Lesch-Nyhan syndrome

Lesch-Nyhan disease is an uncommon genetic disorder that involves a hypoxanthine-guanine phosphoribosyltransferase deficiency. Hypoxanthine-guanine phosphoribosyltransferase is an enzyme in the purine salvage pathway. Purines are bases in DNA that are necessary in every cell. Great amounts of energy are put into maintaining the proper quantity of purines as well as synthesis of new purines for DNA during cell division. Purine salvage is important for the reuse of nucleotides in synthesis and maintenance of DNA. Deficiency in hypoxanthine-guanine phosphoribosyltransferase results in an overproduction of uric acid, leading ultimately to gouty arthritis and nephrolithiasis. During the buildup of uric acid, many other unexplainable complications arise and are seen in Lesch-Nyhan patients. Two complication is neurological and behavioral abnormalities. Symptoms found in all classical Lesch-Nyhan patients have symptoms of hematological disturbances, neurological manifestations, overproduction of uric acid, hyperuricemia-related renal and articular symptoms, these symptoms are not related to the severity of the enzyme defect. There are Lesch-Nyhan variants which present some, but not all, of the classic signs and symptoms. Although the variants present a wide variety of symptoms related to their moderate deficiency, they always have the Hypoxanthine-guanine phosphoribosyltransferaseis deficiency. Due to the presence of the enzyme in every cell in the body a variety of symptoms are seen. From the cellular progression to the clinical signs and symptoms, to treatment options, many complications are seen.

History

In 1962 a young boy of only four years was taken to the Johns Hopkins Hospital. The young boy was spastic and had been diagnosed with cerebral palsy when he was younger. This misdiagnosis of cerebral palsy is still common. The boy’s urine was bright orange, with a tinge of pink and consisted of a gritty texture. The boy had an older brother that had similar symptoms. It seemed that whatever was wrong with these boys was hereditary. The presiding physician and her intern looked at the urine under a microscope and saw unusually beautiful crystals. The intern inquired about the possibility of them being uric acid. Until this point, over production of uric acid was only seen in older men in a disease called gout. The physician took the sample upstairs to the “guru of metabolism”, Bill Nyhan. When Dr. Nyhan and his companion Dr. Lesch went to meet the boy, his hands were wrapped in white gauze. As they removed them, the boy asked them to stop. Upon removal, the doctors noticed his fingertips were missing chunks the boy immediately thrust his hands into his mouth. The doctors noticed that parts of the boys lips had been bitten off as well. This was the first patient to be recorded with Lesch-Nyhan syndrome. In the spectrum of disease, this one is new and almost as much of a mystery now as it was then. However, the gene itself is conserved from bacteria to humans. The catalytic activity has also been retained, but the substrates specificity may have changed. Humans, primates, and New World monkeys lost the enzyme uricase about 20 million years ago. Along with the loss of uricase came the loss of the ability to degrade uric acid into more soluble allantoin.

Etiology and Pathogenesis
Lesch-Nyhan is an X-linked recessive disorder resulting in mutations in the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene. The HPRT gene is located on the long arm of the X chromosome and contains nine exons with a coding sequence of 654 bp\textsuperscript{26}. Due to its location on the X chromosome all most every case of the Lesch-Nyhan defect is recorded in males\textsuperscript{7}. Even though Lesch-Nyhan is a relatively new disease, at least five cases have been reported in females\textsuperscript{26}. It has been approximated by the NIH that 1 in 380,000 people have Lesch-Nyhan syndrome\textsuperscript{10}. HPRT is a housekeeping enzyme that is involved in purine metabolism in practically every cell of the body. The HPRT enzyme recycles purine bases, hypoxanthine, and guanine into usable purine nucleotide pools. Without HPRT hypoxanthine and guanine cannot be recycled and are instead degraded into uric acid, which the body must excrete. A secondary effect of the HPRT defect is a constant synthesis and constant destruction of purines since they can no longer be efficiently recycled. This constant synthesis and degradation process is responsible for the overproduction of uric acid in Lesch-Nyhan patients\textsuperscript{8}.

Purines are heterocyclic aromatic organic compounds that are components of DNA and RNA; they are also involved in energy production, signal transduction with in cells, and neurotransmitters. Purines and pyrimidine bases are normally recycled by salvage pathways. This process is a single reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase. In this reaction, free xanthine and guanine react with 5'-phosphoribosyl-1-pyrophosphate (PRPP) to produce guanine-monophosphate (GMP) adenine-monophosphate (AMP) plus diphosphate, PPi\textsuperscript{14}.

