Control of alkaptonuria with nitisinone and gene therapy: A systematic review

Martin L. Nelwan*

1Nelwan Institution for Human Resource Development, Department of Animal Science – Other, Jl. A. Yani No. 24, Palu 94111, Indonesia. E-mail: mlnelwan2@gmail.com

Abstract

Alkaptonuria (AKU) is a genetic disorder inherited in accordance with Mendel first law. Mutations in the HGA gene result in the AKU disorder. Three major features of this disorder: arthritis, ochronosis, and the presence of Homogentisic Acid (HGA) in the urine. The author searched the PubMed Databases at National Center for Biotechnology Information (NCBI) for articles on AKU published between 2014 and 2019. All articles were open access and in English. In this systematic review, the author included one’s own references and other relevant publications. Search results showed that detection tools for people with AKU can include x-rays and genetic tests. No adequate treatment is available for AKU at present. However, counselors of genetic counseling may help patients with AKU and give counseling to them and their families. Candidate drugs of AKU are nitisinone and genetic manipulation techniques. Research results on the use of nitisinone on AKU have shown remarkable improvements. In the future, genetic manipulation techniques may be beneficial for treating AKU. These techniques are such as modified CRISPR/Cas9 (FokI-dCas9), End-Joining Homology Techniques (EJHTs) and induced Pluripotent Stem Cells (iPSCs).

Keywords: Alkaptonuria (AKU), Alcaptonuria, HGD, Homogentisic Acid (HGA)

1. Introduction

Alkaptonuria (AKU) is a rare genetic disorder with a high level of circulating Homogentisic Acid (HGA) (Masoud et al., 2017; Hakim et al., 2018; and Nickavar and Azar, 2018), in urine (Griffin et al., 2018), blood, and tissues (Gupta et al., 2017). Inheritance of AKU follows an autosomal recessive pattern. However, autosomal dominant pattern occurred in AKU families in minority cases (Rana et al., 2015). AKU is the first disease that in accordance with the law of segregation. Mutations in the HGD gene result in this disorder ((Genetics Home Reference, 2019a; and Nelwan, 2013). AKU affects about one in 100,000 to 250,000 (Masoud et al., 2017) or one in 200,000 to one in 1,000,000 live births (Couto et al., 2018). Other names of AKU are alcaptonuria, HGA oxidase deficiency, and homogentisic aciduria (Genetics Home Reference, 2019b).
Symptoms of AKU include arthropathy of major joints, calcifications of cartilaginous tissue with dark color, deterioration of cardiac valves (Gupta et al., 2017; Karaoğlu et al., 2016; and Nelwan, 2013), and pigmentation of ears, sclera (Rana et al., 2015), eyes, and skin (Atalay et al., 2015). No definite cure for AKU is available (Gupta et al., 2017; Karaoğlu et al., 2016; and Nelwan, 2013). Vitamin C may be used. However, it has been proved that vitamin C is not efficient for a number of reasons and dangerous for use in AKU (AKU Society, 2019). Rana et al. suggested that procedures such as joint surgeries and organ transplantations might be recommended for AKU patients (Rana et al., 2015). Joint surgeries alleviate the symptoms of AKU. Organ transplant, especially the liver transplant, has been shown successful.

Modern trends such as genetic counseling, nitisinone, and genetic manipulation techniques hold potential for the elimination of AKU. Genetic counseling is beneficial to advise patients and their families and reduce the occurrence of AKU among people. If two carriers with no family history of AKU marriage, they can have children with AKU. This occurrence will lower than a couple with family history. Nitisinone may be useful for treating this disorder (Alajoulin et al., 2015; Hakim et al., 2018; Nickavar et al., 2018; and Rathore et al., 2016). The benefit of nitisinone for AKU patients is under evaluation. There have been studies regarding possible AKU treatment with nitisinone for humans. Nitisinone reduces circulating of HGA (Milan et al., 2019; and Nelwan, 2013). However, nitisinone is not yet licensed for use in AKU patients. Genetic manipulation techniques could be a potential tool for treating AKU patients in the future (India AKU Society). The ideas of genetic manipulation techniques are to use End-Joining Homology Techniques (EJHTs), FokI-dCas9, induced Pluripotent Stem Cells (iPSCs), and virus’s delivery such as Adeno-Associated Viruses (AAV) and HSV-1 for AKU treatment. For example, Pan et al. suggested that the FokI-dCas9 can correct the major variant in the PAH gene. It suggests that this tool may be useful for correcting incorrect sequences of other inherited metabolic disorders (Pan et al., 2016) such as AKU. It means that genetic manipulations techniques are useful tools for treating AKU.

