

# The Tyrosinemia Type I

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*This research is self-supported*

## Abstract

Tyrosinemia type 1 is an autosomal recessive disorder which can be detected as early as possible after birth so that it may be treated or alleviated immediately. If untreated, the disorder can cause dysfunctions of liver, kidney, or neurological disease. There are 3 kinds of tyrosinemia; that is, tyrosinemia type 1, 2, and 3. Tyrosinemia type 1 is the most severe of these disorders. To treat or alleviate the disorder, it can be performed using nitisinone drug along with diet management and liver transplantation. Other methods, which may be used to reduce tyrosinemia type 1, are gene therapy, and, of course, genetic counseling.

## 1. Introduction

Hereditary tyrosinemia type 1 (HT1) is an autosomal recessive disorder [1, 2] caused by deficiency of fumarylacetoacetate hydrolase (FAH), the last enzyme of tyrosine degradation, encoded by *FAH* [1]. It is a rare genetic disorder [3]. Worldwide, HT1 affects about 1 in 100,000 to 120,000 births (Mitchell et al, 2001) [2]. This type of tyrosinemia is much more common in Quebec, Canada. The overall incidence in Quebec is about 1 in 16,000 births. In the Saguenay-Lac St. Jean region of Quebec, HT1 affects 1 in 1,846 births [4]. HT1 may be detected after birth, newborn screening.

In physiological conditions [5], FAH hydrolyzes fumarylacetoaceta (FAA) into fumarate and acetoacetate (Lindblad et al, 1977) [5, 6]. Deficiency of FAH leads to cellular accumulation of FAA in many body tissues, and then intracellular FAA is rapidly degraded into succinylacetone (SA) and succinylacetoacetate (SAA). Although FAH can be detected in multiple tissues, such as lymphocytes and fibroblasts, it is mainly expresses in hepatocytes and renal proximal tubular epithelium. Therefore, liver and kidney are the two primary organs affected in patients with HT1 (Mitchell et al., 1999) [5]. If untreated, tyrosine and its byproducts build up in tissues and organs, which leads to serious medical problems [4]. Individuals with this disorder are at risk of liver cancer (hepatocarcinoma). Research studies suggest that elevated levels of FAA in liver cells initiate or promote tumor development [7].

Nitisinone treatment should begin as soon as the diagnosis of HT1 is confirmed. Because increases the blood concentration of tyrosine, dietary management with control intake of phenylalanine and tyrosine should be started immediately after diagnosis to prevent tyrosine crystal from forming in the cornea. Prior to the availability of nitisinone, the only definitive therapy for HT1 was liver transplantation, which now should be reserved for those children who have severe liver failure at presentation and fail to respond to nitisinone therapy or have documented evidence of malignant changes in hepatic tissue [2]. In addition, other therapies such as genetic therapy may be used in order to inhibit the development of this disorder. Genetic therapy may include gene therapy and genetic counseling. Genetic therapy along with nitisinone and liver transplantation therapies may be very helpful to fight HT1.

This article describes regarding HT1, treatments of HT1 using nitisinone, liver transplantation, and genetic therapy approaches. It is expected that this work is helpful for people all over the world, including scholarly communities who have deeply attention to HT1 patients and their family facing their troubles.

## 2. Materials and Methods

Materials, which are figures available in this work, were taken from article and book searched based on the search methods as described below. Figures may be seen in the link provided by the article or book where the figures were taken.

Methods for information collection, which were used in this work, were dug from databases on National Center for Biotechnology Information (NCBI). There were 4 databases used in this work. These include the following: Gene, Online Mendelian Inheritance in Man (OMIM), Books, and Pubmed Central (PMC). The information searches were done several times. For example, after searching the *FAH* gene in Gene Database, it was searched in OMIM Database, too. Use of each database was based on the needs. The work was performed as follows:

Gene Database: Visited NCBI home page, <http://www.ncbi.nlm.nih.gov>. Under All Databases, clicked Gene and typed *FAH* into the search box and then clicked Search. To get more information regarding *FAH* gene, it was clicked HGNC.

PMC Database: Visited NCBI home page, <http://www.ncbi.nlm.nih.gov>. Under All Databases, clicked PMC and typed such as tyrosinemia type 1 into the search box. Clicked Search and clicked the references needed. All of the references, which were sought, were for open access.

OMIM Database: Visited NCBI home page, <http://www.ncbi.nlm.nih.gov>. Under All Databases, clicked OMIM and typed tyrosinemia type 1 into the search box. Clicked Search and under items selected the information needed, and then clicked it. To get the gene needed, found gene/locus under Phenotype Gene Relationship.

Books Database: Visited NCBI home page, <http://www.ncbi.nlm.nih.gov>. Under All Databases, clicked Books and typed a book name into the search box; that is, genetics or molecular genetics. In this work, it was picked up Human Molecular Genetics. It was related to gene therapy.

For Genetic Testing Registry, it was searched by clicking Genetic Testing Registry at the bottom on NCBI home page, <http://www.ncbi.nlm.nih.gov>. It was performed in order to know about genetic testing for genetic recessive disorder. In this work, it was for *FAH* gene.