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\text{Guanine} + \text{PRPP} = \text{GMP} + \text{PPi} \\
\text{Adenine} + \text{PRPP} = \text{AMP} + \text{PPi}
\]

When the salvage pathway fails, purines build up and degradation of purines occurs. Degradation of purines occurs in a step by step manner. The first step is removal of a phosphate by the enzyme 5'-nucleotidase. This occurs in both guanine monophosphate and adenine-monophosphate. This results in guanosine and adenosine. Here the pathway splits in to two and Adenosine is deaminated by adenosine deaminase to produce inosine. Inosine and guanosine are hydrolyzed to guanine and hypoxanthine by nucleosidase. Guanine is deaminated by guanine deminase while hypoxanthine is oxidized to xanthine by the combination of the two products, by xanthine oxidase. Further oxidation results in the formation of uric acid\textsuperscript{14}. A flow chart of this process is pictured below. Due to increased availability of PRPP, the rate-limiting step in \textit{de novo} synthesis is decreased, making \textit{de novo} synthesis a quicker process. The rate limiting step is shown below, and is the second step in the process. Because of the constant degradation of the purines there is no negative feedback inhibition of the PRPP amidotransferase, which results in constant AMP and GMP formation\textsuperscript{14}. 

Clinical Signs and Symptoms

The extra uric acid is usually excreted by the urinary system or deposited in the joints where it causes gouty arthritis in the form of crystals. Extra production in uric acid leads to an increase in urinary excretion of some of the excess. After saturation in the urinary tract system is reached, the uric acid crystals become gritty and sludge like. Saturation is reached quickly due to high starting saturation. High saturation occurs in blood as well so even a little extra uric acid will result in crystals formation. Crystals will precipitate from biological fluids when the concentration is above 6.8mg/dL. When too many uric acid crystals are congregated in one area, actual stones may form and collect in the area. These materials can slow and/or obstruct urine flow, back up urine, and ultimately lead to renal failure. This back up also causes problems with glomerular filtration. Uric acid crystals can also precipitate in the subcutaneous tissues where they form tophi, visible masses of uric acid crystals. Several factors can contribute to uric acid deposition in the joints and other areas including trauma or irritation, lower temperatures in the body, and previous disease have also been contributing factors. In the joint space, synovial lining cells appear to be the first to phagocytize uric acid
crystals. This is why joints are more highly affected by uric acid deposits than other parts of the body. It is also easier to damage these cells and trigger an inflammation response. Pictured below is the synovium between acute gout attacks. It is viewed under polarized light. This picture shows that even between painful episodes uric acids is still deposited and build up.

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Low urinary pH, aciduria, often helps cause uric acid stone formation. These stones are formed they are found within the urinary system in the kidneys, ureters, or bladder. Not only must the pH be low, but it must be consistently low for uric acid stone formation in the urinary system to occur. When meals are ingested and passed through the body, alkalinization, high pH, of the urine occurs and breaks up these stones, making it less likely for their formation. This halts the formation of some actual stones. Uric acid stones will dissolve in a standard body pH, of approximately 7, which can be achieved with oral medical therapy. The renal glutaminase activity of these patients is seemingly normal. Glutaminase catalyzes the hydrolysis of glutamine to glutamate. Persistently low pH in the urine causes nephrolithiasis, or kidney stones. These stones are often found by CT scans or ultrasounds. Patients with uric acid stones have a variety of symptoms including pain, malaise (general discomfort), nausea, and hematuria. Patients most often complain about the severe pain and seek medical attention. If stones are formed and blockage occurs, renal failure may occur. With renal failure many symptoms including hyperkalaemia, too much potassium in the blood, will follow. These side effects of renal failure must be treated or death will occur. Not all patients suffering from Lesch-Nyhan will develop a full case of the disease. In one case study a family had a history of kidney stones and this was the only progression of the disease they developed. This family is a Lesch-Nyhan variant. Pictured below on the left is a Lesch-Nyhan patient with uric acid build up in both left and right kidneys. Shown on the right below shows renal failure of a 24 day old patient. This picture shows abnormally inflamed tubules in the kidney it also shows crystals in the center, and a multinucleated foreign body type surrounding them.
If the urinary system fails to keep excreting all of the excess crystals, they will deposit in the joints. These deposits are often extremely painful for these patients. The innate immune system will detect these crystals as foreign and activate caspase-1, inflammasomes, and eventually proinflammatory cytokines interleukin (IL)-1β and IL-18. NALP3 inflammasome have been implicated in the pain and inflammation associated with gout. Uric acid is present at nearly saturated amounts in body fluids and a higher concentration is located within the cytosol of cells. It is thought that once uric acid comes into the high levels of free sodium present in extracellular environments it forms monosodium urate crystals. It is these monosodium urate crystals that activate the inflammation cascade.

