Lesch-Nyhan Syndrome (LNS) is a lethal, X-linked, recessive disease, in which patients suffer from gouty arthritis, kidney stones, loss of muscle control and cognition, and self-mutilation. Some patients bite off their lips, tongue, and fingers, causing tremendous pain and physical difficulties1. LNS is caused by a severe deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), an enzyme that recycles two purine bases, guanine and hypoxanthine, into nucleic acid precursors used to build DNA and RNA. The HPRT enzyme is encoded by the *hypoxanthine phosphoribosyltransferase 1* (*HPRT1*) gene. The deficiency of HPRT leads to the overproduction of uric acid in the blood and urine, resulting in gouty arthritis and kidney stones, which is well-understood. However, it is unknown how loss of HPRT function leads to other LNS symptoms such as muscle control problems, cognitive defects, and self-injurious behavior.

Studies of human brains have suggested that the neurological symptoms of LNS could be related to dysfunction of the dopaminergic neurotransmitter system2,3. Dopamine is a neurotransmitter that controls reward and pleasure in the brain. One pharmacological rat model supported a relationship between reduction of brain dopamine during development and self-mutilative behavior4. *However, the relationship between the dopamine deficit and HPRT deficiency is still unclear.*

**Hypothesis:** *HPRT1* regulates the development of the dopaminergic neurotransmitter system, important for normal cognition and behavior, through protein interactions in the brain.

**Primary Goal:** Determine the genomic and proteomic changes that contribute to Lesch-Nyhan Syndrome neurological dysfunction as a result of mutations in the *HPRT1* gene.

**Aim 1:** Identify proteins that interact with HPRT and that regulate cognitive development and function.

**Approach:** I will use affinity purification-mass spectrometry to identify direct targets of HPRT in wild-type and *HPRT1-*mutant zebrafish. Zebrafish are useful for large-scale screens and have HPRT proteins with 91% identity to human HPRT5. Next I will use STRING to identify proteins that interact with HPRT and are conserved between humans and zebrafish. I will sort these identified proteins by Gene Ontology (GO) terms to determine the functions of the proteins and any neurological pathways that could be disrupted by *HPRT1* mutations. This will help to uncover potential mechanisms for how the loss of HPRT function leads to LNS neuronal behavior defects.

**Aim 2:** Determine which proteins are over- or under-expressed in ­*HPRT1*-mutant zebrafish.

**Approach:** I will use quantitative mass spectrometry to compare protein levels in wild-type and *HPRT1*-mutant zebrafish. This assay will determine which proteins’ expression levels are affected by the *HPRT1* mutation. I hypothesize that some of these proteins may influence dopaminergic pathway development and could contribute to the neuronal-behavioral problems associated with LNS. My primary focus will be on brain tissue because this is where the neurological symptoms of LNS originate.

**Aim 3:** Characterize how gene expression levels change during neuronal brain development in wild-type and HPRT-mutant zebrafish.

**Approach:** I will use a DNA microarray to quantify the expression levels of all genes in the brains of wild-type and *HPRT1*-mutant zebrafish at each stage of development, from fertilization through adulthood. The transparency of zebrafish embryos allows for easy imaging of neural pathologies that occur during developmental stages5. This assay will indicate *when* loss-of-function mutations in *HPRT1* alter neuronal gene function, indicating how brain development may be altered by *HPRT1* mutation. Analysis of the microarray expression data by GO will reveal alternative biological processes affected by *HPRT1* knockout. I hypothesize that pathways implicated in neurogenesis and dopamine metabolism will be identified.

The experiments in this proposal are expected to indicate how gene expression and protein interactions change as a result of mutations in the *HPRT1* gene. This may give us insight into how mutations in *HPRT1* change the dopaminergic neurotransmitter pathway and cause the neurological phenotypes and self-mutilative behaviors associated with Lesch-Nyhan Syndrome. This work will also likely lead to potential targets for therapeutic intervention of LNS while also advancing our understanding of purine biosynthesis and salvage pathways.

1. Torres RJ and Puig JG. (2007). Hypoxanthine-guanine phosophoribosyltransferase (HPRT) deficiency: Lesch-Nyhan syndrome. *Orphanet Journal of Rare Diseases, 2*, 48.
2. Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Jons PH, Hardy C, Hankerson JG, Doudet DJ, and Cohen RM. (1996). Presynaptic dopaminergic deficits in Lesch-Nyhan disease. *New Engl J Med, 334*, 1568-1572.
3. Wong DF, Harris JC, Naidu S, Yokoi F, Marenco S, Dannals RF, Ravert HT, Yaster M, Evans A, Rousset O, Bryan RN, Gjedde A, Kuhar MJ, and Breese GR. (1996). Dopamine transporters are markedly reduced in Lesch-Nyhan disease in vivo. *Proc Natl Acad Sci USA, 93*, 5539-5543.
4. Breese GR, Criswell HE, Duncan GE, and Mueller RA. (1990). A dopamine deficiency model of Lesch-Nyhan disease – the neonatal-6-OHDA-lesioned rat. *Brain Res Bull, 25*, 477-484.
5. Lieschke GJ and Currie PD. (2007). Animal models of human disease: zebrafish swim into view. *Nat Rev Genet., 8*(5), 353-367.