Assessment of Genetics Mutation Gene HPRT1 in induce Lesch-Nyhan Disease in TABRIZ,IRAN

Shahin Asadi (student of Molecular Genetics)

Mahsa Jamali (student of Molecular Genetics)

Zahra Gholizadeh (student of Molecular Biology Genetics)

Samaneh Sadeh Dell (student of Molecular Genetics)

Mina Niknia (student of Molecular Biology Genetics)



Young Researchers and Elite Club, Tabriz,Branch,Islamic Azad University,Tabriz,Iran.
Tell;+989143188935,+989379923364

Email;shahin.asadi1985@gmail.com

***Author; Shahin Asadi-Molecular Genetics-IRAN-TABRIZ. Director of the Division of Medical Genetics and Molecular Research.**

Abstract

In this study we have analyzed 40 people. 20 Lesch–Nyhan syndrome (LNS) and 20 control group.The gene HPRT1 analyzed in terms of genetic mutation made. In this study, people who have genetic mutation were targeted, with nervous disorders, Lesch–Nyhan syndrome (LNS). In fact, of all people with Lesch–Nyhan syndrome (LNS) 20 Lesch–Nyhan syndrome (LNS) had a genetic mutation in the gene HPRT1 Lesch–Nyhan syndrome (LNS). Any genetic mutations in the target genes control group, did not show.

Key Words: Genetic study, Lesch–Nyhan syndrome (LNS),Mutation The gene HPRT1.

Introducation

Lesch–Nyhan syndrome (**LNS**), also known as **Nyhan's syndrome** and **juvenile gout**,[1] is a rare inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT), produced by mutations in the HPRT gene located on the X chromosome. LNS affects about one in 380,000 live births.[2] The disorder was first recognized and clinically characterized by medical student Michael Lesch and his mentor, pediatrician William Nyhan, who published their findings in 1964.[3]

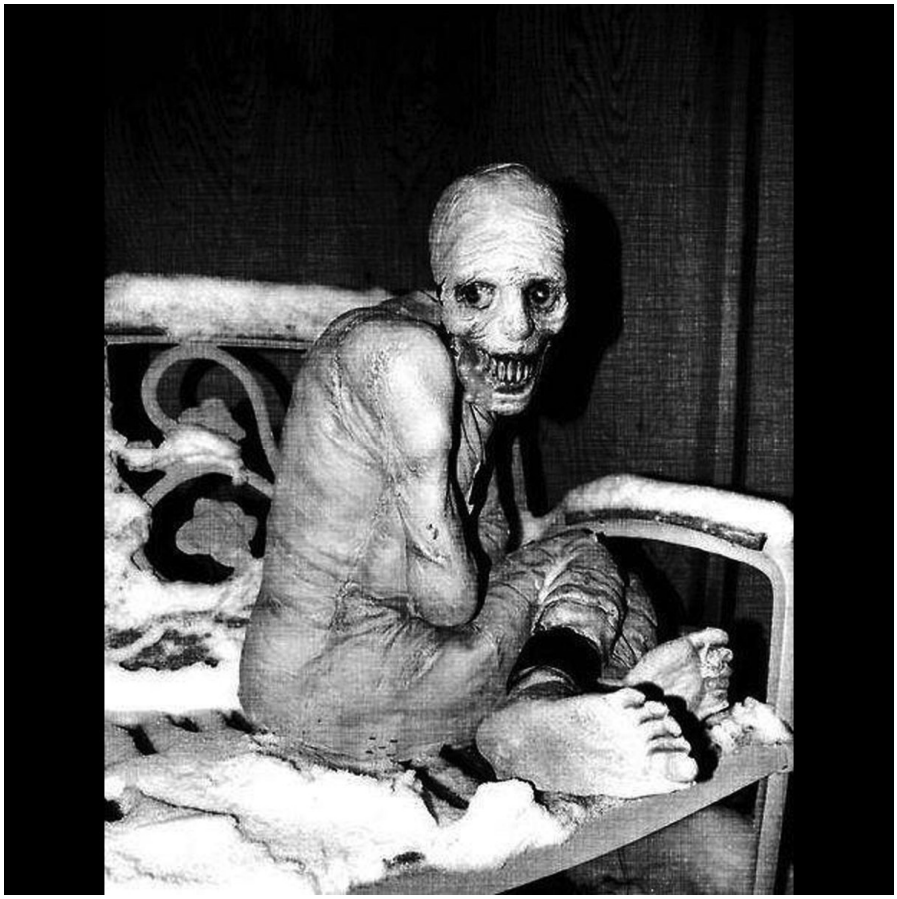


Figure 1: Schematic view of the image first reported case of Lesch-Nyhan syndrome.