Genetic manipulation techniques may cause health risks such as oncogenic transformation into the host genome. For example, the c-Myc retrovirus reactivation boosts tumorigenicity in chimeras. For these reasons, a wide array of delivery methods has been examined; safely integrating AAV vectors to non-integrating vectors as Sendai virus. In addition, non-viral and episomal reprogramming approaches have been developed. For example, Kamath et al has developed iPSCs with virus-free, Myc--free, and Lin28-free (Nelwan, 2017b). It suggests that genetic manipulation techniques are safe tools for treating metabolic disorders such as AKU.

In this study, the author describes the progress in a study of AKU that focused on the genetic aspects, and treatments of AKU. The genetic aspects include the HGD gene, mutations in the HGD gene, and treatments: genetics counseling, nitisinone, and genetic manipulation techniques.

2. Methods

2.1. Systematic review

The present report follows the guidelines of the PRISMA extension statement for systematic review (Nelwan, 2018b). These guidelines also correspond to PROSPERO guidelines for such as review question, searches, and primary outcomes (Liberati et al., 2009).

2.2. Searches

The author searched the PubMed Databases at National Center for Biotechnology Information (NCBI) for AKU articles. These included free PMC articles for CC-BY-4.0 and CC-BY-NC-ND licenses in English published from 2014 to 2019. Each search consisted of the first 40 articles. Other articles on the list were not considered. Keywords included “alkaptonuria,” “alkaptonuria history,” “alkaptonuria and heart disease,” “alkaptonuria and kidney diseases,” “alkaptonuria and arthrities,” “alkaptonuria and diagnosis,” “alkaptonuria and nitisinone,” and “alkaptonuria and gene therapy.” In some cases, articles published before 2014 were also included. In addition, the author included own articles and other relevant publications in this study.
2.3. Exclusion criteria
Criteria for the exclusion of the literature included analysis of subgroups or subsets, conference proceedings, letter to the editor, and opinions publications, and publications other than English.

3. Results
The author took 90 articles from the PubMed Databases searches and took 15 articles from other relevant publication searches (Figure 1). After screening titles and abstracts, the author rejected 36 articles and took 69 articles for full-text review. These articles met the criteria for data extraction. After reviewing, the author put 60
3.1. Genes in alkaptonuria

**HGD** is the gene formal symbol. Other names of the **HGD** gene consists of AKU, HGD_HUMAN, HGO, homogentisate 1,2-dioxygenase (homogentisate oxidase), HGA oxidase, and homogentisicase (Genetics Home Reference, 2019a). The **HGD** gene consists of 14 exons and encodes a 445 amino acid polypeptide with high homology to the Aspergillus hmgA (OMIM #60747) (Fernandez et al., 1996). This gene occupies chromosome 3q in the chromosome map, 3q13.33 (OMIM #60747) (Groz, 2014). The **HGD** gene includes base pairs 120,628,168 to 120,682,571 (Genetics Home Reference, 2019a; and NCBI Gene). The gene provides instructions for making the HGD enzyme. The enzyme involves the catabolism of phenylalanine and tyrosine. HGD enzyme converts HGA to maleyacetoacetic acid during phenylalanine and tyrosine catabolism (Genetics Home Reference, 2019b; Hakim et al., 2018; and Rana et al., 2015).