High circulating uric acid, hyperuricemia, is associated with a few inflammatory diseases including hypertension and cardiovascular disease. Cardiovascular disease is often associated with Lesch-Nyhan patients. This may be due to the recruitment of the inflammatory system to uric acid deposits. By studying cardiovascular disease in gout patients we may further understand the effects in Lesch-Nyhan patients. Evidence in gout patients suggests that uric acid inflammation may not be limited to joints, but may also have an effect on the vascular walls and endothelial. Uric acid has also been seen to promote vascular endothelial cell proliferation, causing vascular occlusion and downstream ischemia, decreased blood supply. Endothelin-1 (ET-1) is an important vasoconstricting peptide and is involved with maintaining basal vascular tone and blood pressure. ET-1 does this by binding to receptors on vascular smooth muscle cells.

An increase in ET-1 production was produced in mice treated with uric acid for 24 hours. These mice also demonstrated and increase in cell proliferation. Although
reactive oxygen species (ROS) are often used for good in the body, too many may cause damage. These ROS may also be the cause of vascular smooth muscle hypertrophy, “an increase in the size of an organ or tissue due to an increase in the size of individual cells”3. ROS can promote vascular leakage in the inflamed environment. ROS production is also associated with activation of factor-κB which is a regulator of inflammation21.

Pathophysiology of Neurological Symptoms

The brains of Lesch-Nyhan patients are slightly smaller than others of their age. This shrinkage is minimal and often overlooked in routine checkups. However, patients brains appear structurally normal. The neurological and behavioral problems point to dysfunction of the basal ganglia and the dopamine system7. The basal ganglion is involved in voluntary motor control, procedural learning, eye movements and genitive emotional functions. The basal ganglion is also involved in action selection22. Reduction of fluorodopa into monoaminergic axons in the striatum by 60-70% was found in one case study. Monoaminergic axons are involved in the visual cortex18. WIN 35 428 to dopamine uptake sites were reduced by a similar number, showing that transport of dopamine is also decreased. WIN35 428 is a commonly used radio-ligand for dopamine transporter9. In adults a profound loss of striatal dopamine often causes parkinsonism, but in children it most often causes dystonia, sustained muscle contractions causing twisting or repetitive movements, which is common in Lesch-Nyhan patients. It has been shown in rats that the age at which striatal dopamine depletion occurs has an in affective influence on motor function. When this depletion occurs in younger rats it results in spontaneous hyperactivity and aggressiveness7. Pictured below is a brain of Lesch-Nyhan patient, right, and a normal, left. You can see the shrinkage of the brain stem in the Lesch-Nyhan patient.
Lesch-Nyhan patients will appear normal at birth, but soon thereafter appears the first signs of the disease, orange crystals in their diapers. The next sign of the disease is psychomotor delay, which usually become evident within three to six months after birth. This will be seen as a delay in the acquisition of sitting and head support with hypotonia and athetoid⁴, nonstop slow writhing in voluntary movements seen especially in the hands²⁶. Hypotonia is a term used to describe decreased muscle tone. Hypotonia is not the same as muscle weakness. Children with hypotonia appear “rag doll”, like their arms and legs are limp by their sides and they have little to no head control. Hypotonia also includes problems with poor reflexes, lethargy, breathing, speech difficulties, and ligament and joint laxity. Hypotonia does not affect intellect so other factors must be accounted for in the mental retardation of Lesch-Nyhan¹³.

Lesch-Nyhan patients are the first classification of HPRT deficiency, hypoxanthine-guanine phosphoribosyltransferase, which includes complete defect of the hypoxanthine-guanine phosphoribosyltransferase. Lesch-Nyhan patients are fully dependent on others for daily activities as well as personal needs²⁶.