Also, there were other home pages used in this work such as Hugo Gene Nomenclature Committee (HGNC). For example, HGNC were used to get symbol gene. It was done by visiting HGNC home page, <http://www.genenames.org> and put cursor on Search Gene tab. Clicked Quick Gene Search, for example, typed *FAH* and then clicked search.

Searches were without date. The main searches were conducted from July to August of 2013. Searches were also conducted beyond the main searches in order to support this work. Search results were related to abstract and/or extraction of articles and books. Also, articles were related to definition for tyrosinemia, for example. Information on analytical processes was made on results and discussion section.

The information, which was obtained from the NCBI and other sources, was directed in order to get: 1) *FAH* gene functions as results of the work, for example; and 2) Discussion of the work. This work is related to computational and systems biology, health sciences, genetics, and information technology. The paper includes Introduction, Materials and Methods, Results and Discussion, Conflict of Interests, Acknowledgement, References, and Figures.

### 3. Results and discussion

#### 3.1 *FAH* gene functions

The official name of this gene is “fumarylacetoacetate hydrolase” (fumarylacetoacetate). *FAH* is the gene’s official symbol. The *FAH* gene is also known as beta-Diketonase, FAA, FAA\_HUMAN, and fumaryacetoacetase [7]. The Human *FAH* gene is localized to the q23-q25 region of chromosome 15 (Phaneuf et al., 1991) [5, 6, 8], contains 14 exons, and covers ~35 kilobases of DNA (Labelle et al., 1993; Awata et al., 1994) [5, 9]. Cytogenetic location of *FAH* gene is on chromosome 15q25.1. More precisely, the *FAH* gene is located from base pairs 80,445,232 to 80,478,923; Figure 1 [7]. For more information regarding *FAH* gene, visit <http://www.ncbi.nlm.nih.gov/gene/2184>. To see MapViewer, for example, on the site, click MapViewer under Genomic context section. To calculate how similar nucleotide or protein sequences are among the same or different kinds of organisms, it is used BLAST (Basic Local Alignment Search Tool). For more information about BLAST, refer to the NCBI Handbook (<http://www.ncbi.nlm.nih.gov/books/NBK21097/>).

The *FAH* gene provides instructions for making an enzyme called FAH. This enzyme is abundant in the liver and kidney, and smaller amounts are found in many tissues through the body. FAH is the last in series of five enzymes needed to break down the amino acid tyrosine, a protein building block found in many foods [7]. Specifically, FAH converts tyrosine byproducts called FAA into smaller molecules that are either excreted by the kidneys or used in reactions that produce energy [4, 7].

FAH is mainly expressed in mammalian liver. It is also expressed, in lesser amounts, in cell from a wide range of tissues such as kidneys, adrenal glands, lungs, hearts, bladder, intestine, stomach, pancreas, lymphocytes (Tanguay et al., 1990), skeletal muscle, placenta, fibroblasts, chorionic villi (Berger et al., 1987), and some glial cells of the mammalian brain (Labelle et al., 1993) [5].

### 3.2 Mutations in *FAH* gene

*FAH* is the only gene in which mutation is known to cause HT1 [2, 4, 7]. Mutations in *FAH* gene cause a shortage of one of the enzymes in the five-step process. The resulting enzyme deficiency leads to a toxic accumulation of tyrosine and its byproducts, which can damage the liver, kidneys, nervous system, and other organs and tissues [4].

Nearly 50 mutations in *FAH* gene have been identified in different races around the world (Human Genome Mutation Database, <http://www.hgmd.cf.ac.uk>). Mutations in *FAH* included missense, nonsense, and splice-site mutations [6]. These mutations are evenly spread along the *FAH* gene but with a slightly higher frequency in some parts of exons 8 and 13 (Bergeron et al., 2001) [5, 6]. One mutation (R<sub>341</sub>W) causes a pseudodeficient phenotype with a reduce amount of FAH immunoreactive material (Rootwelt et al., 1994) [10]. Different ethnic patients with HT1 have different common mutations in *FAH* gene. For example, mutation of IVS12+5G>A is the most prevalent in French and Canadian populations (Grompe et al., 1994), and mutation of IVS6-1G>T, W262S, and P251L are common in the Mediterranean area, Finland and Ashkenazi Jew populations respectively (Himsworth, 1950; Rootwelt et al., 1994; Elpeleg et al., 2002; and De Braekeleer, Larochelle, 1990). Mutation of R237X was detected in a Turkish proband with HT1 (Ploos Amstel et al., 1996) and 7 different families with HT1 in Saudi Arabia (Imtiaz et al., 2011). It was also found a Chinese patient with compound heterozygous mutation (R237X and L375P) [6].

Jorquera and Tanguay (2001) reported that a subapoptogenic dose of FAA, the mutagenic metabolite accumulating in HT1, included spindle disturbances and segregational defects in both rodent and human cells. A sustained activation of the extracellular signal-regulated protein kinase (ERK) was also observed. Primary skin fibroblasts derived from HT1 patients not exogenously treated with FAA showed similar mitotic-derived alterations and ERK activation. Replenishment of intracellular glutathione (GSH) with GSH monoethylester abolished ERK activation and reduced the chromosomal instability induced by FAA by 80%. Jorquera and Tanguay (2001) speculated that this tumorigenic-related phenomenon may rely on the biochemical/cellular effects of FAA as a thiol-reacting and organelle/mitotic spindle-disturbing agent [11, 12].