A gene is the primary physical and functional unit genetic. Genes serve as instructions to construct molecules of protein and form DNA. Mutations can arise in a gene; a permanent change in the DNA. Gene mutations result in damage of protein. A genetic disorder is a condition caused by mutations in at least one gene (Nelwan, 2017a) such as Friedreich ataxia and AKU. Selvakumar et al. reported that there have been more than 80 mutations in the **HGD** gene in patients with AKU (Selvakumar et al., 2018). Most of these mutations change single amino acids used to create the HGD enzyme. Substitution of the amino acid valine for the Met368Val is

| Table 1: Mutations in the HGD Gene |
|-----------------------------------|
| **Molecular Consequence** | **Total** | **References** |
| Frameshift | 15 | NCBI ClinVar |
| | 3 | Beltran-Valero |
| | | de Bernabe et al. 1998 |
| Intronic | 2 | Beltran-Valero |
| | | de Bernabe et al. 1998 |
| Missense | 40 | NCBI ClinVar |
| | 16 | Beltran-Valero |
| | | de Bernabe et al. 1998 |
| Nonsense | 2 | NCBI ClinVar |
| Splice site | 10 | NCBI ClinVar |
| | 1 | Beltran-Valero |
| | | de Bernabe et al. 1998 |

| **Variation Type** | **Total** | **References** |
|-------------------|----------|----------------|
| Deletion | 23 | NCBI ClinVar |
| Duplication | 20 | NCBI ClinVar |
| Indel | 2 | NCBI ClinVar |
| Insertion | 7 | NCBI ClinVar |
| Single nucleotide | 94 | NCBI ClinVar |
the most common HGD gene mutation in European populations (Genetics Home Reference, 2019a). Mutations in the HGD gene can include duplication (NCBI ClinVar), missense (Beltran-Valero de Bernabe, 1998; and NCBI ClinVar) and the mutations are in a 54,363 bp gene (Rana et al., 2015), frameshift (Beltran-Valero de Bernabe, 1998; and NCBI ClinVar), intronic (Beltran-Valero de Bernabe, 1998), nonsense (NCBI ClinVar), and splice site (Beltran-Valero de Bernabe, 1998; and NCBI ClinVar) (Table 1). These mutations damage the HGD enzyme’s role to break down the amino acids phenylalanine and tyrosine (Genetics Home Reference, 2019b).

| No. | Old | References       | Old | References       |
|-----|-----|------------------|-----|------------------|
| 1   | 6   | Karaolu et al. 2016 | 8   | Nickavar et al. 2018 |
| 2   | 39  | Damarla et al. 2017 | 48  | Gupta et al. 2017 |
| 3   | 39  | Alajoulin et al. 2015 | 50  | Bhattar et al. 2015 |
| 4   | 39  | Elciglu et al. 2003 | 51  | Phornphutkul et al. 2002 |
| 5   | 45  | Alaya and Mzabi, 2017 | 57  | Li et al. 2016 |
| 6   | 46  | Rana et al. 2015 | 59  | Phornphutkul et al. 2002 |
| 7   | 48  | Rathore et al. 2016 | 59  | Biler et al. 2015 |
| 8   | 48  | Mayatepek et al. 1998 | 60  | Rathore et al. 2016 |
| 9   | 50  | Karaolu et al. 2016 | 60  | Harun et al. 2014 |
| 10  | 55  | Karaolu et al. 2016 | 62  | Mazoochy and Razi, 2018 |
| 11  | 60  | Tekgoz et al. 2018 | 64  | Azami and Maleki 2015 |
| 12  | 63  | Chatzis et al. 2016 | 65  | Couto et al. 2018 |
| 13  | 65  | Hakim et al. 2018 | 65  | Cunningham et al. 1989 |
| 14  | 70  | Carrier and Harris, 1990 | 72  | Isa et al. 2014 |
| 15  | 72  | Atalay et al. 2015 |
| 16  | 72  | Selvakumar et al. 2018 |

T-Test: Two-Sample Assuming Unequal Variances

| Variable 1 | Variable 2 |
|------------|------------|
| Mean       | 51.0625    | 54.64285714 |
| Variance   | 281.129    | 225.1703297 |
| Observations | 16          | 14             |
| Hypothesized Mean Difference | 0          |
| df          | 28          |
| t-Stat      | -0.6172     |
| P(T < t) one-tail | 0.27105    |
| t Critical one-tail | 1.70113    |
| P(T < t) two-tail | 0.54211    |
| t Critical two-tail | 2.04841    |

Carrier and Harris et al; Cunningham et al; Elciglu et al; Mayatepek et al;

