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Overcome Alkaptonuria

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Abstract
Alkaptonuria (AKU) is an autosomal recessive inborn error metabolism resulting from deficiency of homogentisic acid oxidase (homogentisate 1, 2-dioxygenase) that is required in the metabolism of phenylalanine and tyrosine during the step when homogentisic acid is converted to maleylacetoacetate. AKU is a rare disease affecting approximately 1 in 250,000 people. In certain areas such as Jordan, parts of South India and Slovakia, AKU may be up to ten times higher. It is caused by mutations in the *HGD* gene. Treatment of AKU may be performed using drugs such as nitisinone and anti-inflammatories. Researches are underway using nitisinone in order to treat AKU. Other methods, which may be used to reduce alkaptonuria, are therapy gene and, of course, genetic counseling.
Keywords: Alkaptonuria, alcaptonuria, AKU, HGD

1. Introduction

Alkaptonuria (AKU) was the first genetic disease ever identified as such, Dr Archibald Garrod 1901 in London (Garrod, 1908) [1]. AKU is due to deficiency of homogentisate 1, 2-dioxygenase that is required in the metabolism of phenylalanine and tyrosine during the step when homogentisic acid is converted to maleylacetoacetate [2]. Deficiency of homogentisate 1, 2-dioxygenase causes homogentisic acid, which is produced as phenylalanine and tyrosine are broken down, accumulated in the body. Excess homogentisic acid and related compounds are deposited in connective tissues, which causes cartilage and skin to darken. Over time, a buildup of this substance in the joints leads to arthritis [3]. Homogentisic acid is excreted in urine, too, making the urine turn dark when exposed to air [3, 4, and 5].

Ochronosis, a buildup of dark pigment in connective tissues such as cartilage and skin, is also characteristic of AKU [3]. The ochronotic pigment can be found in the sclera, conjunctiva, limbic cornea, cardiac valve particularly aortic valve, intervertebral disc, muscles and other tissues. Fatal complication may occur in older age (Cobos and Morelo, 2002; Rayana et al., 2008) [2]. Ochronosis occurs after age 30 years [3, 4]; arthritis often begins in the third decade [3]. Other manifestations include pigment deposition, aortic or mitral valve calcification or regurgitation and occasionally aortic dilatation, renal stones, and prostate stones [4].

There is no definite therapy for AKU [2]. Treatment with vitamin C to enhance homogentisate 1, 2-dioxygenase degradation has not proved helpful (La Du, 2001; Wolff et al., 1989) [4]. However, it has been known that Orfadin (nitisinone) may be used as potential therapy to treat AKU (Anikster, Nyhan, Gahl, 1998) since it inhibits the enzyme that produces homogentisate 1, 2-dioxygenase, i.e., 4-hydroxyphenylpiruvat dioxygenase (Lindstedt et al., 1992) [6, 7]. 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 treatment using nitisinone, for example, may be very helpful to fight AKU.

This research paper describes regarding overcoming alkaptonuria using drugs such as nitisinone and genetic therapy approaches. It is expected that this work is helpful for people all over the world, particularly people in developing countries, including scholarly communities who have deeply attentions to AKU patients and their family facing their troubles.

2. Methods

Methods, which were used in this work, were from databases on the National Center for Biotechnology Information (NCBI). There were four databases used in this work. These included 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 *HGD* gene in Gene Database, it could be searched in OMIM Database, too. Use of each database was based on the need. The work was performed as follows:

Gene: Visited NCBI home page, http://www.ncbi.nlm.nih.gov. Under All Databases, clicked Gene and typed HGD in the box. To get more information regarding HGD, clicked HGNC.

PMC: Visited NCBI home page, http://www.ncbi.nlm.nih.gov. Under All Databases, clicked PMC and typed the information such as alkaptonuria in the box. Clicked search and clicked the references needed. All of the references, which were sought, were for open access.

