# NNPDF-Funded Research Fellowship # 2

## TITLE: The Role of NPC2 (Niemann-Pick Disease Type C2) Protein in Lysosomal Cholesterol Trafficking

**PROJECT INVESTIGATOR: Heng-Ling Liou, Ph.D.**

## PERIOD: 3/15/2003 - 3/14/2005

**PROJECT DESCRIPTION**

Cholesterol is a major constituent of the Western diet, and it is important for wide variety of physiological functions. It is the essential component of cell membranes and is the precursor to form steroids (steroid hormones) and other compounds that may be involved in controlling nerve activity. In the normal cells, cholesterol is taken up into cells and to be reutilized, and the excess cholesterol is transported out of cells. In Niemann-Pick disease type C (NP-C), cholesterol is taken up into cells but the transport out of cells is blocked and this accumulation eventually kills the cells. Recently, Dr. Ioannou's laboratory have discovered that the Niemann-Pick C1 protein (NPC1) is a membrane protein and appears to transport fatty acids and other lipids but does not directly bind cholesterol, therefore the link between the NPC1 protein and the cholesterol blockage in NP-C1 patients is not yet clear. Dr. Lobel's laboratory have identified the second gene that is responsible for NPC disease type C2 (NPC2). NPC2 is a lumenal lysosomal cholesterol binding protein. Given that NPC1 protein did not directly bind to cholesterol, it is possible that NPC1 requires another protein to function in cholesterol trafficking. Our hypothesis is that NPC2 may be involved in the same pathway of cholesterol transport (for example, NPC2 protein may physically interact with NPC1 protein) as mutations in either protein cause an indistinguishable cellular and disease phenotype. The specific aims of my proposal are 1) to produce and characterize functional NPC2 protein for structural and biochemical studies; 2) to explore the potential for protein-protein interactions for NPC2 using biochemical and immunological approaches; 3) to investigate the potential binding of ligands other than cholesterol to NPC2 using fluorescent-based binding assays. For Aim 1, I will produce soluble NPC2 protein and characterize its function, particularly in determining its three-dimensional (3D) structure. The 3D conformation of NPC2 protein with cholesterol binding (cholesterol bound) and without cholesterol binding (cholesterol unbound) will be both determined. The three-dimensional structure of NPC2 is very important as it may reveal the cholesterol binding sites and the potential structural elements of NPC2 protein that may interact with other proteins. For Aim 2, I will specifically investigate the potential proteins that may interact with NPC2 in cholesterol transport. Protein-protein interactions are increasingly recognized as playing a major role in the transport of lipids within cells due to lipids' poor solubility in aqueous milieu. The identical cellular phenotypes in NP-C1 and NP-C2 patients suggest that these two proteins, at least, are closely linked at the functional level. This may translate into physical interactions such as that the NPC2 protein, which binds cholesterol, may receive its cholesterol from another protein(s) (e.g. acid lipase – the enzyme that produce free cholesterol from cholesteryl esters), and/or may deliver the cholesterol to

another protein(s) (e.g. NPC1) in normal cells. I will use our purified functional NPC2 protein and anti-NPC2 antibodies as the tools to detect the potential NPC2-partner protein interactions. For Aim 3, I will examine the potential binding of ligands other than cholesterol to NPC2 protein. Although it is likely that cholesterol represents a true physiological ligand for NPC2, there well may be additional ligands that accumulate in NP-C disease and contribute to the disease process. For instance, in NPC brain, glycosphingolipids are elevated, and the relationship between lipid accumulation and neuronal death remains unclear. Research on NPC2-deficient mice, currently being conducted by others in our lab and by our collaborators, may reveal elevations in other sterols and lipids that may represent potential NPC2 ligands. In addition, it will be of great interest to determine if NPC2 may be the target of sterols and hydrophobic amines that induce an NPC-like phenotype in cultured cells. Finally, once the structure of the NPC2 holo protein is determined (see Aim 1), we will be able to predict which interactions are important for ligand binding and test this by measuring the relative affinity of NPC2 for different sterol derivatives. These studies may provide information about the structural determinants of ligand binding, which will enhance our overall understanding in Niemann-Pick C disease.

Cholesterol is a major constituent of the Western diet, and it is important for wide variety of physiological functions. In addition, it is the essential component of cell membranes and is the precursor for steroids hormones and compounds involved in controlling nerve activity. In Niemann-Pick disease type C (NP-C), cholesterol is abnormally accumulated in cells and this eventually led to the cell death. It has been discovered that the two genes are directly responsible for NPC disease. Niemann-Pick C1 protein (NPC1) is a endosomal membrane protein. NPC2 is a lumenal lysosomal cholesterol binding protein. Recently, it has been shown that NPC1 and NPC2 work in collaboration to function in cholesterol trafficking, and yet the mechanism of this transport still remain elusive. My main focus is to investigate the molecular function of NPC2 and thus to provide us insight in the entire mechanism of cholesterol transport.

In the past few months, I have purified NPC2 protein regularly and utilized the protein for threedimensional (3D) structure. Both cholesterol-bound and cholesterol-unbound forms were studied. The 3D structure of NPC2 is very important as it may reveal the cholesterol binding sites and the potential structural elements of NPC2 protein that may interact with other partners. In order to solve the structure, larger size of the crystal is needed. Thus far, we were able to improve the size of the crystals. Further, we have found several compounds that have very similar structures to cholesterol also bind to NPC2. Particularly, the sulfonated cholesterol seems to give us an interesting result. We will continue to use cholesterol and other compounds to obtain a structure of NPC2-ligand complex that may give us insight in understanding the physiological function of NPC2.

**FINAL STATUS REPORT**

The identical cellular phenotypes in type C1 and C2 patients suggest that these two proteins, at least, are closely linked at the functional level. This may translate into physical interactions such that the NPC2 protein may receive its cholesterol from another protein(s) and/or may deliver the cholesterol to another protein(s) in normal cells. I used purified functional NPC2 protein to generate an affinity column

to detect the potential interacting proteins. Two strategies were used simultaneously. Either tissue homogenates or subcellular fractionated endosomes/lysosomes were prepared and used from wild type and NPC2-deficient mice. In this study, several proteins appeared to be interesting candidates and the analysis is in close investigation.

Although it is likely that cholesterol represents the only physiological ligand for NPC2, there well may be additional ligands that accumulate in NP-C disease and contribute to the disease process. For example, the level of glycosphingolipids is elevated in NPC patients. However, the relationship between their accumulation in brain and neuronal death is not yet completely understood. To enhance our overall understanding in NPC disease, we examined the lipid extracts from our purified NPC2 protein, as well as an in vitro binding assay to confirm their binding. Our studies confirmed that cholesterol is an endogenous ligand. Additionally, some compounds with extremely similar structure also binds to NPC2 protein including cholesterol precursors (e.g. lathosterol and desmosterol), plant sterols (e.g. stigmasterol and b-sitosterol), and cholesterol sulfate. This indicates that ligand binding of NPC2 is very specific, and the glycosphingolipid is likely not the direct ligand for NPC2.

**PUBLICATIONS:**

http://www.jbc.org/cgi/content/full/281/48/36710
http://www.jbc.org/cgi/reprint/282/32/23525