How Does Mutant Proprotein Convertase Neural Apoptosis-Regulated Convertase 1 Induce Autosomal Dominant Hypercholesterolemia?

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I
n 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). 1 Seven members of the SPC family are known. One, the subtilisin kexin isoenzyme-1/site-1-protease (SKI-1/S1P), has a key role in cholesterol homeostasis through processing of the sterol regulatory element-binding proteins (SREBPs). 2,3 Another member of the SPC family, neural apoptosis-regulated convertase 1 (NARC-1), which was recently linked to a novel form of autosomal dominant familial hypercholesterolemia, 4 is downregulated by cholesterol and upregulated by SREBP-2. 5,6 These findings indicate that NARC-1, like SKI-1/S1P, is an important player in lipid metabolism.

Autosomal dominant hypercholesterolemia (ADH) is characterized by an increase in low-density lipoprotein (LDL) cholesterol levels and premature cardiovascular disease. ADH is associated mainly with mutations in the genes encoding the LDL receptor (ie, familial hypercholesterolemia) 7 and apolipoprotein B100 (apoB100; ie, familial defective apoB100). 8 These mutations directly or indirectly reduce the ability of the LDL receptor to mediate tendocytosis of plasma lipoproteins containing apoB or apoE. Analysis of large cohorts of patients with heterozygous familial hypercholesterolemia indicated that inherited defects in genes other than those encoding the LDL receptor and apoB can cause hypercholesterolemia. This was first proven when Garcia et al showed that mutations in the phosphotyrosine-binding domain protein ARH cause autosomal recessive hypercholesterolemia. 9,10 A novel locus associated with ADH has been mapped to 1p34.1-p32. 11 To identify this locus, Abifadel et al identified mutations in PCSK9 (the gene encoding NARC-1) in 12.5% of the families with ADH that were tested. To date, 3 point mutations in PCSK9 causing amino acid changes have been reported. The mutations S127R and F216L were detected in different French families, 4 and D374Y was detected in Utah pedigrees. 12 In all cases, the LDL cholesterol level was 2- to 5-fold higher than normal. Genetic variants in PCSK9 also affect the cholesterol level in the general population in Japan. 13

How Do NARC-1 Mutations Cause Familial ADH?
The molecular mechanisms that underlie the hypercholesterolemia phenotype are unknown, and it is unclear whether the mutations affect the enzymatic activity of NARC-1 or cause a gain of nonenzymatic function. The fact that the syndrome is dominant could indicate that mutations result in a gain of function. NARC-1 is synthesized as a soluble zymogen that undergoes autocatalytic intramolecular processing in the endoplasmic reticulum. 14 Prosegment cleavage is necessary for NARC-1 to exit from the endoplasmic reticulum. Thus, the amino acid substitutions in NARC-1 might affect the zymogen processing of the enzyme. However, this is less likely because the prosegment cleavage site has been indirectly but not conclusively identified at leucine 82 in NARC-1, and all of the mutations are located carboxyl terminally of this site. 14

The aim of the studies by Ouguerram et al in this issue of Atherosclerosis, Thrombosis, and Vascular Biology 15 was to investigate how the S127R mutation in NARC-1 influences the secretion of apoB100-containing lipoproteins and their metabolic fate. Using the stable isotope [1H3] leucine, they assessed the in vivo kinetics of apoB100-containing lipoproteins in a mother and her son carrying the S127R mutation and compared the findings with those in healthy subjects and patients with familial hypercholesterolemia who had known mutations in the LDL receptor gene. Tracer models can be used to generate direct information on lipoprotein synthetic rates, and the combination of tracer models and multicompartiment analysis made it possible to estimate the apoB100 production rate and the catabolism and transfer rates of very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL.