OMIM: Visited NCBI home page, http:///www.ncbi.nlm.nih.gov. Under All Databases, clicked OMIM and typed HGD in the box. Clicked Search and under items selected the information needed, and then clicked. To get the gene needed, found gene/locus under Phenotype Gene Relationship.
Books: Visited NCBI home page, http://www.ncbi.nlm.nih.gov. Under All Databases, clicked Books and typed a book name needed in the 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 alkaptonuria.
Also, there were other home pages used in this work, among other things, 1) Genetic Home Reference (GHR) and Hugo Gene Nomenclature Committee (HGNC). GHR was used in order to get definition(s) and information needed for this work. HGNC was used in order to get symbols for gene, for example. For GHR, visited GHR home page, http://ghr.nlm.nih.gov and typed alkaptonuria or HGD in the box, then clicked Search. Searched the information needed. For HGNC, visited HGNC home page, http://www.genenames.org and put cursor on Search Gene tab. Clicked Quick Gene Search. Typed HGD and then clicked Search.
The information, which was obtained from the NCBI and other resources, was directed in describing this work; that is, Overcome Alkaptonuria. It included Natural history, Mutations in *HGD* gene, Clinical diagnosis, Genetic counseling, Gene therapy, Treatment of alkaptonuria, and A clinical trial using nitisinone.

3. Results and Discussion

3.1 Natural history

Alkaptonuria occurs worldwide. At least 1000 cases of AKU have been identified by 2012 [1]; this is likely underestimate (LaDu, 2001). The incidence of AKU in the US is estimated as 1 in 250,000 to 1,000,000 live births. A high prevalence has been observed in the Dominican Republic (Milch, 1960) and near the Slovakian-Bohemian border, likely of the result of a founder effect (Srsen, 1993) [4]. The prevalence of AKU in Slovakia is estimated as 1 in 19,000 (Zatkova et al., 2003) [3, 4]. AKU is one of four conditions hypothesized by Garrod to be inborn error of metabolism. Mutations responsible for other conditions are albinism, cystinuria, and pentosuria. All of them have been identified.
The clinical findings of AKU include darkening of urine upon standing as a result of the presence of homogentisic acid and its oxidation products, connective tissue ochronosis, and arthritis of the spine and larger joints. Homogentisic acid excretion and disease severity can vary significantly within the same family. In some cases, the diagnosis of AKU is made only after the individual seeks medical attention because of chronic joint pain or after black articular cartilage is noted during orthopedic surgery [4].
Urine in an infant's diaper may darken and can turn almost black after several hours. However, many persons of this condition may not know they have it until mid adulthood [5]; around 30 to 40 years old. Symptoms of AKU may include: arthritis (especially of the spine) that get worse over time, darkening of the ear, and dark spots on the white of the eye (sclera and cornea) [5]. AKU does not cause developmental delay or cognitive impairment and does not generally reduce the life span of affected individuals [4].
Phornphutkul et al. (2002) provided a review of the natural history of AKU. They based the review on an evaluation of 58 patients with the disorder ranging in age from 4 to 80 years. They found that joint replacement was performed at a mean age of 55 years and the renal stones developed at 64 years, cardiac-valve involvement at 54 years, and coronary artery calcification at 59 years. Linear regression analysis indicated that the radiographic score for the severity of disease began increasing after the age of 30 years, with a more rapid increase in men than in women [6, 7]. In the 58 patients reviewed by Phornphutkul et al. (2002), kidney stones were documented in 13 male and 3 female patients. Of the 27 men who were 31 to 36 years old, 8 had prostate stones. The development of prostate stones was not associated with the development of kidney stones. Three patients, each over the age of 50 years, had undergone aortic valve replacement (OMIM 203500) [6].
Stenn et al. (1977) provided evidence that the Egyptian mummy Harwa, dating from 1500 B.C., had alkaptonuria, AKU disorder [6]. Harwa was the first known patient with AKU. Previous reviews of the history of AKU (O'Brien et al., 1963) have shown cases throughout history, beginning in 1584 with the examination of a young boy whose urine turned black when exposed to air [8].