Post-mortem biochemical tissue analysis shows some dysfunction in neurotransmitters in the brain of Lesch-Nyhan patients, including dopamine and serotonin. Dopamine is a neurotransmitter that has many responsibilities in the body. In the striatum in the brain it is responsible for outgoing messages²⁴. Dopamine levels in Lesch-Nyhan patients appears to be down 60-80%. In the striatum dopamine-neuron terminals were decreased while serotonin and 5-hydroxyindolacetic were increased²⁴. Serotonin also a neurotransmitter has been shown to be regulated by dopamine release in pre-synaptic channels. It is involved in decreasing the magnitude and frequency of synaptic depression. This results in less signal control and the inability to stop sending messages for movement. This is seen in the dystonia and uncontrolled spastic movements. Depletion of serotonin results in high-frequency stimulation, which is seen in Lesch-Nyhan patients¹². In the cerebrospinal fluid Lesch-Nyhan, patients exhibit decreased levels of the dopamine metabolite homovanillic acid. Homovanillic acid is a dopamine metabolite. In vivo studies have been done to confirm the dopaminergic system alterations in affected Lesch-Nyhan patients. In an animal knockout study the mice did not show neurological alterations, but did present with an age-related decrease in dopamine in their brains. In a pharmacological rat model, there was a correlation between self-injurious behavior and dopamine deficit. This is the thought to be the connection to the self-injurious behavior often seen in Lesch-Nyhan patients. Self-mutilation can appear as soon as teeth are present and often include biting the lip or chewing on fingers. In HPRT-deficient cell cultures dopamine deficit has been confirmed with the presence of hypoxanthine-guanine phosphoribosyltransferase enzyme deficit. A combination between effects is taking place in Lesch-Nyhan patients making it difficult to study the pathology in laboratory animals.
Many Lesch-Nyhan patients exhibit a characteristic neurobehavioral syndrome. All patients have a severe motor handicap and generalized dystonia, sustained muscle contractions, which cause twisting repetitive movements or abnormal postures. Most patients have cognitive disability, but it is usually not severe. Patients show patterns of recurrent self-injury, especially self-biting. Patients also exhibit other behaviors such as impulsive acts of aggression, spitting, or use of foul or sexually charged language. Affected individuals usually have short, delayed or absent puberty. Macrocytic asymptomatic anemia is also common. Gastroesophageal reflux and recurrent vomiting in these patients may be so severe that malnutrition may occur. These symptoms vary from severe to absent in patients this is known as Lesch-Nyhan variants.

Motor and mental impairment occurs in Lesch-Nyhan patients. Dystonia is not generalized to a part of the body. Severe cases may lead to the inability to stand or walk, leaving patients often confined to a wheelchair. Involuntary movements are usually present but not at rest. They are increased with excitement and anxiety. Often seen is Dysarthria, difficulty pronouncing words; dysphagia, difficulty with swallowing; opisthotonus, abnormal body posture. Hyperreflexia is generally reported in later years of their life.

Lesch-Nyhan patients cognitive impairments include motor difficulties, and mild to moderate mental retardation. They have attention deficit disorders and exhibit non-verbal intelligence in the majority of patients. Compulsive self-injurious behavior is only shown in patients with complete enzyme defect and some never show auto-destructive behavior. Patients who do develop self-mutilating behaviors begin to bite their lips, tongue and fingers without restrictions. Mutilation is not the due to the lack of sensation, but is more of an obsessive-compulsive behavior. Patients have full feeling capabilities. Patients begin self-mutilation between 2 and 16 years of age. When patients are restrained from themselves they are relieved, happy, and engaging. Neuro-behavioural disorders may be induced by numerous environmental factors, education is the most relevant. Megaloblastic anaemia and microcitc anaemia are usually present and associated with hiatus hernia.

Patients also have a megaloblastic anemia. This occurs in the bone marrow and is thought to be due to increased folic acid consumption in de novo purine synthesis pathway. However, the anemia is not corrected by B12 or folinic acid therapy. Folinic acid is the active form of folic acid. However, when given adenine orally reticulocytes improved and red-blood-cell volume decreased. There was also a disappearance of megaloblasts in the bone-marrow. Other effects of megaloblastic anemia seen are somatic growth, the maturation of bone marrow stem cells into erythrocytes and on gastrointestinal motility.

Hypoxanthine excess has been shown to have toxic effects. These toxic effects have been implicated in the pathogenesis of neurological dysfunction by altering the adenosine transport and Na+K+ATPase activity. In Lesch-Nyhan patient’s adenosine transport is an average of 32% lower.