Both subjects displayed a 3-fold increase in apoB100 production that was related to direct overproduction of VLDL (3-fold), IDL (3-fold), and LDL (5-fold). The high VLDL
release primarily reflected production of smaller VLDL_{2} particles and did not affect the size of the triglyceride pool. Interestingly, VLDL from subjects with the S127R NARC-1 had a high cholesteryl ester to triglyceride ratio. Because this ratio correlates negatively with affinity for lipoprotein lipase (LPL),^{16} VLDL enriched with cholesteryl esters are probably not well recognized by LPL and contribute little to LDL production. Indeed, the kinetics data showed lower conversion rates of VLDL and IDL in the 2 affected subjects (10% to 30% of the rates in controls), and VLDL and IDL accounted for only 28% of LDL formation versus 75% in controls. Thus, direct synthesis was the principal pathway for LDL production.

The mechanism underlying the elevated ratio of cholesteryl ester to triglyceride associated with the NARC-1 mutation is not known. The data also showed a slightly decreased LDL fractional catabolic rate. Because subjects with S127R NARC-1 display normal LDL receptor activity,^{11} the decrease was likely caused by reduced LPL affinity of the cholesteryl-enriched LDL particles. These results indicate that the S127R mutation in NARC-1 induces hypercholesterolemia through overproduction of cholesterol-enriched apoB100-containing lipoproteins accompanied by a slight decrease in their removal.

**How Does Mutant NARC-1 Influence ApoB100-Containing Lipoprotein Secretion?**

Assembly of apoB-containing lipoproteins (a complex process that occurs in the lumen of the secretory pathway) consists of 2 relatively well-identified steps. In the first step, 2 VLDL precursors are formed simultaneously and independently: an apoB-containing VLDL precursor (a partially lipidated apoB) and a VLDL-sized lipid droplet that lacks apoB.^{17,18} In the second step, the 2 precursors fuse to form a mature VLDL particle. The apoB-containing VLDL precursor is formed during translation of the protein and its concomitant translocation to the lumen of the endoplasmic reticulum.^{19} The VLDL precursor is completed shortly after the protein is fully synthesized. The process is dependent on the microsomal triglyceride transfer protein (MTP).

Although the mechanism by which lipid droplets are formed is unknown, recent observations indicate that the process is dependent on MTP.^{20} Fusion of the 2 precursors is not dependent on MTP, but the mechanism remains to be elucidated. Conversion of the apoB-containing precursor to VLDL seems to be dependent on the ADP ribosylation factor 1 and its activation of phospholipase D.^{21} During their assembly, nascent apoB chains undergo quality control and are sorted to proteasomal degradation.^{22}

Sorting to degradation also appears to occur at additional levels throughout the secretory pathway.^{22} Data from several laboratories support the occurrence of nonproteasomal apoB degradation, but much remains to be determined, including the number of distinct processes, the factors targeting apoB to this pathway, and the proteolytic activities involved.^{22} The LDL receptor may also participate in this intracellular sorting of apoB-containing lipoproteins to degradation.^{23} It is possible that partially lipidated forms of apoB (the tentative VLDL precursor) are removed in this fashion unless they are converted to VLDL (Figure).

Thus, the biosynthesis of apoB-containing lipoproteins is complex, and NARC-1 could tentatively be important at different levels throughout the assembly process. Although the physiological substrates of NARC-1 are not known, functional characterization of NARC-1 in vitro has demonstrated unusual substrate specificity.^{24} Identification of the physiological substrates of NARC-1 will likely yield important information about the role of NARC-1 in the assembly and secretion of apoB-containing lipoproteins. In the meantime, we can only speculate that NARC-1 could be involved in the post-translational degradation of nascent VLDL. However, proprotein convertases usually are not involved in protein degradation; rather, they perform specific cleavages that activate proprotein substrates.^{1,25} It is also possible that the mutations in NARC-1 affect the lipidation of nascent VLDL. That the secreted VLDL is highly enriched in cholesteryl esters could indicate that the mutant NARC-1 directly or indirectly increases the biosynthesis of cholesteryl and thereby decreases the post-translational degradation of nascent VLDL. Further studies of NARC-1 will likely shed light on the assembly and secretion of apoB-containing lipoproteins.

**References**


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