The HGPRT deficiency causes a build-up of uric acid in all body fluids. This results in both hyperuricemia and hyperuricosuria, associated with severe gout and kidney problems. Neurological signs include poor muscle control and moderate intellectual disability. These complications usually appear in the first year of life. Beginning in the second year of life, a particularly striking feature of LNS is self-mutilating behaviors, characterized by lip and finger biting. Neurological symptoms include facial grimacing, involuntary writhing, and repetitive movements of the arms and legs similar to those seen in Huntington's disease. The etiology of the neurological abnormalities remains unknown. Because a lack of HGPRT causes the body to poorly utilize vitamin B_{12}, some boys may develop megaloblastic anemia.[4]

LNS is an X-linked recessive disease; the gene mutation is usually carried by the mother and passed on to her son, although one-third of all cases arise *de novo* (from new mutations) and do not have a family history. LNS is present at birth in baby boys. Most, but not all, persons with this deficiency have severe mental and physical problems throughout life. There are a few rare cases in the world of affected females.[5]

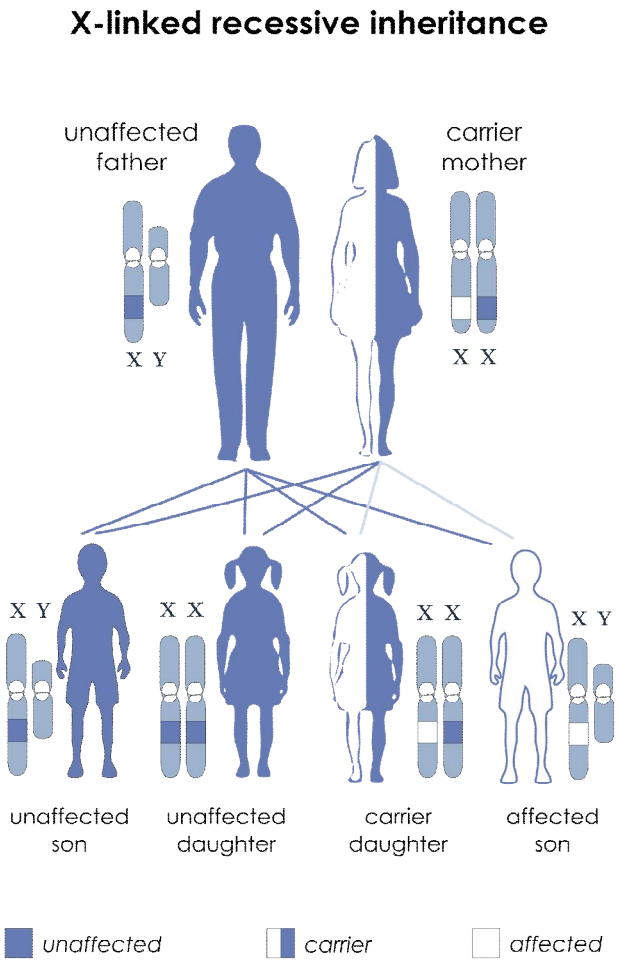


Figure 2: Schematic view of inheritance pattern Lesch-Nyhan syndrome.

The symptoms caused by the buildup of uric acid (gout and renal symptoms) respond well to treatment with drugs such as allopurinol that reduce the levels of uric acid in the blood. The mental deficits and self-mutilating behavior do not respond well to treatment. There is no cure, but many patients live to adulthood. Several new experimental treatments may alleviate symptoms.