Genetic correction, a reversion, in HT1 could occur. Kvittingen et al. (1994) found that this genetic correction for 3 different tyrosinemia-causing mutations. In each case, a mutant AT nucleotide pair was reverted to a normal GC pair [1]. One of the mutations that showed reversion was the splice site mutation [13]. Another was the glu357-to-ter mutation due to G-to-T transversion at nucleotide 1069 [14]. Chemical mutagenesis, reversion the disease-causing mutation, could result from the metabolites accumulating in tyrosinemia [1].

For more information regarding mutations in *FAH* gene, visit references [1] and [11].

### 3.3 Genotype-phenotype correlations

One mechanism that explains this clinical variation is gene reversion. Hepatic nodules removed from livers of individuals with chronic form of HT1 have been shown to have cells that are immunologically positive for FAH protein and to have enzymatic activity for FAH (Kvittingen et al., 1994; Grompe, 2001). These seemingly "normal" cells appear to have arisen by gene reversion; that is, the spontaneous self-correction (i.e., back-mutation) of germline mutation to the normal gene sequence during somatic cell division. Spontaneous somatic mutation that suppresses the effects of the pathologic mutations and allows for normal or near-normal gene expression in these cells has also been reported (Blikrud et al., 2005). This is a true reversion of the mutant sequence and not the result of maternal cell colonization or maternal cell fusion (Bergeron A et al., 2004). The

“normal” (i.e., reverted) cells have a selected growth advantage since they are no longer at risk for apoptosis from the accumulation of the FAA. These foci of revertant “normal” cell colonies comprise many of the liver nodules in untreated individuals with chronic HT1 who have a milder biochemical and clinical phenotype (Kim et al., 2000; Demers et al., 2003). However, the continued production of SA and FAA by the non-revertant mutant cells places the individual at continued risk for hepatocellular carcinoma (Kim et al., 2000) [2].

If enough cells have the reverted gene, some FAH activity is restored. Researches have found a correlation between the severities of symptoms and the extent of reversion in liver cells. People with sever symptoms of HT1 have few reverted cells, while those with milder symptoms have many cells with the reverted *FAH* gene [7].

No correlation is observed between clinical presentation and genotype. Acute and chronic forms have been seen in the same families, as well as in unrelated individuals with the same genotype (Poudrier et al., 1998) [2].

Theoretically, genetic correction shows that a genetic disorder, including HT1, may be treated. In this case, treatments for HT1 may be conducted using both drugs and genetic therapies. Use of these both approaches may neutralize the HT1. Researches, which have been conducted in order to overcome the disorder, have been performed since 1992. It has shown success so far.

### 3.4 Diiagnosis

#### 3.4.1 Natural history

The natural history of the typical disease is an evolution to liver failure, cirrhosis with hepatocellular carcinoma, end stage renal failure, acute neuropatic pains and hypertrophic cardiomyopathy [17]. HT1 displays phenotypic heterogeneity with both acute and chronic forms (Mitchel et al., 2001; Poudrier, 1998) [9].

Untreated HT1 usually presents either in young infants with severe liver involvement or later in the first year with liver dysfunction and significant renal involvement, growth failure, and rickets. Growth failure results from chronic illness with poor nutritional intake, liver involvement, and/or chronic renal disease. Death in the untreated child usually occurs before age ten years, typically from liver failure, neurologic crisis, or hepatocellular carcinoma [2].

Children with HT1 may have a characteristic odor of “boiled cabbage” or “rotten mushrooms.” Infants occasionally have persistent hypoglycemia; some have hyperinsulinism (Baumann et al, 2005). Others have chronic low-grade acidosis (Scott, unpublished data) [2]. An acute form of HT1 results in death during the first month of life because of hepatic failure, whereas a chronic form leads to gradual development of liver disease often accompanied by childhood rickets, renal dysfunction, neurological crisis, and hepatocellular carcinoma [8, 10].

Untreated children before age six months typically have acute liver failure with initial loss of synthetic function of clotting factors (Coffe et al., 1999). Prothrombine time (PT) and partial thromboplastine time (PTT) are markedly prolonged and not corrected by vitamin K supplementation: factor II, VII, IX, XI, and XII levels are decreased; factor V and factor VIII levels are preserved. Paradoxically, serum transaminase levels may be only modestly elevated; serum bilirubin concentration may be normal or only slightly elevated, in contrast to most forms of severe liver disease in which marked elevation of transaminases and serum bilirubin concentration occur **concomitantly** with prolongation of PT and PTT. Resistance of affected liver cells to cell death may explain the observed discrepancy in liver function (Vogel et al., 2004) [2]. HT1 is characterized by severe liver injury [18].

Acute patients present before the age of six months, the most severe between 0-2 months (van Spronsen et al., 1994). These patients are particularly susceptible to infections (Epoy et al., 2010). de Laet et al (2013) introduced about sub-acute patients. These patients present with progressive liver disease usually in the first year of life that is usually less severe than the younger patients. The main features are coagulopathy, failure to thrive, hepatosplenomegaly and rickets [19].

