduces plasma PCSK9 levels by slightly >50%.[7–9,12,13] This is mainly, but not exclusively, due to the removal of apoB-bound PCSK9.[9] The combined loss of PCSK9 and LDL during apheresis may thus synergize to keep LDL-C levels down between LA treatments.

Another line of evidence underpinning PCSK9 association with LDL comes from studies of the two common PCSK9 forms: (i) the intact 62 + 13 kDa heterodimer, and (ii) the furin-cleaved 55 + 13 kDa heterodimer, a product of cleavage of the intact PCSK9 by furin in the circulation or at the surface of hepatocytes.[9,14–16] Most of apoB-bound PCSK9 is intact (62 + 13 kDa), and may therefore be the most physiologically active form of PCSK9, since furin-cleaved PCSK9 is generally considered inactive.[10] But *in vitro* binding of intact PCSK9 to LDL appears to inhibit PCSK9 action.[11] The significance of PCSK9 binding to LDL and its effect on the distribution of PCSK9 molecular forms and function therefore require further investigation.

Given the heterogeneity of circulating PCSK9, it is not surprising that correlations between plasma PCSK9 and LDL-C levels in cohorts are so low.[17] The PCSK9 ELISAs developed in research laboratories or those commercially available lack the ability to discriminate between the various forms of PCSK9 present in the plasma, such as the gain-of-function and loss-of-function variants, the intact and furin-cleaved forms, the forms differentially modified post-translationally through sulfation or phosphorylation, and the lipoproteins-associated and apoB-free forms.

It is not known whether apoB-bound and apoB-free PCSK9 equally affect the LDL receptor. Thus, new methods to quantify plasma PCSK9 form are needed, especially in the light of recent data showing that total plasma PCSK9 levels do not predict CVD event.[18]

PCSK9 inhibitors lower Lp(a), but how?

Lp(a) consists of a unique protein homologous to plasminogen, apolipoprotein (a), that is covalently linked to the apoB100 moiety of an LDL size particle by a unique disulphide bond. Lp(a) is widely regarded as extremely atherogenic.[19] Both statins and PCSK9 inhibitors act by increasing the abundance of LDLR at the surface of hepatocytes, thus lowering LDL-C levels.[20] But unlike statins,[21] PCSK9 inhibitors also promote a uniform as-yet unexplained 25–30% reduction in circulating Lp(a) levels.[22–24]

The molecular and cellular pathways governing apo(a)/Lp(a) hepatic production and Lp(a) cellular uptake and degradation are not well understood.[19] The potential role of the LDLR in Lp(a) clearance remains extremely controversial.[25,26] For instance, hepatoma cell lines use the LDLR for Lp(a) clearance,[25] whereas primary hepatocytes (Lambert *et al.* unpublished observation) and *in vivo* studies indicate that the LDLR is not involved in Lp(a) clearance.[27] In addition, PCSK9 was shown to affect apoB-containing lipoprotein production,[28–30] and it was suggested that PCSK9 inhibition would thereby reduce Lp(a) levels.

Finally, if PCSK9 associates with Lp(a) in the circulation, this could provide a possible mechanism for the reduction in Lp(a) caused by PCSK9 monoclonal antibodies via target-mediated or reticuloendothelial clearance. There is currently a clear need to fully elucidate how, unlike statins, PCSK9 inhibitors reduce circulating levels of Lp(a).

Is PCSK9 inhibition safe for the brain?

Because cholesterol is an essential component of the developing and adult brain, it has been suggested that very-low LDL-C levels could impact on brain functions and cognition.[4] The FDA advised for neurocognitive impairments assessment in phase III clinical trials of Praluent® and Repatha®. A slight but not significant increase in neurocognitive troubles such as amnesia, memory loss, and confusional states was observed in patients treated with both drugs.[22,24]

Given that (i) brain cholesterol mainly originates from endogenous synthesis, (ii) people with loss-of-function PCSK9 mutations do not apparently exhibit cognitive impairments,[31] and (iii) PCSK9 inhibitors are monoclonal antibodies that should not cross the blood–brain barrier, a significant impact of PCSK9 inhibitors on brain health

or cognition remains extremely unlikely. By improving arterial health, PCSK9 inhibitors could even potentially ameliorate cognition and reduce dementia.

