

COMMENTARY

A role for NPC1 and NPC2 in intestinal cholesterol absorption – the hypothesis gutted

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Dietary and biliary cholesterol are taken up by intestinal epithelial cells and transported to the endoplasmic reticulum. At the endoplasmic reticulum, cholesterol is esterified, packaged into chylomicrons and secreted into the lymph for delivery to the bloodstream. NPC1L1 (Niemann–Pick C1-like 1) is a protein on the enterocyte brush-border membrane that facilitates cholesterol absorption. Cholesterol's itinerary as it moves to the endoplasmic reticulum is unknown, as is the identity of any cellular proteins that facilitate the movement. Two proteins that play an important role in intracellular cholesterol transport and could potentially influence NPC1L1-mediated cholesterol uptake are NPC1 and NPC2 (Niemann–Pick type C disease proteins 1 and 2). In

this issue of the *Biochemical Journal*, Dixit and colleagues show that the absence or presence of NPC1 and NPC2 has no effect on intestinal cholesterol absorption in the mouse. Thus neither protein fills the gap in our knowledge of intra-enterocyte cholesterol transport. Furthermore, the NPC1/NPC2 pathway would not be a good target for limiting the uptake of dietary cholesterol.

Key words: absorption, cholesterol transport, Niemann–Pick C1-like 1 (NPC1L1), Niemann–Pick type C disease (NPC), Niemann–Pick type C disease proteins 1 and 2 (NPC1 and NPC2).

THE NPC (NIEMANN–PICK TYPE C DISEASE) PROTEIN FAMILY

NPC is an autosomal recessive lysosomal lipid storage disease that catapulted from relative obscurity to centre stage when the major disease-causing gene was cloned by Carstea and colleagues in 1997 [1]. NPC was little studied at the time because of its rarity; NPC is diagnosed in 1 in every 150 000 births. However, biomedical scientists took notice when the *npc1* gene was shown to encode a large membrane protein with features shared by several key regulators of cholesterol homeostasis. Identification of the NPC2 protein by Lobel's laboratory in 2000 [2] revealed a small soluble glycoprotein that likely partners with NPC1 to transport lipids. Subsequent identification of the *npc3* gene and demonstrated involvement of the NPC1L1 (Niemann–Pick C1-like 1) protein in intestinal cholesterol absorption intensified the spotlight on this gene family [3,4].

In this issue of the *Biochemical Journal*, Dixit and colleagues have tested the hypothesis that intestinal cholesterol absorption, although mediated by NPC1L1, is facilitated by NPC1 and NPC2 [5]. The study serves to delimit the physiological roles of the NPC protein family.

NPC – THE DISEASE

Clues about the normal physiological roles of NPC1 and NPC2 have mostly come from analysing primary fibroblasts from individuals with NPC [6]. The unifying feature of all NPC cells is the cellular storage of cholesterol and glycosphingolipids. In normal cells, endocytic vesicles bring fluid-phase cargo and membrane constituents to late endosomes and lysosomes, where they are digested. Many digestion products recycle out

of endosomes and lysosomes and are re-utilized. In NPC cells, however, the egress of cholesterol and glycosphingolipids is impaired, and these lipids accumulate in storage bodies. Cells compensate for the loss of these recycling lipids by increasing the biosynthesis of the lipids.

Although all NPC cells show cholesterol and glycosphingolipid accumulation, the major clinical impact is in the liver and brain [7]. Many affected individuals have liver disease at birth. Most begin to show symptoms of neurodegeneration as young children, with learning difficulties and motor co-ordination problems paramount. These individuals typically die in their teen years. There is also an infantile form of the disease; these infants show hepatosplenomegaly, fail to thrive and die within 2–3 years. Other individuals with a milder form of the disease enjoy a normal childhood and are diagnosed as adults, with early dementia as the predominant symptom.

NPC1 AND NPC2 – PARTNERS IN INTRACELLULAR CHOLESTEROL TRANSPORT

Despite a decade of work on NPC1, little insight has been gained into the protein's function. It has been determined that NPC1 is a large glycoprotein with 13 transmembrane-spanning domains and resides in late endosomes [8]. NPC1 captured broad scientific attention because it contains a five-transmembrane domain, called the 'sterol-sensing domain', that it is found in multiple other proteins hypothesized to sense the cholesterol content of their surroundings. A large hydrophilic N-terminal domain and two hydrophilic loops extend into the endosome lumen, but any functions and/or binding partners are unknown. At steady state, most NPC1 protein is in late endosomes, but the protein is present

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in tubules and vesicles that bud off of endosomes, traffic across the cell and then return [6]. The physiological importance of the NPC1 protein is underscored by its conservation; yeast, insects, worms and mammals all have NPC1, although these organisms diverge considerably in their need for, and handling of, sterols.