In the more chronic form of the untreated disorder, symptoms develop after age six months; renal tubular involvement is the major manifestation. The renal tubular dysfunction involves a Fanconi-like renal syndrome

with generalized aminoaciduria, phosphate loss, and, for many, renal tubular acidosis. The continued renal loss of phosphate is believed to account for rickets; serum calcium concentrations are usually normal [2]. The chronic form is generally diagnosed in early childhood and presents with a milder phenotype by such as secondary renal tubular dysfunction leading to hypophosphatemic rickets [6].

Chronic patients are more than one year old and present mainly with liver and/or renal disease. The illness may be complicated by cardiomyopathy (Arora et al., 2006) and neurological problems such as porphyria-like episodes. Cirrhotic liver changes are generally already present [19].

Untreated children may have repeated neurologic crisis similar to those seen in older individuals with acute intermittent porphyria. These crises include change in mental status, abdominal pain, peripheral neuropathy, and/or respiratory failure requiring mechanical ventilation. Crisis can last one to seven days. Repeated neurologic crisis often go unrecognized. Mitchell et al (1990) reported that 42% of untreated French Canadian children with HT1 had experience such episodes. In an international survey, van Spronsen et al (1994) reported 10% of deaths in untreated children occurred during a neurologic crisis [2].

Untreated infants diagnosed before age two months had a two-year survival rate of 29% (van Spronsen et al., 1994). Those diagnosed between ages two and six months had a 74% two-year survival rate; those diagnosed after age six months had a 96% two-year survival rate. After more than five years the survival rate of the group diagnosed between ages two and six months dropped to approximately 30% and that of the group diagnosed after age six months dropped to approximately 60% [2]. Patients surviving beyond infancy are at considerable risk for the development of hepatocellular carcinoma, and a high level of chromosomal breakage is observed in HT1 cells, suggesting a defect in the processing of DNA [20].

### 3.4.2 Newborn Screening

Newborn screening is best performed using SA as a primary marker because it is sensitive and specific. Blood spot tyrosine is neither specific nor sensitive. Although biochemical abnormalities may be identified shortly after birth (Schlum, Mayatepek, and Spiekerloutter, 2010), babies with HT1 are rarely symptomatic in the first days of life. Newborn screening enables treatment of children who are not yet clinically ill. However, newborn diagnosed by screening have markedly raised  $\alpha$ -fetoprotein levels (Hostetter et al., 1983) [19]. Sniderman et al (2011) indicated that SA is now a routine biomarker for HT1 in newborn screening laboratories. SA is measured directly from the newborn blot spot by tandem mass spectroscopy (Allard et al., 2004; Rashid et al., 2005; Al Dirbashi et al., 2008). In Quebec, Canada, ALA dehydratase (PBG synthase) enzyme activity is measured in the newborn screening program (Gigure et al., 2005). SA is then measured in the urine of infants with apparent  $\delta$ -ALA dehydratase deficiency (Schulze et al., 2001) [2]. Cassiman et al (2009) indicated that the diagnosis of HT1 is based on presence of SA in urine and blood and SAA in urine, and increased tyrosine and methionin [17].

Another method, which can be used, is blood tyrosine or methionin concentration. Elevated concentration of tyrosine or methionin in the blood suggests liver disease; the diagnosis of HT1 should be further evaluated by quantification of plasma or urinary SA. In this case, (1) infants with HT1 may have only modestly of tyrosine and methionin when the first newborn screening sample is collected. (2) Elevated tyrosine concentration on newborn screening can be the result of transient of the newborn, tyrosinemia type II or III, or other liver diseases. (3) Elevated methionin concentration can indicate liver dysfunction, defects in methionin metabolism, or homocystunuria [2]

In addition to SA and SAA, the presence of 4-oxo-6-hydroxyheptanoic acid in urine has also been described pathognomonic (Lindblad, Steen, 1982). The presence of SA and SAA is considered pathognomonic for the disease. Until now, no HT1 without SA or SAA in urine has been described (Scott, 2006). The diagnosis of HT1 is confirmed by measurement of FAH enzyme activity in cultured fibroblasts (or on liver tissue) and/or detection of disease-causing mutations in *FAH* gene [17].

### 3.4.3 Testing [2]

In FAH deficiency, FAA:

(1) Appears to accumulate in hepatocytes, causing cellular damage and apoptosis (identified in animal models by Endo and Sun (2002) and (2) it is diverted into SA and SAA. SA interferes with the activity of the following

enzymes: (a) parahydroxyphenylpyruvic acid dioxygenase (*p*-HPPD), resulting in elevation of plasma tyrosine concentration and (b) PBG synthase, and resulting in 1) reduced activity of the enzyme  $\delta$ -ALA dehydratase and circulating red blood cells; 2) reduced heme synthesis; 3) increased  $\delta$ -aminolevulinic acid ( $\delta$ -ALA), which may induce acute neurologic episodes; and 4) increased urinary excretion of  $\delta$ -ALA.

HTI is characterized by the following chemical findings:

There is increased SA concentration in the blood and excretion in the urine. It needs to be known that (1) Plasma tyrosine concentration in affected infants can be normal in cord blood and during the newborn period. (2) Elevated plasma tyrosine concentration can also be a nonspecific indicator of liver damage or immaturity; for example, in infants taking a high-protein formula (Techakittiroj et al. 2005), including undiluted goat milk (Hendriksz and Walter, 2004).