3.2 Mutations in the HGD gene

Homogentisate 1, 2-dioxygenase is composed of 445 amino acids and is expressed predominantly in the liver and kidney, with some expression in the small intestine, colon, and prostate (Fernadez-Canon et al., 1996). Homogentisate 1, 2-dioxygenase functions in the metabolism of homogentisic acid by catalizing an oxidative cleavage of the benzene ring to yield maleylacetoacetic acid. It requires oxygen, ferrous iron, and sulfhydril groups [4].

The *HGD* gene provides instructions for making an enzyme called homogentisate 1, 2-dioxygenase. The enzyme helps break down the amino acids phenylalanine and tyrosine, which are important building blocks protein. Mutations in the *HGD* gene cause alkaptonuria. The mutations impair the enzyme's role in this process [3]. The AKU locus was mapped to human chromosome 3q2 [2]. The gene is located on the long (q) arm of chromosome 3 at position 13.33. More precisely, the *HGD* gene is located from base pair 120, 347, 014 to base pair 120, 401, 417 on chromosome 3 (Figure 1). Mutations in the *HGD* gene inactivate homogentisate 1, 2-dioxygenase by changing its structure [9].

Fernandez-Canon et al. (1996) identified missense mutations in the *HGD* gene that consegrated with the disease, and provided biochemical evidence that at least one of these missense mutations is a loss function mutation. Gehrig et al. (1997) found 2 novel mutations in the *HGD* gene in 4 PKU patients from Slovakia. In Turkey, Elcioglu et al. (2003) described a 39-year-old male patient with typical features of AKU. In addition to the typical changes of the skin at many sites and in the pinnae and sclerae, there were grayish-blue longitudinal rigging of his fingernails and bluish-gray pigment deposition on the tympanic membrane. He was found to be compound heterozygous for 2 mutations in the *HGD* gene: gly270 to arg in exon 11 and 342delA in exon 3 leading to frameshift after arg58 and a subsequent premature stop codon. Beltran-Valero de Bernabe et al. (1998, 1999), Muller et al. (1999), Rodriguez et al. (2000), Zatkova et al. (2000), and Phornphutkul et al. (2002) identified other mutations in patients with AKU, too [6].

Normal allelic variants of homogentisate 1, 2-dioxygenase is 54.3 kb in length and has 14 exons coding for a 1715-bp transcript (Garadino et al., 1997). At least 67 mutations have been identified in people with AKU and are found in different allelic combinations (Phornphutkul et al., 2002) [4]. Most of these mutations change single amino acids used to build the homogentisate 1, 2-dioxygenase enzyme. For example, a substitution the amino acid valine for the amino acid methionine at protein position 368 (also written at Met368Val) is the most common *HGD* mutation in European population [9]. The mutations are distributed throughout the homogentisate 1, 2-dioxygenase sequence. The majority of mutations are missense, but nonsense, frame shift, and splice site do occur. Most mutant's alleles are predicted to result in complete loss of enzymatic activity [4].

3.3 Clinical diagnosis

Diagnosis of AKU is made by examining the urine for the presence of homogentisic acid. X-rays may also be taken to decide if the patient's joints and bones show arthritic changes [10]. Testing for AKU may be performed using biochemical testing. It is based on the detection of a significant amount of homogentisic acid in the urine by gas chromatography-mass spectrometry analysis. The amount of homogentisic acid per day in individuals with AKU is usually between 1 and 8 grams (LaDu, 2001 and Phornphutkul et al., 2002). A normal 24-hour urine sample contains 20-30 mg of homogentisic acid. Biochemical testing cannot detect the carrier state [4].If ferric chloride is added to the urine, it will turn the urine to black color in patients with PKU disorder [5].

Diagnosis typically either happens in early childhood or much later in life. In early childhood, diagnosis is suspected due to the urine turning black when exposed to air. Diagnosis later in life is usually first suspected to back pain and joint pain. In both cases, the diagnosis is confirmed through urine tests [8], as described above.