The *npc2* gene encodes a protein with the reassuring features of a *bona fide* lipid-transport protein. NPC2 is a soluble glycoprotein that is delivered to lysosomes by virtue of its mannose phosphate moiety [2]. It is also secreted, and is found in epididymal fluid, bile and milk. The secreted protein was purified in apo and sterol-bound forms, and the crystal structure was solved by Stock's laboratory [9]. Apo-NPC2 was found to have an incipient ligand-binding pocket, which expands to accommodate cholesterol. NPC2 was shown, in *in vitro* assays, to rapidly transport cholesterol from donor to acceptor membranes via a collisional mechanism [10]. As might be expected for a lysosomal protein, transfer activity was greater in an acidic environment and was enhanced by the presence of the lysosome/late-endosome-specific lipid lysobisphosphatidic acid.

How do NPC1 and NPC2 work together to transport lipids? The most favoured hypothesis is that, as lipid cargo is brought to the late endosomes and lysosomes, the lipids are digested into their constituent molecules. NPC2 facilitates the transfer of cholesterol, and perhaps other lipids, to the delimiting membrane of the organelle. NPC1 senses the rising membrane cholesterol content and signals for membrane to bud, carrying cargo to destinations throughout the cell. Alternatively, NPC1 may directly transport cholesterol across the membrane, or be tightly linked to cholesterol's vectorial transport, with NPC1L1 having an analogous function at the enterocyte brush-border membrane.

NPC1L1 – RESPONSIBLE FOR INTESTINAL CHOLESTEROL ABSORPTION

In 2000, the NPC protein family was joined by NPC1L1, which has 42 % identity with, and 51 % similarity to, NPC1 [4]. Identification of NPC1L1 came at a time when the cardiovascular division of the Schering-Plough Research Institute (Kenilworth, NJ, U.S.A.) had a lead compound that reduced intestinal cholesterol absorption but which lacked a drug target. Using a genomics–bioinformatics approach, they prepared cDNA libraries from jejunum and searched for transcripts with potential features of a cholesterol transporter. NPC1L1 emerged as a jejunum-enriched protein that is localized to brush-border membranes of absorptive enterocytes [3]. Evidence quickly mounted linking NPC1L1 to intestinal cholesterol absorption. NPC1L1 knockout mice had reduced cholesterol absorption, were insensitive to the lead compound, ezetimibe (U.S. trademark name Zetia) and were resistant to diet-induced hypercholesterolaemia [3,11]. Final confirmation that NPC1L1 is the direct molecular target of ezetimibe came from [³H]ezetimibe binding studies [12].

NPC1L1 and NPC1 are both transmembrane proteins that have sequence similarities to each other and to the RND (resistance–nodulation–cell division) permease family, which transports drugs and other lipophilic compounds across bacterial membranes. Although potentially catalysing similar processes at the molecular level, the phenotypes of NPC1L1 and NPC1 mouse mutants are distinct, indicating that they do not have overlapping physiological roles. By contrast, the phenotype of individuals affected with NPC disease, as well as studies on mutant mice, suggest that NPC1 and NPC2 function in a common pathway; however, the two proteins are structurally unrelated and thus catalyse distinct molecular events.

NPC1, NPC2 AND NPC1L1 – PARTNERS IN CHOLESTEROL ABSORPTION?

The mechanism by which NPC1L1 mediates cholesterol absorption is unknown, as is the pathway that absorbed cholesterol takes to the endoplasmic reticulum for esterification and chylomicron assembly. The rationale for examining the role of NPC1 and NPC2 is many-fold. It is possible that the NPC2 that is secreted in the bile and brought to the intestinal lumen plays a role in solubilization of dietary and biliary cholesterol. NPC2 could enhance delivery of cholesterol to NPC1L1 in the brush-border membrane. It is also possible that the absorbed cholesterol traffics through the endocytic pathway to the NPC1/NPC2 compartments. Furthermore, NPC1 appears to influence cholesterol uptake in fruitflies (*Drosophila melanogaster*). Knockout of *D. melanogaster* dNPC1a, the NPC1 orthologue, increases cholesterol uptake [13]. This increase is also seen in the double knockout with the *D. melanogaster* NPC1L1. Does NPC1 affect cholesterol uptake in mammals?

Enter Dixit and colleagues. They have tested the hypothesis that NPC1 and NPC2 play a role in mammalian intestinal cholesterol absorption and transport to the endoplasmic reticulum using NPC1-deficient and NPC2 hypomorph mouse models [5]. Their study showed that fractional cholesterol absorption was equal in control and mutant mice. Furthermore, the incorporation of absorbed cholesterol into chylomicrons and appearance in the bloodstream and liver was equal in control and mutant mice. The study by Dixit et al. helps to define the cholesterol transport pathways in which NPC1 and NPC2 participate. Neither NPC1 nor NPC2 appear to play a role in intra-enterocyte transport of cholesterol absorbed from the intestinal lumen. Thus the NPC1/NPC2 pathway would not be a good target for limiting the uptake of dietary cholesterol.

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