There is elevated urinary concentration of tyrosine metabolites. *p*-hydroxyphenylpyruvate, *p*-hydroxyphenyllatate, and *p*-hydroxyphenylactate detected on urine organic acid testing.

Increased urinary excretion of the compound  $\delta$ -ALA secondary to inhibition of the enzyme  $\delta$ -ALA dehydratase by SA in liver and circulating red blood cells (Sassa & Kappas, 1983).

Untreated HTI is characterized by the following changes in liver function: (1) markedly elevated serum concentration of  $\alpha$ -fetoprotein (average 160,000 ng/mL) (normal <1000 ng/mL for infants age 1-3 months and <12 ng/mL for children age 3 months to 18 years; and (2) prolonged prothrombin and partial thromboplastin times. It should be noted that (a) Changes in serum of  $\alpha$ -fetoprotein and PT/PTT are more severe in HTI than in nonspecific liver disease and are often the presenting findings in HTI. (b) Transaminases and bilirubin are only modestly elevated, if at all. (c) Presence of normal serum concentration of  $\alpha$ -fetoprotein and normal PT/PTT in an individual with liver disease has a low probability of being from HTI.

Assay of FAH enzyme activity is possible in skin fibroblasts but is not readily available. Affected individuals have very low or undetectable FAH enzyme activity; specific reference ranges vary among laboratories.

It should be noted that homozygosity for the pseudodeficiency allele (p.AArg341Trp) or compound heterozygosity for the pseudodeficiency allele and a pathologic allele results in low FAH enzyme activity but not clinical symptoms and normal serum concentration of tyrosine, thus potentially complicating the interpretation of FAH enzyme activity particularly in prenatal testing. This potential difficulty is now avoided because assay of FAH enzyme activity is no longer in routine use.

### 3.5 Genetic counseling

HTI is inherited in an autosomal recessive manner. At conception, each sib of an affected individual has a 25% chance of being affected (recessive homozygote), a 50% chance of being an asymptomatic carrier (heterozygote), and a 25% chance of being unaffected and not a carrier. This is fit for the first Mendel's law called "Segregation of Allelic Genes." Once an at-risk sib is known to be unaffected, the chance of his/her being a carrier is 2/3. All of the autosomal recessive inheritances are fits with the above information.

The appearance of HTI would be lower if the parents are not relative. If the parents are relative, called consanguinity, the appearance would be higher. The equation for this is  $(q^2 + pqCc/q^2)$  [22]. Cc is the inbreeding coefficient. The equation for the inbreeding coefficient is  $F(Cc) = \Sigma(1/2)^{p+m+1}$  [23]. The prevalence of HTI is 100,000 to 120,000. If the prevalence of 100,000 is used, then, the probability of an expression of HTI for CcI would be about as follows:

$$\begin{aligned} Cc &= (1/2)^{2+2+1} + (1/2)^{2+2+1} = 0.0625 \\ q^2 &= 1/100,000 = 0.00001 \\ q &= \sqrt{0.00001} = 0.0032 \\ p &= 1-0.0032 = 0.997 \end{aligned}$$

If the accurate equation is used, the result is:

$$(q^2 + pqCc/q^2) = 20.70$$

Thus, the HTI would be about 20.70 times higher if the parents were cousins (consanguinity) than if the parents were unrelated. It seems that it is important to get married without unrelated. Unrelated marriages may help to reduce the increase of HTI in the areas where marriages are common. It needs to give education through genetic counseling in the elementary schools in those areas regarding HTI. It may help people in those areas know about HTI as early as possible. However, the decision should be given to the people in those areas whether or not they would like to get married with unrelated.

In the general US population, the carrier frequency is estimated at 1 : 150 to 1 : 100 [2].

Carrier detection and prenatal diagnosis are possible if the diseases-causing mutations for HTI have been identified in the family. The testing can be done on the DNA extracted from chorion villus sampling (CVS) at 10-12 weeks gestation or on DNA extracted from fetal cells obtained by amniocentesis at 15-18 weeks gestation. Preimplantation genetic diagnosis (PGD) for at-risk pregnancies is also possible in principle.

Prenatal diagnosis for pregnancies at 25% risk is possible by detection of SA in amniotic fluid obtain by amniocentesis usually performed at approximately 15-18 weeks gestation. Detection of SA in amniotic fluid is diagnostic; however, because false negative have been reported this method should only be used by laboratories consistently able to identify SA at low levels by stable isotope detection [2].

### 3.6 Gene therapy

The term gene therapy describes any procedure intended to treat or alleviate disease by genetically modifying the cells of a patient. It encompasses many different strategies and the material transferred into patient cells may be genes, gene segments, or oligonucleotides. The genetic materials may be transferred directly into cells within a patient (*in vivo* gene therapy), or cells may be removed from the patient and the genetic material inserted into them *in vitro*, prior to transplanting the modified back into the patient (*ex vivo* gene therapy). Because the molecular basis of the diseases can vary widely, some gene therapy strategies are particularly suited to certain type of disorder, and some to others. Major disease classes include: infectious disease, inherited disorders, cancers, and immune system disorders (Strachan and Reap, 1999) [23]. For HTI, it seems that gene therapy for inherited disorders may be used to treat or alleviate the disorder.