What else do not we know?

The recent discovery of GRP94, a PCSK9 inhibitory binding protein that prevents LDLR premature degradation in hepatocytes[3] clearly underlines the possibility that PCSK9 function may be physiologically regulated by additional protein partners intracellularly or in the plasma. Besides the potential relevance of the various circulating forms of PCSK9, and besides the elusive molecular pathway by which PCSK9 inhibitors reduce Lp(a), there are several unanswered questions pertaining to PCSK9 function and to PCSK9 inhibition.

One series of questions relate to the putative consequences of very low LDL levels achieved with PCSK9 inhibitors not only on brain function but also on the risk of haemorrhagic stroke, cancer, and new-onset diabetes.

A second series of questions relate to the potential adverse effects of a massive upregulation of LDLR in tissues such as pancreatic β cells and hepatocytes where the LDLR may serve as an entry route for viral infections.

Finally, the physiology of PCSK9 in extra hepatic tissues (brain, kidney, intestine, pancreas, and steroidogenic tissues) in adults and during development needs to be fully addressed. The comprehensive study of PCSK9 gain-of-function and loss-of-function mutations carriers beyond just cardiovascular health appears invaluable in that respect.


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References

References are available as supplementary material at *European Heart Journal* online.

Professor Ulrich Laufs MD

Recently appointed new International Associate Editor of the *European Heart Journal* is currently researching the cellular actions of PCSK9

Ulrich Laufs was born in Göttingen and studied philosophy and medicine in Bochum. He completed his training at the University of Hamburg, where he did his medical thesis at the Institute of Pharmacology. After his residency at the University of Köln, he worked for 2 years at the Cardiovascular Division of the Brigham and Women's Hospital in Boston. In the lab of Jim Liao, he described the cholesterol-independent regulation of endothelial nitric oxide synthase by statins, a mechanism that contributes to the effects of statins in ischaemic stroke. Under the mentorship of Michael Böhm, he undertook his Fellowships at the University of Köln and the University of Saarland in Germany.

Ulrich Laufs is Professor for Clinical and Experimental Medicine and Vice Chairman of the Department of Internal Medicine, Cardiology, Angiology, and Intensive Medicine at the University of Saarland, Homburg, Germany. He has received numerous honours including a first place of the Young Investigator Award of the American College of Cardiology, the Population Sciences Award of the European Society of Cardiology, the Paul-Martini-Award, and the Albert-Fränkel-Award of the German Cardiac Society.

Ulrich Laufs serves on several editorial boards and as a member of the Drug Commission of the German Medical Association (Arzneimittelkommission der Deutschen Ärzteschaft) and the Federal Union of German Associations of Pharmacists (Arzneimittelkommission der Deutschen Apotheker). He is chairman and member of the nucleus of the working groups on heart failure, heart and brain and on prevention of the German Society of Cardiology.

Prof. Laufs works as an interventional cardiologist. He runs a specialized clinic for lipid disorders and heads a research group with special focus on the cellular and molecular mechanisms and clinical studies for cardiovascular prevention. Ulrich Laufs enjoys the combination of acute and interventional patient care with cardiovascular prevention and translational research. His current research is focused on:

- Vascular biology and cardiovascular prevention. His group has a special interest in the study of the molecular effects of physical activity, such as the molecular regulation of cellular senescence. He has a longstanding interest in the vascular biology of ischaemic stroke.