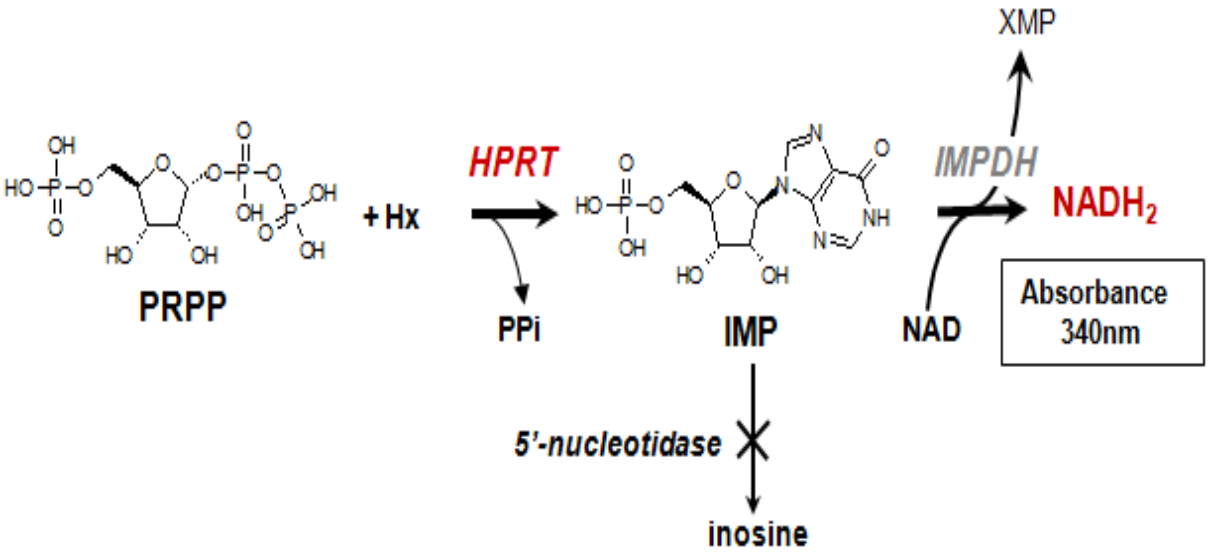


Figure 3: Schematic view of the molecular structure of genes HPRT1 and PRPP Lesch-Nyhan syndrome.

Signs and symptoms

LNS is characterized by three major hallmarks: neurologic dysfunction, cognitive and behavioral disturbances including self-mutilation, and uric acid overproduction (hyperuricemia). Damage to the basal ganglia causes sufferers to adopt a characteristic fencing stance due to the nature of the lesion. Some may also be afflicted with macrocytic anemia. Virtually all patients are male; males suffer delayed growth and puberty, and most develop shrunken testicles or testicular atrophy. Female carriers are at an increased risk for gouty arthritis but are usually otherwise unaffected.

Overproduction of uric acid

One of the first symptoms of the disease is the presence of sand-like crystals of uric acid in the diapers of the affected infant. Overproduction of uric acid may lead to the development of uric acid crystals or stones in the kidneys, ureters, or bladder. Such crystals deposited in joints later in the disease may produce gout-like arthritis, with swelling and tenderness.

The overproduction of uric acid is present at birth, but may not be recognized by routine clinical laboratory testing methods. The serum uric acid concentration is often normal, as the excess purines are promptly eliminated in the urine. The crystals usually appear as an orange grainy material, or they may coalesce to form either multiple tiny stones or distinct large stones that are difficult to pass. The stones, or calculi, usually cause hematuria (blood in the urine) and increase the risk of urinary tract infection. Some victims suffer kidney damage due to such kidney stones. Stones may be the presenting feature of the disease, but can go undetected for months or even years.

Nervous system impairment

The periods before and surrounding birth are typically normal in individuals with LNS. The most common presenting features are abnormally decreased muscle tone (hypotonia) and developmental delay, which are evident by three to six months of age. Affected individuals are late in sitting up, while most never crawl or walk. Lack of speech is also a very common trait associated with LNS.

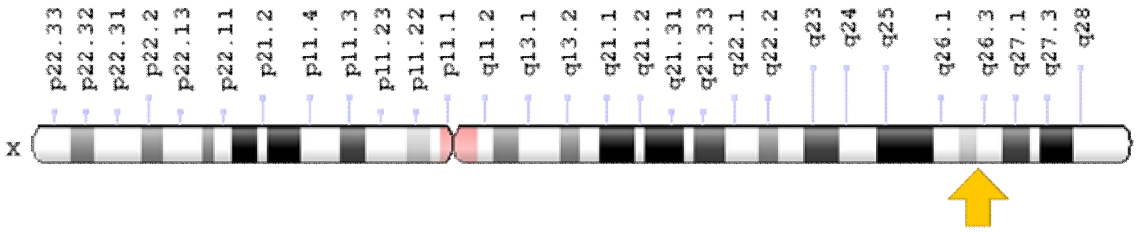


Figure 4: Schematic view of the HPRT1 gene on the long arm of chromosome X in Lesch-Nyhan syndrome.