One of the gene therapy techniques is called as gene augmentation therapy (GAT). GAT has been used to treat several inherited disorders caused by genetic deficiency. GAT is targeted at clinical disorders where the *pathogenesis is reversible*. It also helps to have no precise for expression levels of the introduced gene and a clinical response at low expression levels. GAT has been particularly applied to autosomal recessive disorders. Where even modest expression levels of the introduced gene may make a substantial different [24].

The type of DNA molecules used for gene therapy is crucial but “DNA delivery vehicles” are also very important. Gene therapy defined as the transfer of nucleic acid molecules (usually DNA) to patient somatic cells in order to prevent, treat, or alleviate a specific condition. Different gene therapy strategies have been design to suit different type of diseases, the most “classical” of which involves gene delivery to target cells in order to obtain optimal expression of the gene introduced. This therapeutic approach is particularly well suited for inherited diseases that are causes by recessive mutations, since these are typically associated with the absence of a functional gene product or the drastic decrease in the expression of gene. In these cases, the “therapeutic gene” must be inserted within a DNA molecule (usually a bacterial plasmid) along with its entire essential regulatory sequences in order to ensure the correct expression of the gene in the target cells. To facilitate the adequate cellular of the uptake molecules, they must be packed within appropriate “gene delivery vehicles” [25]

One of the first vectors to be developed was pBR322 (Bolivar et al., 1977), which was constructed by ligating restriction fragments from 3 naturally occurring *E. coli* plasmids: R1, R6.5 and pMB1 [26]. The pBR322 plasmid consists of 4362 base pairs (bp). It carries genes for resistance to two antibiotics and cannot grow when either of the two antibiotics is present [23].

Some of the different types of viruses used as gene therapy vectors: (1) Retroviruses - a class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus. (2) Adenoviruses - a class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans.

The virus that causes the common cold is an adenovirus. (3) Adeno-associated viruses - a class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 9. (4) Herpes simplex viruses - a class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores [26].

Lentiviruses belong to the retrovirus family. They are able to transduce both proliferating and nonproliferating cells, augmenting the range of target cells (Naldini et al., 1996; Reiser et al., 1996). These viruses are currently the most widely used gene therapy vectors, as they sometimes permit prolonged gene expression, although gradually silencing of gene expression has also been reported in occasion (Pannell et al., 2000) [27].

Gene therapy may be useful in genetic diseases where there is genetic aberration that causes the absent of expression of a certain gene such as in HT1 due to FAH deficiency and  $\beta$  thalassemia. A study has developed an induced pluripotent stem cell (iPSC) from an FAH deficiency mouse, corrected the genetic aberration by transduction of FAH cDNA using lentiviral vector, and successfully generated healthy mice from corrected iPSC (Wu and Liu, 2011) [25]. It seems that lentiviral vector may be used to fight HT1 on human.

Except lentiviral vector [25], delivery system based on adeno-associated virus (AAV) vectors have attracted significant attention (Youjin and Lun, 2009) [28]. Perhaps, AAV vectors may be developed in order to fight HT1 on human. In mice albinism, AAV vectors had been used in order to treat the disorder. Gargiulo et al (2009) indicated that mice that received sub retinal injection of AAV 2/1-CMV-hTYR-mediated delivery at birth and adult Tyr<sup>c2j</sup> retina resulted in *ex novo* melanin biosynthesis in retinal pigment epithelium and in the choroid, suggesting that deposition of melanin and consecutive pigmentation of the eye are reversible when treated, regardless of the age of the animals [23, 29]]. Therefore, it is reasonable to suggest that AAV vectors may be used in order to treat HT1 on human.

### 3.7 Treatment of HT1 with nitisinone and transplantation

Sniderman et al (2011) suggested that nitisinone should be prescribed as soon as the diagnosis of HT1 is confirmed. A diet low in phenylalanine and tyrosine can lower plasma tyrosine concentration [2]. It would need the combination of NTBC (nitisinone) therapy, liver transplantation, and gene therapy to fight tyrosinemia type 1. At present, the biggest hope to treat the disorder is with using nitisinone therapy. Nitisinone is indicated as an adjunct to dietary restriction of tyrosine and phenylalanine in the treatment of patients with HT1.

Nitisinone was first approved in the US on 30 October 2002, and subsequently in the European Union (EU) via centralized procedure on 21 February 2005. Orfadin is also available for "named-patient" used in other countries. Nitisinone was designed as an Orphan Drug for the treatment of HT1 the Therapeutic Goods Administration (TGA) Health Safety Regulation of Australian Government on 30 October 2008. The drug is not registered in Canada or New Zealand [30].