Phornphutkul et al were taken from MIM #203500 (Edit history: 07/09/2016).
3.2. Alkaptonuria on the globe

AKU occurs worldwide and the prevalence is one in 250,000 to 1,000,000 births (Genetics Home Reference, 2019b). This disorder has a very low prevalence. However, AKU has a high prevalence in China (Bhattar et al., 2015), the Dominican Republic, Jordan, parts of South India (Nelwan, 2013), Singapore (Bhattar et al., 2015), Slovakia (Nelwan, 2013), and Thailand (Bhattar et al., 2015). In Slovakia, the prevalence is one in 19,000 (Genetics Home Reference, 2019b; and Nelwan, 2013). The prevalence in China, Jordan, the Dominican Republic, India, Singapore, and Thailand are unknown. Either male or female has the same chances of inheriting AKU (Table 2). In this systematic review, of the 30 case reports, 15 (50%) were adult males, 13 (43.33%) were adult females, 1 (3.33%) was a boy, and 1 (3.33%) was a girl. Of the 30 case reports of AKU, 16 (53.33%) were males and 14 (46.67%) were females. Ages of male and female were not significantly different (Table 2). The author did not find any reference for the prevalence of AKU with autosomal dominant.

Phornphutkul et al. reviewed 58 patients with AKU aged four to 80 years (MIM #203500) (Phornphutkul, 2002). The authors found that joint replacement was at a mean age of 55 years and the development of the renal stone at 64 years. In addition, cardiac-valve involvement was at 54 years and coronary artery calcification at 59 years. Linear regression analysis showed that the radiographic score for the severity of AKU commenced increasing after the age of 30 years. Males increased more rapidly than females. Phornphutkul et al. found that kidney stones occurred in 13 male and three female patients. Of the 27 males who were 51 to 36 years, eight were prostate stones. The development of prostate stones did not have an association with the development of kidney stones (NCBI ClinVar; Nelwan, 2013; and Phornphutkul, 2002). Kidney stones occur by 64 years old in 50% of individuals with AKU (Introne and Gahl, 2019). Three patients, each greater than 50 years old, were aortic valve replacement (NCBI ClinVar; Nelwan 2013; and Phornphutkul, 2002). Aortic valve stenosis occurs at a high frequency in the sixth and seventh decades of life (Introne and Gahl, 2019).

AKU causes urine to turn black (Hakim et al., 2018; Genetics Home Reference, 2019a; and Masoud et al., 2017) on alkalization or when exposed to air (Hakim et al., 2018; Genetics Home Reference, 2019a; Introne and Gahl, 2019; and Li et al., 2016). In children, there are no symptoms of AKU other than the urine turning black. Pain associated with AKU starts around the second decade of life (Nelwan, 2013). Ochronosis builds up the blackish blue pigment in cartilage, connective tissues, and skin (Genetics Home Reference, 2019b). Ochronosis occurs after 30 years old (Genetics Home Reference, 2019; and Introne and Gahl, 2019b). Patients with AKU typically develop arthritis, particularly in the spine and large joints, beginning in early adulthood (Nelwan, 2013). Arthritis begins in the third decade. Joint symptoms involving the spine appear in that decade. In one large series, low back pain occurred prior to 30 years old in 49% and prior to 40 years old in 94% (Introne and Gahl, 2019). Selvakumar et al. introduced the idea that a patient with arthritis reached 72 years old without being detected as an AKU sufferer. Ochronosis occurs in the fifth decade for the pigment to accumulate in valvular tissue (Selvakumar et al., 2018). HGA and its oxidation products accumulate in arteries and pancreas, heart valves, hyaline cartilage, ligaments, renal tubule epithelial cells, skin, tendons, the cartilage of the ears and nose, and the sclera (Karaoğlu, 2016).

3.3. Diagnosis tools for alkaptonuria

Diagnosis of AKU can be at any age; early childhood and older people. In early childhood, the urine is turning black after exposure to the air. Diagnosis in later in life can include such as back pain and joint paint (Nelwan, 2013). Several methods are available for diagnosis of AKU. These can include computed tomography (CT), gas chromatography-mass spectrometry (GCMS), liquid chromatography tandem mass spectrometry (LC-MS/MS), magnetic resonance imaging (MRI), radiographic examinations (X-rays), and genetic tests (Table 3).