AKU has 3 major features [4]. These include the following: Homogentisic acid in the urine, arthritis, and ochronosis. Oxidation of the homogentisic acid excreted in the urine produces a melanin-like product and causes the urine to turn black upon standing. Individuals with alkaptonuria usually have dark urine or urine that turns dark on standing or exposure to an alkalin agent. However, darkening may not occur for several hours after avoiding and many individuals never observe any abnormal color to their urine. Arthritis often begins in the spine and resembles ankylosing spondylitis in its large-joint distribution. Radiograph of the spine showing flattened calcified intervertebral disks are pathognomonic. Findings include the generation of the intervertebral disks followed by disks calcification and eventually fusion of the vertebral bodies. Osteophyte formation and calcification of the intervertebral ligaments are minimal. Radiograph of the large joints may show joint space narrowing, subchondral cysts, and infrequent osteophyte formation. Enthesopathy can be seen at the muscle insertions (Mannoni et al., 2004). Ochronosis may include:

(1) Brown pigmentation of the sclera is observed midway between the cornea and the outer and inner canthi at the insertion of the recti muscle. Pigment deposition may also be seen in the conjunctiva and cornea. The pigmentation does not affect vision (Chevez Barriors & Font, 2004).

(2) Each cartilage pigmentation is first seen in the concha and antihelix, and later in the tragus. The cartilage is slate blue or gray and feels irregular or thickened. Calcification of the ear cartilage may be observed on radiographs.

(3) Pigment also appears in cerumen and in perspiration, causing discoloration of clothing.

(4) A deep purple discoloration may be seen on the skin of the hands, corresponding to the underlying tendons, or in the web between the thumb and index finger.

During childhood, there are no symptoms of AKU other than the urine turning black when left to stand for a view minutes. Typically, the pain associated with AKU starts around the second decade of life. Joint pain

typically starts in the weight-bearing joints, especially the lower back, hips and knees (Phornphutkul et al., 2002; Helliwell et al., 2008; Schumacher and Holdsworth, 1977; and O'Brien et al., 1963). By the fifth decade of life, many patients will have had at least one joint replaced through surgery [8].

3.4 Genetic counseling

AKU 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 asymptomatic carrier (heterozygote), and a 25% chance of being an unaffected and not a carrier, dominant homozygote. 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.

The appearance of AKU 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 $(q^2 + pqCc)/q^2$ [11]. Cc is the inbreeding coefficient. The equation for the inbreeding coefficient is $F(Cc) = \Sigma(1/2)^{p+m+1}$. The prevalence of PKU is 1 in 250,000. Then, the probability of an expression of AKU for CcI would be about as follows:

$Cc = (1/2)^{2+2+1} + (1/2)^{2+2+1} = 1/16$

$q^2 = 1/250000 = 0.000004$

$q = \sqrt{0.000004} = 0.002$

$p = 1 - 0.002 = 0.998$

If the accurate equation is used the result is:

$(q^2 + pqCc)/q^2 = 32.1875 = 32.19$

Thus, the AKU would be about 32.19 times higher if the parents were cousins (consanguinity) than if the parents were unrelated.

In countries, notably, Slovakia (Zatkova et al., 2000), the Dominican Republic (Milch, 1960), and the Middle East (Al-Shou and Mwafi, 2010) the frequency is closer to 25 in 500000. Recent work from Jordan identified 9 cases within one family (Al-Shou and Mwafi, 2010). The reason for this seems to be the consequences of consanguinity; that is, marriage among close relative such as within kinship [8]. It seems that it is important to get married without unrelated.

Carrier detection and prenatal diagnosis are possible if the diseases-causing mutations for AKU have been identified in the family. The testing can be done on 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.

Request for prenatal testing for conditions such as AKU that do not affect intellect or life span and have some treatment available are not common. However, most centers would consider decisions about prenatal testing to be the choice of the parents, discussion of these issues is appropriate [4].