Nitisinone (Orfadin<sup>®</sup>), 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3 cyclohexanedione (NTBC) was approved by the Food and Drug Administration in April 2002 for treatment of tyrosinemia type 1 (Schewets, 2002) [2]. Nitisinone is competitive inhibitor of 4-hydroxyphenyl-pyruvate dioxygenase, an enzyme upstream of FAH in the tyrosine catabolic pathway. By inhibiting the normal catabolism of tyrosine in patients with HT1, nitisinone prevents the accumulation of the catabolic intermediates maleylacetoacetate and fumarylacetoacetate. In patients with HT1, these catabolic intermediates are converted to the toxic metabolites SA and SAA, which are responsible for the observed liver and kidney toxicity [23]. SA can also inhibit the porphyrin synthesis pathway leading to the accumulation of 5-aminolevulinic acid, a neurotoxin responsible for the porphyric crises characteristic of HT1 (Orfadin: [www.accessdata.fda.gov/drugsatfda\\_docs/label/2013/021232s0101bl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021232s0101bl.pdf)). The tyrosine catabolic pathway can be seen at Figure 2.

The recommended dosage of nitisinone is 1 to 2 mg/kg divided into two daily doses. The initial dose is 1 mg/kg/day divided for morning and evening administration; individual requirements may vary. In patients whose porphobilinogen (PBG) synthase activity and urine 5-ALA and urine SA are not normalized within one month after the start of nitisinone treatment, the nitisinone dose may be increased to 1.5 mg/kg/day. In patients receiving 1.5 mg/kg/day, whose erythrocyte PBG-synthase activity and urine 5-ALA and urine SA remain elevated and whose plasma SA is not normalized after three months, the dose may be increased to up a maximum dose of 2 mg/kg/day.



If plasma nitisinone concentration, plasma SA, urine 5-ALA and erythrocyte PGB-synthase activity are not available, clinical laboratory assessments should include urine SA, liver function tests,  $\alpha$ -fetoprotein, and serum tyrosine and phenylalanine level. During initiation of therapy and during acute exacerbations, it may be necessary to follow more closely all available biochemical parameters.

Schlump et al. (2010) reported that a significant increase in SA was observed within the first 12 hours of life in a newborn baby with HT1 without treatment. It may be necessary to make an early diagnosis and start the treatment. It appeared that nitisinone slowed progression of the disease [16].

Nitisinone is typically given in two divided doses; however, because of the long half-life (50-60 hours), affected individuals who are older and stable may maintain adequate therapy with once-per-day dosing [2].

As soon as the diagnosis is confirmed, or even suspected because of liver disease and appropriate investigations, have been sent, start nitisinone in a dose of 1 mg/kg/day once a day as the half life is 54 hours (Hall MG et al., 2001; Schlune a et al., 2012). A dose of 2 mg/kg/day should be given for 48 hours for those in acute severe liver failure. An alternative approach is to give all patients in liver failure nitisinone at a dose of 2 mg/kg/day from the start and allow the dose to fall with growth to 1 mg/kg/day before increasing it. Nitisinone can only be given orally (or by naso-gastric tube). It is imperative to do so quickly to prevent further liver and kidney damage and avoid potentially major complications such as haemorrhage. The risk of long term complication is also reduced (Holme E and Lindstedt S, 1998) [19].

The safety of nitisinone in the treatment in pregnancy, and the accompanying fetal and maternal hypertyrosinemia, has not been firmly established. However, three patients on nitisinone have had babies who were normal on examinations in the newborn period. Early follow up was also normal [19].

A low phenylalanine and tyrosine diet for HT1 patients was introduced by Halvorsen and Gjessing (1964) and for a long time was the only treatment available. It had a beneficial effect on renal tubular defects, but did not cure the liver disease [19].

Nitisinone increases blood concentration of tyrosine, necessitating a low-tyrosine diet to prevent tyrosine crystals from forming in the cornea. Dietary management should be started immediately upon diagnosis and should provide a nutritional complete diet with controlled intakes of phenylalanine and tyrosine using a vegetarian diet with low-protein foods and a medical formula such as Tyrex<sup>®</sup> (Ross) or Tyros-1<sup>®</sup> (Mead Johnson) [2].

Infants with HT1 should be placed on a low protein diet that contains limited amounts of phenylalanine and tyrosine. In some cases, affected infants have exhibited improvement of liver and kidney abnormalities with dietary management alone. However, progression to cirrhosis, liver failure and potential liver cancer is still possible. Therefore, affected individuals must observe a very strict diet using special medical foods through their lifetime [3].

Phenylalanine and tyrosine requirements are interdependent and vary from individual to individual and within the same individual depending on growth rate, adequacy of energy and protein intakes, and state of health. With appropriate dietary management, plasma tyrosine concentration should be 200-500  $\mu$ mol/L, regardless of age; plasma phenylalanine concentration should be 20-80  $\mu$ mol/L (0.3-1.3 mg/dL). If the blood concentration of phenylalanine is too low (<20  $\mu$ mol/L), additional protein should be added to the diet from milk or foods [2]. Liver transplantation may be required in cases where affected infants or children develop end stage liver failure, fail to respond to therapy with nitisinone, or have evidence of liver cancer [3], hepatocarcinoma.

In an overview of HT1 by Kvittingen in 1986, the case of a patient with HT1 is described who was treated in 1978 with a liver transplant performed by Fisch *et al.* The patient died three months later but the biochemical derangements apparently improved. Subsequently, the use of liver transplants in HT1 cases have increased and the benefit appear to be confirmed. Paradis *et al.* reported in 1990 that six out of seven patients who received liver transplants survived with normal liver function and growth on a normal diet. Thus, liver transplantation seems to be an effective treatment and it may reduce the risk of hepatocellular carcinoma (Ueda *et al.*, 2005). However, long-term follow-up of the patients with HT1 treated with liver transplantation may be necessary to confirm the results [16].