– Clinical and experimental research in lipid metabolism. The group has characterized several cholesterol-independent effects of statins mediated by isoprenylation of small G proteins. Currently, his group is interested in the cellular actions of triglycerides, plant sterols, and PCSK9.
– Pathophysiology of myocardial remodelling and fibrosis. Ulrich Laufs heads a research consortium funded by the Deutsche Forschungsgemeinschaft that elucidates the signalling during adaptive and maladaptive myocardial remodelling, a topic that is closely related to his projects addressing small G protein function and physical activity.
– Cardiovascular pharmacotherapy. The clinical trial unit of his research group conducts investigator initiated studies with a special interest in topics related to medication adherence and participates in multi-centre trials.

Andros Tofield

The annual Northwestern Cardiovascular Young Investigators' Forum in Chicago

For many years, the Feinberg School of Medicine of the Northwestern University in Chicago, Illinois (*Figure 1*) has organized a cardiovascular forum for young investigators. The research prize of the Forum is awarded to the best young investigators engaged in either basic science or clinical research, respectively. A distinguished faculty of established basic scientists and cardiologists from different universities, mainly from the United States, select the best candidates from a large number of presenters.

The 11th Annual Northwestern Cardiovascular Young Investigators' (CVYI) Forum held at the Wallerton Hotel in Chicago on 15–18 October, 2015, was again chaired by Dr Robert O. Bonow, Goldberg Distinguished Professor of Cardiology at Northwestern University Feinberg School of Medicine in Chicago. To select the best candidates of the year, three professors of medicine George L. Bakris, Elisabeth M. McNally, and Clyde W. Yancy from the University of Chicago were part of the faculty and assisted by colleagues from other US Universities such as C. William Balke and Douglas L. Mann both from St. Louis, Joseph A. Hill from the University of Texas Southwestern Medical Center in Dallas, Eric T. Peterson from Duke and F. Thomas Elly from Baltimore. In 2015, for the first time ever, a European cardiologist, Thomas F. Lüscher, editor-in-chief of the *European Heart Journal*, was invited to join the faculty. (*Figure 2*).

Forty-four Fellows and junior faculty from diverse US universities, mainly from Northwestern, John Hopkins, Duke, Harvard, and Vanderbilt among others, presented and also for the first time four European young investigators from the Czech Republic and Italy were part of the competition. Furthermore, several of the presenters were from Europe and had spent their postdoctoral time at one of the prestigious US universities.

The *Best Presenter Wards* were given to the top three Fellows in basic science and in clinical science. The 2015 winners in basic science were Kevin R. King, Boston (1st prize; 1500 $), Vimal Ramjee, Philadelphia (2nd prize; 1000 $), and Geoffrey W. Cho, Dallas (3rd prize; 500 $). In clinical science, these were Pradeep Natarajan, Boston (1st prize), Anneline S.J.M. te Riele, Baltimore (2nd prize), and Carey E. Tabit, Chicago (3rd prize). The junior faculty winners in basic science were Lisa Wilsbacher, Chicago (1st prize), Ravi Karra, Durham (2nd prize), and Ravi Ranjam, Salt Lake City (3rd prize) and in clinical research David Zidar, Cleveland (1st prize), Tara I. Chang, Stanford (2nd prize), and Melissa A. Daubert, Durham (3rd prize).

Figure 1 The Feinberg Northwestern University Medical School.

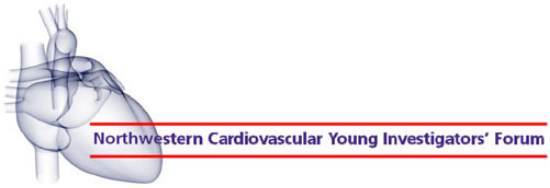
Figure 2 Cardiovascular Young Investigators Forum Faculty 2015.

In addition, the traditional Jeremiah Stamler Award was presented. Jeremiah Stamler is a distinguished professor of preventive medicine and epidemiology and a pioneer in hypertension and obesity research and nutrition at Northwestern University. The winners of this year's Jeremiah Stamler Award in the amount of 10 000 $ were Kary J. Levine from St. Louis and Sammy Elmariah from Boston.