3.5 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 material may be transferred directly into cells within a patient (*in vivo* gene therapy), or cells may be removed from the patient and the genetic 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 types of disorder, and some to others. Major disease classes include: infectious disease, inherited disorders, cancers, and immune system disorders (Stratchan and Reap, 1999) [12]. For alkaptonuria, it seems that gene therapy for inherited disorders may be used to treat the disease.

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 an introduced gene may make a substantial different [12].

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 tyrosinemia type 1 due to fumarylacetoacetate (FAH) deficiency and β 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 lentiveral vector, and successfully generated healthy mice from the corrected iPSC (Wu and Liu, 2011) [13].

The type of DNA molecules used for gene therapy is crucial but the "DNA delivery vehicles" are also very important. Gene therapy is 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 designed 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 a 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 uptake of DNA molecules, they must be packed within appropriate "gene delivery vehicles" [14].

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 [15]. The pBR322 plasmid consists of 4362 base pairs (bp). It carries genes for resistance to two antibiotics: ampicillin and tetracycline. Normal *E. coli* cells are sensitive to these two antibiotics and cannot grow when either of the two antibiotics is present.

Viral vectors have become the preferred "gene delivery vehicles" in the field of gene therapy due to their extremely high efficiency in gene transfer in somatic cells. Recently, delivery systems based on adeno-associated virus (AAV) vectors have attracted significant attention (Youjin and Lun, 2009) [15]. According to the current dogma, AAV can efficiently package cargo up to 4.7 kilo base (kb), AAV2/1-5 are capable of packaging up to 6 kb and rAAV vectors with AAV5 caspids are capable of packaging up to 8.9 kb of single-stranded DNA more efficiently than other stereotypes (Alloca et al., 2008) [16]. It shows that package cargo based on AAV vectors have been developed almost two times of wild-type AAV contains a 4.7 kb genome, and perhaps it may be developed of using large fragments in gene therapy.

Perhaps AAV vectors may be used to treat alkaptonuria disorder. A research using AAV vectors had been done in order to treat albinism. The result of the research showed that albinism in mice can be treated. 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^{c2j} 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 [17]. Therefore, it is reasonable to suggest that AAV vectors should be used in order to treat AKU a long with nitisinone and genetic counseling.

3.6 Treatment of alkaptonuria

There is no cure for AKU. The biggest hope for a future treatment of alkaptonuria lies with a drug, nitisinone (NTBC, Orfadin). Nitisinone blocks the homogentisate 1, 2-dioxygenase enzyme, thereby blocking production of homogentisic acid (Hall et al., 2001). It is expected that it should provide an effective treatment (Suwannarat et al., 2005). Patients on this drug report an improvement in their condition; however, this drug is not licensed for use in AKU since it is still under clinical trial. The AKU Society estimates 1 % of the AKU population are taking the drug off-label [8]. However, nitisinone is approved for use in tyrosinemia type 1; its registration does not include AKU as an indication [4].

Nitisinone is competitive inhibitor of 4-hydroxyphenyl-pyruavate dioxygenase (NTBC), an enzyme upstream of fumarylacetoacetate hydrolase (FAH) in the tyrosine catabolic pathway. By inhibiting the normal catabolism of tyrosine in patients with HT-1, nitisinone prevents the accumulation of the carbolic intermediates maleylacetoacetate and fumarylacacetoacetate. In patients with HT-1, these catabolic intermediates are converted to the toxic metabolites succinylacetone and succinylacetoacetate, which are responsible for the observed liver and kidney toxicity. Succinylacetone can also inhibit the porphyrin synthesis pathway leading to the accumulation of 5-aminolevulinate, a neurotoxin responsible for the prophyric crises characteristic of HT-1. Chemically, nitisinone is 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione (Orfadin; www.acessdata.fda.gov/drugsatfda_docs/label/2013/021232s010lbl.pdf).