There are three items important that related to liver transplantation [2]:

1) liver transplantation should now be reserved for those children who (a) have severe liver failure at clinical presentation and fail to respond to nitisinone therapy or (b) have documented evidence of the malignant changes in hepatic tissue (Mohan et al., 1999).

2) Transplant recipients require long-term immunosuppression. Mortality associated with liver transplantation in young children is 10%.

3) Transplant recipients may also benefit from low-dose nitisinone therapy to prevent continued renal and glomerular dysfunction resulting from SA generated in renal tissue (Pierik et al., 2005).

It seems that nitisinone, diet management, and liver transplantation are the best way in order to treat the disorder at the present time. The drug is helpful for both rich countries and development countries. It is needed to register the drug for countries that have not yet registered it, particularly the development countries. Nitisinone is beneficial in order to fight HT1.

### 3.8 A Clinical trial

A sample of clinical trial using nitisinone is as follow [31]:

Title: Taste and Palatability of Orfadin Suspension. An Open, Non-controlled 3 Day in Pediatric Patients with Hereditary Tyrosinemia Type 1 Treated with Orfadin

Purpose: The purpose of this study is to verify that pediatric patients, especially those who are not old enough to swallow capsules, accept the taste and palatability of a new suspension.

Detailed description: This is an open, non-randomized, non-controlled, multiple-dose study in 18 pediatric patients. The treatment period is 3 days, and during the study the subject will rate the taste and palatability of the suspension or (for younger children) their parents will rate the child's acceptance of the suspension.

The study consists of a screening period, a 3 day treatment period and a 1 week follow-up period.

Sponsors and collaborators: Swedish Orphan Biovitrum

For more information, visit reference [31].

### Conflict of interests

The author declares that there is no conflict of interests regarding the publication of this article.

### Acknowledgment

Thanks to Editor-in-Chief who has given the opportunity for publication of this article.

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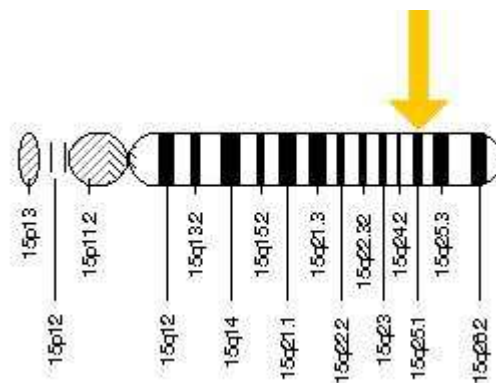
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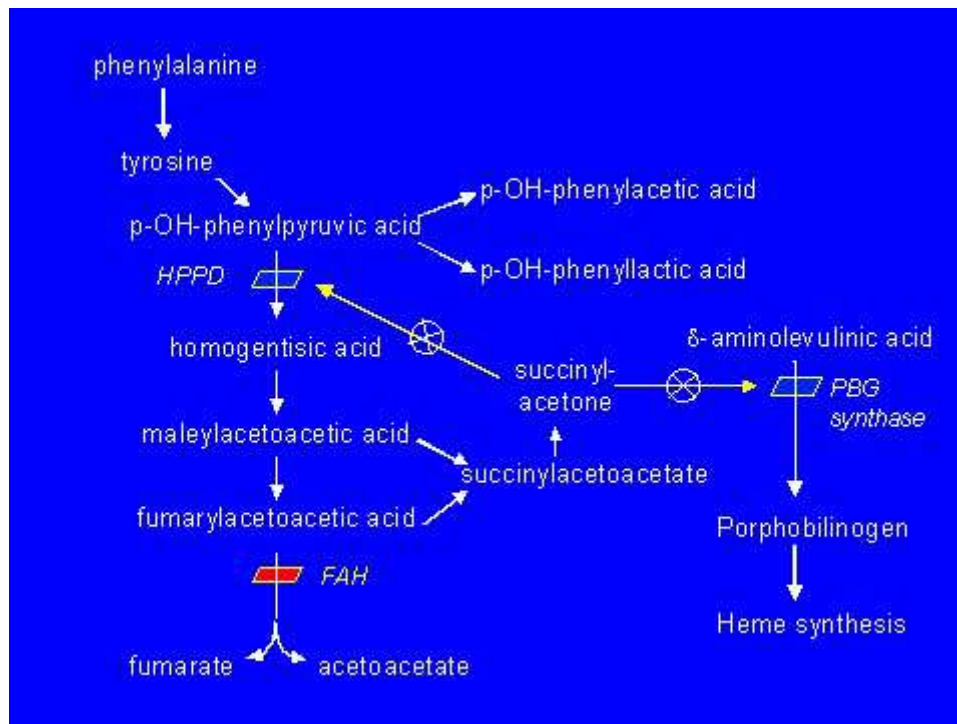
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Figure 1. *FAH* gene location on chromosome 15



Taken from Genetics Home Reference

Figure 2. The Tyrosine Catabolic Pathway



From Sniderman KL e al., *Tyrosinemia Type 1*, University of Washington, Seattle; 1993-2013

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