Irritability is most often noticed along with the first signs of nervous system impairment. Within the first few years of life, extrapyramidal involvement causes abnormal involuntary muscle contractions such as loss of motor control (dystonia), writhing motions (choreoathetosis), and arching of the spine (opisthotonus). Signs of pyramidal system involvement, including spasticity, overactive reflexes (hyperreflexia) and extensor plantar reflexes, also occur. The resemblance to athetoid cerebral palsy is apparent in the neurologic aspects of LNS. As a result, most individuals are initially diagnosed as having cerebral palsy. The motor disability is so extensive that most individuals never walk, and become lifelong wheelchair users.

Materials and Methods

In this study,20 patients with Lesch–Nyhan syndrome (LNS) and 20 control group were studied. Peripheral blood samples from patients and parents with written permission control was prepared.After separation of serum, using Real Time-PCR technique of tRNA molecules were collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solutionin ratio of 1to5with the peripheral blood of patients and controls were mixed.After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 400 Gera. The cells ediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrateratio of 1to2onficole (Ficol) was poured in the 480G was centrifuged for 34 minutes.Mono nuclear Neuroglial cells also are included,has alower density than ficole and soon which they are based.The remaining erythrocytes has a molecular weight greater than fico leand depositedin test tubes.

The supernatant, which contained the mono nuclear cells was removed, and the 400 Gera was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after34 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription gene HPRT1,and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose,approximately 4-5 × 10^3 Neuroglial cells were transfer red to1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7minutes at atime. Remove the supernatant culture medium and there maining sediment, 100µl of PBS buffer was added. After adding 5-10µl PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and readimme diately by flow cytometry. For example,rather than control anti body Neuroglial cells PE,IgG1 negative control solution was used.

Total mRNA extraction proced urein cludes:
1) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperaturefor 5 minutes. Then 200µl chloroform solution to target mix, then transfer the micro tubes were added,and the shaker wellwas mixedfor 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 minat 4°Can was centrifuged at 13200 rpmera. Remove theupperphaseproductweretransferredtoa new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hoursat -20°C were incubated.

2)Then for 45min at 4°C an was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C an by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placedat room temperature until completely dry deposition. The precipitate was dissolved in 20µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs,the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and1µl oligoprimers18 (dT) was performed. Following the PCR reaction2µM dNTP, 1µg cDNA,Fermentas PCR buffer1X, 0 / 75µM MgCl2, 1.25 U / µL Tag DNAat 95°C for 4min, 95°C for 30s, annealing temperature 58°C for 30s, and 72 °C for 30 seconds, 35 cycles were performed. Then1.5% agarose gel,the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color were evaluated.