In a murine model of alkaptonuria that had been created with ethylnitrosurea by Mongagutelli et al. (1994), they observed a dose-dependent reduction in urinary output of homogentisic acid with administration of nitisinone.[6]. It should be known that no long-term studies in animals have been performed to evaluate the carcinogenic potential of nitisinone (Orfadin; www.acessdata.fda.gov/drugsatfda_dogs/label/2013/021232s010lbl.pdf).

No preventive or curative treatment is available. Treatment of prostate stones and renal stones may include surgical intervention. Knee, hip, and shoulder replacement surgeries are options for managing of significant arthritis. In general, the goal of joint replacement is pain relief rather than increased range of motion. Joint replacement in individuals with alkaptonuria is associated with prosthetic survival comparable to that found in individuals with osteoarthritis (Spencer et al., 2004). Maintaining joint range of motion through moderate non-weight-bearing exercise such as swimming, non-contact and lower impact sports for younger individuals may have beneficial effects. Physical and occupational therapy are important to promote optimal muscle strength and flexibility [4]. In addition, a low protein diet that restricted diet severely is not recommended. However, it is best to avoid eating too much red meat, instead replace it with white meats, such as chicken. There is no evidence that increasing vitamin C dietary in individuals with AKU is beneficial. It may actually contribute to the formation of homogentisic acid [4, 8]. No therapy is proven to prevent or correct the pigmentation changes of ochronosis [4].

3.7 A Clinical trial using nitisinone

More recently, low-dose nitisinone reduced urinary homogentisic acid by up to 95% in 9 individuals with AKU. In the same study, 7 individuals were treated for up to 15 weeks while receiving normal protein intake; all had elevated plasma tyrosine concentrations. No ophthalmic, neurologic, or severe dermatologic complications were observed. Two individuals had transient elevations in liver transaminase levels that returned to normal after stopping nitisinone (Suwannarat et al., 2005) [4].

A study is currently recruiting participant as follows [18].

Title: Nitisinone (NTBC) in Different Age Groups of Patients with Nitisinone

Purpose: Nitisinone is a potent inhibitor of the enzyme that catalyzes the formation of homogentisic acid, and should be an even more logical treatment for alkaptonuria than for tyrosinemia, for which has been approved by the FDA. The objective of this research is to explore reported age related differences in toxicity of nitisinone and its pharmacokinetic underpinnings and to develop an optimal therapeutic requirement for a targeted population of presymptomatic patients. The additional effect of mixtures of amino acids excluding tyrosine will be explored to take advantage of protein synthesis to avoid elevations of tyrosine that would otherwise limit the optimal dosage of nitisinone. The study is designed to treat patients and find the optimal dosage of nitisinone to obtain maximal reduction in levels of homogentisic acid and maintain safe levels of tyrosine.

The long term objective in the target population of pre-symptomatic patients is the prevention of the characteristic effects on joint cartilage and tendons.

Study Type: Interventional

Study Design: Endpoint classification: Safety/Efficacy Study
Intervention Model: Single Group Assignment
Masking: Open Label
Primary Purpose: Treatment

Drug: Nitisinone. Taken orally. Supplied as a 2 mg tablet. The starting dose is 2 mg once daily.

Sponsor: University of California, San Diego

Dates: January 2011 – June 2021

References

1. Sireau NT. (2012). Developing a cure for Black Bone Disease. *Orphanet Journal of Rare Diseases,* 7(Sppl 2): A37, http://www.ojrd.com/content/7/52/A37.

2. Datta AR et al. (2008). Alkaptonuria diagnose in a 4-month-old-baby girl: a case report. *Case Journal*, 1 : 308. doi: 10.1186/1757-1626-1-308, http://www.casesjournal.com/content/1/308.

3. Genetic Home Reference. Alkaptonuria. Available: http://ghr.nlm.nih.gov/condition/alkaptonuria.

4. Introne WS, Kayaer MA, Gahl WA,. (2003). *Alkaptonuria.* [Updated 2011 Mar 10]. In: Pagon RA, Adam MP, Bird ID, et al., editors. GeneReview™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013. Available: http://www.ncbi.nlm.nih.gov/books/NBK1454/.