Testing
A diagnosis for Lesch-Nyhan should be suspected within the first few years of life when hyperuricemia, orange crystals in the diaper and neurological impairment appear. A high serum urate concentration is usually the biochemical finding that prompts testing and specific diagnosis\textsuperscript{26}. There is genetic testing for mutations available. Testing for HPRT enzyme activity is the main diagnostic tool\textsuperscript{16}. The HPRT gene is a housekeeping gene expressed in peripheral blood. Therefore testing for enzyme activity is very easy. A sample of the patients blood is obtained and tested for the presence of the HPRT transcript of the gene. The HPRT gene is tested for by preforming RNA extraction reverse transcription-PCR to make complementary DNA, more commonly called cDNA, and then another PCR for the genomic DNA is preformed\textsuperscript{25}. This process is done in this particular way because if there is a totally lack of the enzyme in the cells no RNA will be made and the results will show nothing. More often than not, however, there is a mutation in the RNA sequence which changes the amino acid sequence or the protein. In the formation of the cDNA, the RNA is transcribed to the DNA sequence which is more stable can then be further amplified by the second PCR reaction. This amplified DNA can then be sent for sequencing. From here, bioinformatics specialists will take the resulting sequence and compare it to the normal sequence. They will analyze if the mutations in the DNA result in a truncated protein or a protein that is simply incapable of preforming its duty properly. If the protein is there and just not preforming properly this may be due to mutations in the active site, the part of the protein responsible for its function, or binding site, the site that binds the precursors in our case xanthine and guanine. More than 300 disease-associated mutations have been found most of which are point mutations. Decreased HPRT activity and an increase in adenine phosphoribosyltransferase are typical of patients. The variation of expression in these two enzymes helps distinguish the grades of 1-4. Prenatal diagnosis can be performed on amniotic cells from 15-18 weeks as well as on chorionic villus cells from 10-12 weeks in gestation\textsuperscript{26}.

**Treatment**

Allopurinol is given to almost all patients for its ability to inhibit the conversion of hypoxanthine and guanine to uric acid. Allopurinol inhibits this conversion with the enzyme xanthine oxidase. Hydration is necessary to wash out all purine metabolites. This does not however, help with other clinical neurological symptoms, such as motor impairments, discomfort from increased muscle tone, and behavioral problems. Increased muscle tone can be somewhat alleviated with muscle relaxants such as benzodiazepines or baclofen. Behavioral problems are managed with therapy and medications if required. When self-biting cannot be controlled, dental extraction is required\textsuperscript{8}.

To control or manage the motor spasticity and dystonia benzodiazepines and gamma-aminobutyric acid inhibitors are often used. These do not control the muscular problems but do help with anxiety. Physical rehabilitation and use of tools are also used to help manage the disease. Special devices to enable hand control of objects walkers, and posture management are used and are found to be helpful for patients. To control self-injurious behavior, a combination of pharmaceutical treatments, behavioral therapy, and physical restraints are used. When patients are stressed there
is an increase in self-injurious behavior. Physical restraint is needed daily to protect
patients from themselves. Elbow restraints and dental guards allow use of hands
without possibility of finger or cheek mutilation. Patients often request restraints and
become anxious when unrestrained. With treatment, life expectancy of these patients
may reach to the second or third decade (Torres, 2007)

Conclusion

Compared to other diseases, Lesch-Nyhan is fairly new. It has a slow
progression but symptoms can be seen relatively soon after birth. From the first
appearance of crystals to the slow neurological progression to the self-destructive
behavior, nothing is certain. Many patients have variations in severity. All patients
however, have one thing in common, the lack of the HPRT enzyme. For treatment the
many symptoms must be treated individually. Constant care must be given to these
patients. And for those who suffer from self-destructive behavior they must be
restrained from themselves. Although there are many variables associated with Lesch-
Nyhan disease and its progression everyday more is understood about it. Due to many
factors including lack of knowledge of symptoms, decreased care for sick, and inability
to constantly watch animals this has never been seen. Lesch-Nyhan would be very
difficult to diagnosis in an animal because many people would not know what to look for
and the animal would probably not survive very long due to crystal formation in the
kidney.
Resources


12. Rav-Acha M, Bergman H, Yarom Y. Pre- and postsynaptic serotonergic


Figures