Such research competitions are important to motivate young scientists and clinicians to pursue an academic career and to receive the recognition they deserve for their work. Besides the winners, all other participants profit from such meetings, as they have to prepare and present their work in an optimal and educational manner and meet contemporaries of their age and stage in their career. Such a tradition is more common in the USA, but would also be welcomed in Europe where such major competitions at universities are still quite rare.

However, the *European Society of Cardiology* holds a young investigators competition every year at their Annual Congress (see box).

A. Tofield



Northwestern Cardiovascular Young Investigators' Forum, 2015 Winners

There are 14 awards for this programme:
11th Annual Northwestern University Cardiovascular Young Investigators' Forum – Awards

Junior Faculty	Fellows
Jeremiah Stamler Award	
1 Basic Science Research – $10,000	
1 Clinical Research – $10,000	
Basic Science Research	**Basic Science Research**
1st Place: $1,500	1st Place: $1,500
2nd Place: $1,000	2nd Place: $1,000
3rd Place: $500	3rd Place: $500
Clinical Research	**Clinical Research**
1st Place: $1,500	1st Place: $1,500
2nd Place: $1,000	2nd Place: $1,000
3rd Place: $500	3rd Place: $500

Grants will be provided to institution for distribution to the winner.

Each 1st, 2nd and 3rd place winner in the basic science and clinical research categories will receive an engraved plaque memorializing this accomplishment. The two Jerimiah Stamler Award recipients will receive a trophy for their accomplishment.

All participants will receive a Certificate of Recognition that they should pick up this evening before they leave the restaurant.

Scoring: The maximum score possible is 16. Each expert faculty member provided their scores and all faculty abstained from scoring when a researcher was from their institution.

The winners for this competition are as follows:

FELLOWS BASIC SCIENCE GRANT AWARD WINNERS

Third Place – $500 grant
Abstract title: Rescue of Diabetic Cardiomyopathy via Cardiomyocyte-Restricted Silencing of FoxO1
Geoffrey W. Cho, MD
University of Texas Southwestern Medical Center
Dallas, TX
Score: 14.53 out of 16

Second Place – $1000 grant
Abstract title: The Epicardium Orchestrates an Immune Suppressive Response Following Myocardial Infarction to Attenuate Adverse Remodeling
Vimal Ramjee, MD
University of Pennsylvania, Perelman School of Medicine
Philadelphia, PA
Score: 14.7 out of 16

First Place – $1500 grant
Abstract title: Self-DNA Fuels a Fatal STING- and IRF3-Dependent Innate Immune Response to Myocardial Infarction
Kevin R. King, MD, PhD
Brigham and Women's Hospital
Boston, MA
Score: 15.74 out of 16

Runner ups:

Abstract title: *In Vitro* Characterization and *In Vivo* Feasibility of an in Situ Light-Activated Vascular Scaffold for Endovascular Therapy
Mazen Albaghdadi, MD, MS
Massachusetts General Hospital
Boston, MA
Score: 14.28 out of 16

Abstract title: Hypertrophic Cardiomyopathy-Causing Mutations in Converter Domain of Human β-Cardiac Myosin Do Not Cause Gain of Function *In Vitro*
Masataka Kawana, MD
Stanford School of Medicine
Stanford, CA
Score: 14.23 out of 16

Remainder of Fellows scored below 14 in this category.

FELLOWS CLINICAL GRANT AWARD WINNERS

Third Place – $500 grant
Abstract title: Critical Role of Angiopoietin-2 in Mediating Abnormal Angiogenesis and Gastrointestinal Bleeding in Patients With Continuous-Flow Left Ventricular Assist Devices
Corey E. Tabit, MD, MBA, MPH
The University of Chicago Medical Center
Chicago, IL
Score: 14.59 out of 16

Second Place – $1000 grant
Abstract title: Exome Sequencing, Functional Analysis and Super Resolution Imaging Identify a Pathogenic Role for SCNSA Mutations in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy
Anneline S.J.M. te Riele, MD
Johns Hopkins University School of Medicine

Baltimore, MD
Score: 15.48 out of 16

First Place – $1500 grant
Abstract title: Human Knockout Project in a Cohort With a High Rate of Consanguinity
Pradeep Natarajan, MD, MMSc
Massachusetts General Hospital
Boston, MA
Score: 15.72 out of 16

Runner ups: No fellow was close; the remainder of fellows in this category were all below 14 points.