5. MedlinePlus. Alkaptonuria. Available: http://nlm.nih.gov/medlineplus/ency/article/001200.htm.

6. Online Mendelian Inheritance in Man. Alkaptonuria. Available: http://omim.org/entry/203500.

7. Phornphutkul C. et al. (2002). Natural History of Alkaptonuria. Available: http://www.ncbi.nlm.nih.gov/pubmed/12501223?dopt=Abstract.

8. AKU Society. Alkaptonuria. Available: http://www.rarediseasesindia.org/aku.

9. Genetic Home Reference. *HGD*. Available: http://ghr.nlm.nih.gov/gene/HGD.

10. Madisons Foundation. Alkaptonuria. Available: http://www.madisonsfoundation.org/idex.php?option=com_mpower&task=disease&diseaseID=378.

11. Genetic Counseling. Available: http://atlasgeneticsoncology.org/Educ/consangID30039ES.html.

12. Stratchan T and Reap AP. (1999). *Human Molecular Genetics.* 2nd edition. New York: Wiley-Liss. Chapter 22, Gene therapy and other molecular genetic-based therapeutic approaches. Available: http://www.ncbi.nlm.nih.gov/books/NBK7569/.

13. Pawitan JA. (2012). Prospect of Induced Pluripotent Stem Cell Genetic Repair to Cure Genetic Diseases. *Stem Cells International*, Volume 2012, 7 pages. doi: 10.1155/2012/498197.

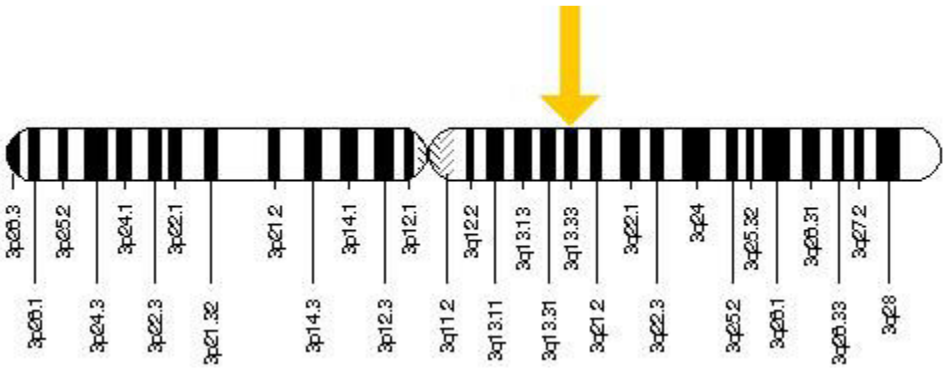
14. Perez-Luz S and Diaz-Nido J. (2010). Prospect for the Use of Artificial Chromosome and Minichromosome-Like Episomes in Gene Therapy. *Journal of Biomedicine and Biotechnology*, Volume 2010, 16 pages. doi: 10.1155/2010/642804.

15. Brown TA. (2002). *Genomes*. 2nd edition. Oxford: Wiley-Liss. Chapter 4, Studying DNA. Available: http://www.ncbi.nlm.nih.gov/books/NBK21129/.

16. Alloca M et al. (2008). Serotype-dependent packaging of long genes in adeno-associated viral vectors effective gene delivery in mice. *J. Clin. Invest.* 118: 1955-1964. doi: 101172/JC134316.

17. Gargiulo A et al (2009). AAV-mediated Tyrosinase Gene Transfer Restores Melanogenesis and Retina Function in a Model of Oculocutaneous Albinism Type 1 (OCA1). *Molecular Therapy vol.* 17 no. 8, 1347-1354.
18. Clinical Trials. Available: http://clinicaltrials.gov/ct2/show/NCT01390077?cord=22alkaptonuria22&rank=3.

Figure 1. *HGD* gen location on chromosome 3



Taken from Genetic Home Reference.