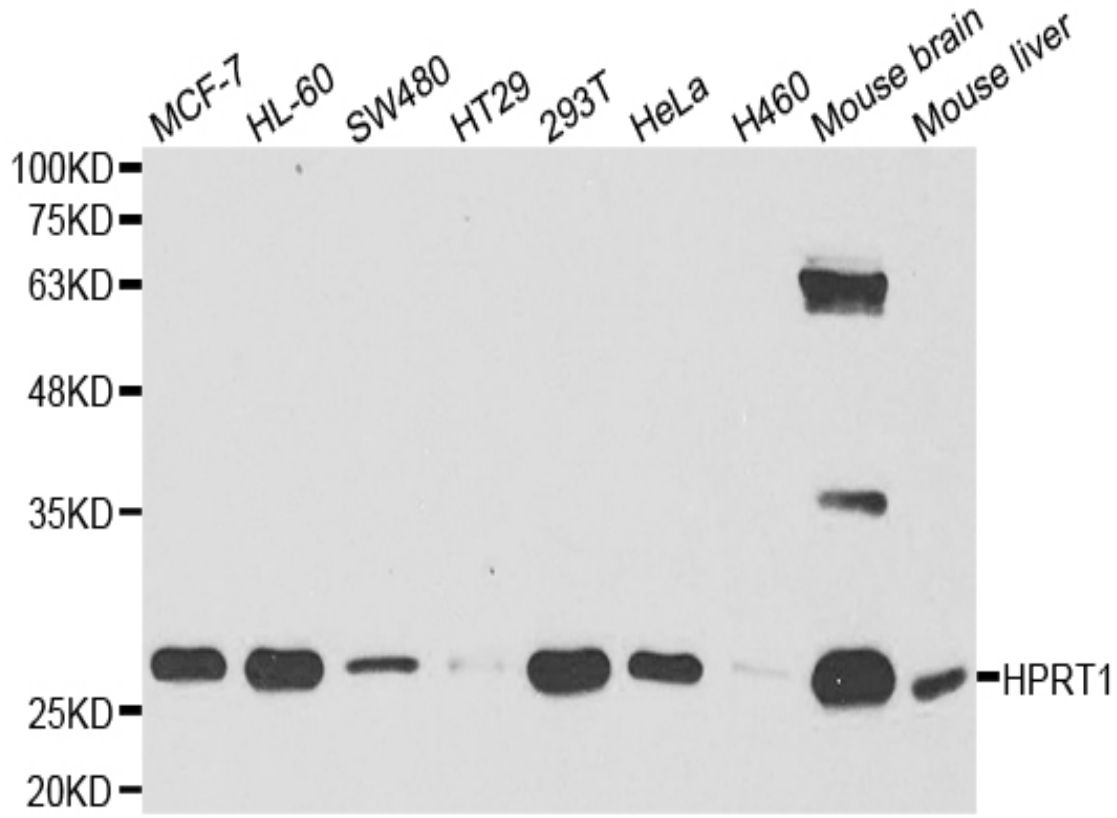


Figure 5: Schematic view of the pattern formed in the band gene HPRT1.