JUNIOR FACULTY BASIC SCIENCE GRANT AWARD WINNERS

Third Place – $500 grant
Abstract title: High Frequency Driving Sites Are Anchored by Fibrotic Regions in Chronic Atrial Fibrillation
Ravi Ranjan, MD, PhD
University of Utah
Salt Lake City, UT
Score: 14.29 out of 16

Second Place – $1000 grant
Abstract title: Myocardial NF-κβ Activation is Essential for Zebrafish Heart Regeneration
Ravi Karra, MD, MHS
Duke University Medical Center
Durham, NC
Score: 14.42 out of 16

First Place – $1500 grant
Abstract title: Sphingosine 1-Phosphate Receptor-1 Regulates Ventricular Compaction and Fibrotic Response
Lisa Wilsbacher, MD, PhD
Northwestern University Feinberg Cardiovascular Research Institute
Chicago, IL
Score: 14.66 out of 16

Runner ups: All other Junior Faculty in this category scored less than 14 points

JUNIOR FACULTY CLINICAL GRANT AWARD WINNERS

Third Place – $500 grant
Abstract title: Prognostic Significance of Positive Exercise Electrocardiography With Normal Stress Echocardiography
Melissa A. Daubert, MD
Duke University Medical Center
Durham, NC
Score: 14.05 out of 16

Second Place – $1000 grant
Abstract title: Drug-Eluting Stents Versus Bare-Metal Stents in Patients on Dialysis
Tara I. Chang, MD, MS
Stanford University School of Medicine
Stanford, CA
Score: 14.06 out of 16

First Place – $1500 grant
Abstract title: Exhaustion of CD8 T Cells in Patients Presenting With Acute Coronary Syndromes: A Novel Effect of Oxidized Lipoproteins
David A. Zidar, MD, PhD
Case Western Reserve University School of Medicine
Cleveland, OH
Score: 14.09 out of 16

Runner ups:

Abstract title: Oral Anticoagulant Therapy Prescription in Patients With Atrial Fibrillation Across the Spectrum of Stroke Risk: Insights From the NCDR® PINNACLE Registry
Jonathan C. Hsu, MD, MAS
University of California, San Diego
San Diego, CA
Score: 13.96 out of 16

Abstract title: Differences in Natriuretic Peptide Levels by Race/Ethnicity in the Multi-Ethnic Study of Atherosclerosis
Deepak K. Gupta, MD
Vanderbilt University School of Medicine
Nashville, TN
Score: 13.76 out of 16

Abstract title: Safety of Intravenous Tissue Plasminogen Activator in Acute Ischemic Stroke Patients Taking Novel Oral Anticoagulants (NOACs)
Ying Xian, MD, PhD
Duke Clinical Research Institute
Durham, NC
Score: 13.25 out of 16

Remainder of Junior Faculty were scored less than 13 in this category

JUNIOR FACULTY STAMLER GRANT AWARD WINNERS

Junior Faculty Basic Science – $10,000 grant
Abstract title: Distinct Lineages of Embryonic-Derived Macrophages Populate the Heart and Are Essential Mediators of Coronary Angiogenesis During Development of Heart Failure
Kory J. Lavine, MD, PhD
Washington University School of Medicine

St Louis, MO
Score: 15.71 out of 16

Junior Faculty Clinical – $10,000 grant
Abstract title: Metabolite Profiles Predict Acute Kidney Injury and Mortality in Patients Undergoing Transcatheter Aortic Valve Replacement

Sammy Elmariah, MD, MPH
Massachusetts General Hospital
Boston, MA
Score: 14.65 out of 16

Total grants awarded: $32,000

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