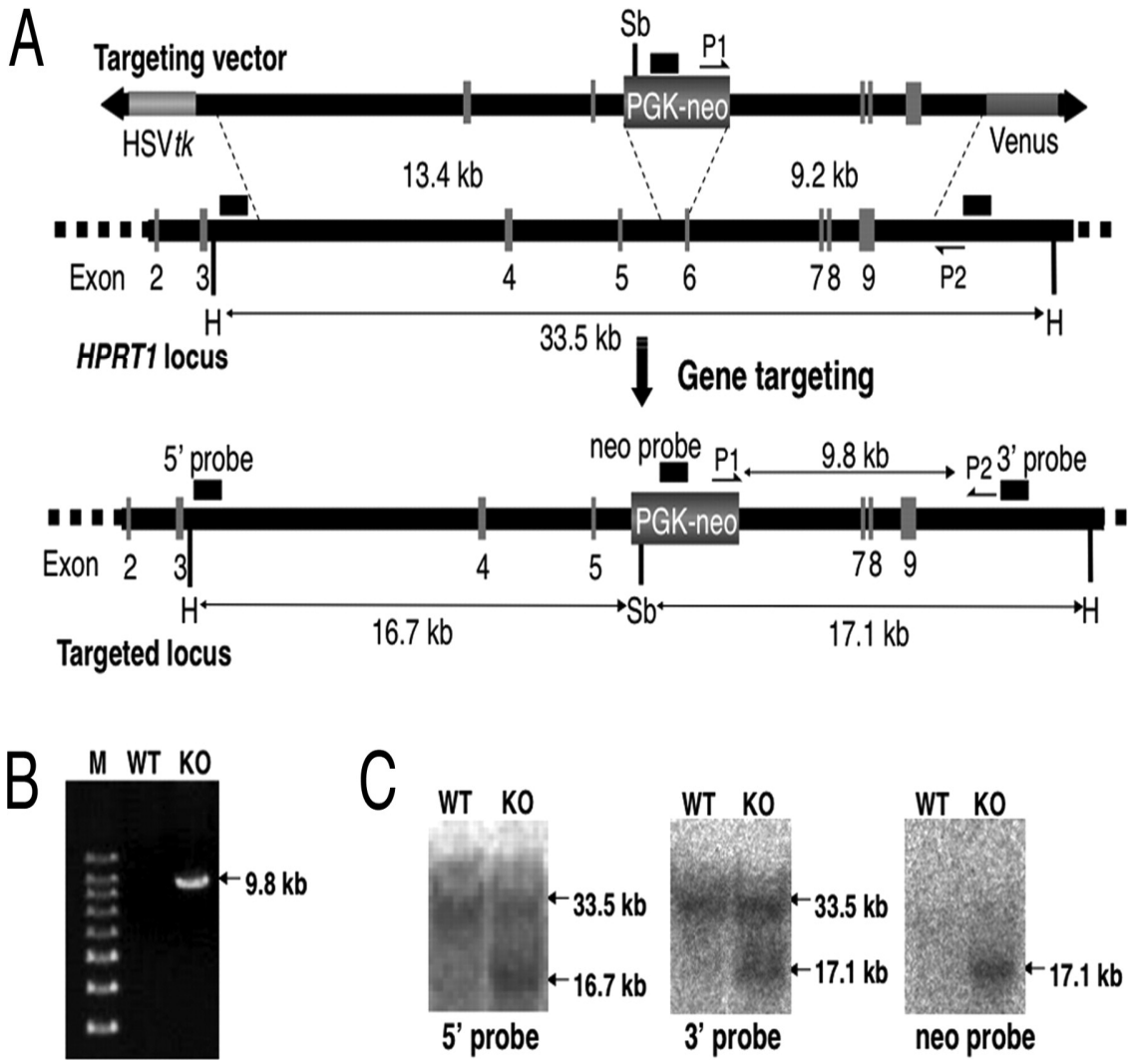


Figure 6: Schematic view exon and gene locus in HPRT1.

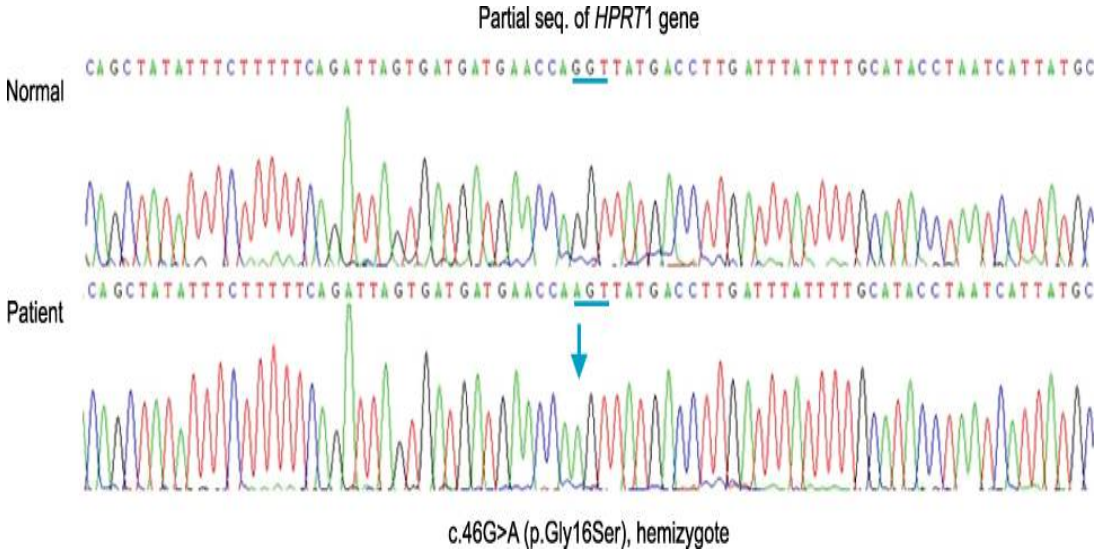


Figure 7: Schematic view of the nucleotide sequence of the mutated gene HPRT1.

Discussion and conclusion

According to the results of sequencing the genome of patients with Lesch–Nyhan syndrome (LNS), and the genetic mutation HPRT1 gene found that about 100% of patients with Lesch–Nyhan syndrome (LNS), they have this genetic mutations. Patients with Lesch–Nyhan syndrome (LNS), unusual and frightening images in the process of Lesch–Nyhan syndrome (LNS), experience. Lot epigenetic factors involved in Lesch–Nyhan syndrome (LNS). But the most prominent factor to induce Lesch–Nyhan syndrome (LNS), mutation is HPRT1 gene.This gene can induce the birth and can also be induced in the adulthood.

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