Li et al. used CT scans and MRI to a 62-year-old woman to indicate AKU. With this method, the authors found multilevel degenerative disc disease from cervical to lumbar spine, including calcification, osteophytosis, and vacuum phenomenon. MRI showed the spinal cord compression at multi-level. It occurred primarily from the unnecessary ligamentum flavum posteriorly. The patient’s urine turned to dark after exposure to the air (Li et al., 2016). Mazoochy and Razi examine radiographic a 57-year-old woman to indicate ochronosis. The right hip X-ray in the patient showed progression in ochronotic arthropathy (Mazoochy and Razi, 2018). Hakim et al. (2018) used radiographic evaluation to display degenerative arthritis in both hips and knees with the left side being more affected than the right in a 65-year-old man. To confirm this, the authors used DNA sequencing. The authors detected an uncommon genomic deletion of 69 bp of exon 2 and surrounding DNA sequences in flanking introns (Hakim et al., 2018). The diagnosis of AKU could base on the detection of a significant amount of HGA in the urine by GCMS analysis (Introne and Gahl, 2019; and Nickavar et al., 2018) (Table 3). The
amount of HGA excreted per day was between one and eight grams (Introne and Gahl, 2019). However, GCMS is not routine approach for diagnosis of AKU. LC-MS/MS is largely used. Davison et al. used LC-MS/MS to confirm the elevated HGA in patients with AKU (Davison et al., 2019). Introne and Gahl suggested that identification of biallelic pathogenic variants in the HGD gene on molecular genetic testing confirmed the diagnosis and allowed family studies. MRI method can detect abnormalities in the tendon in patients with AKU (Introne and Gahl, 2019).

Table 3: Diagnosis tools for alkaptonuria

| Tools      | Diagnose                      | References        |
|------------|-------------------------------|-------------------|
| CT         | Degeneration disc             | Introne and Gahl, 2019 |
|            | Coronary artery clas.         | Damarla et al. 2017 |
|            | Prostate stones               |                   |
| GCMS       | Detection of HGA              | Introne and Gahl, 2019 |
| GS         | Exom sequencing               | Damarla et al. 2017 |
|            | Genome sequencing             |                   |
|            | Mitoch. Sequencing            |                   |
| LC-MS/MS   | Detection of HGA, tyrosine    | Davison et al. 2019 |
| MRI        | Arthritis                     | Damarla et al. 2017 |
|            | Ochraronosis                  |                   |
| MSI        | Detection of nitisinone       | Davison et al. 2019 |
| SG         | Sequence of HGD               | Damarla et al. 2017 |
| X-rays     | Arthritis                     | Hakim et al. 2018  |
|            | Ochraronosis                  | Li et al. 2016    |
|            | Prostate stones               |                   |

**Note:** CT = computed tomography; GCMS = gas chromatography-mass spectrometry; GS = genome sequencing; LC-MS/MS = liquid chromatography tandem mass spectrometry; MRI = magnetic resonance imaging; and MSI = mass spectrometry imaging

Introne and Gahl suggested molecular genetics methods to detect AKU; single-gene testing (SG) and genome sequencing (GS). In SG, it first performs sequence analysis of HGD gene, followed by gene-targeted deletion/duplication analysis, if only one or no pathogenic variant is found. This testing may be performed first in individuals of high-risk ancestry such as the Dominican Republic, Jordan, and Slovakia. The author stated that GS consists of exom sequencing, genome sequencing, and mitochondrial sequencing. These testing may be used if serial SG testing (and/or use of multigene panel) fails to confirm a diagnosis in an individual with features of AKU (Introne and Gahl, 2019).

Other methods for diagnosis of AKU are physical examinations and laboratory tests. These methods can be used for aortic valve replacement. Physical examinations for identification of blackish blue pigment can include head (nose, ear, and jaw), knees, and shoulder. Laboratory examinations can include blood counts and blood pressure. Selvakumar suggested that symptoms of an AKU patient could include such as dyslipidemia and hypertension (Selvakumar et al., 2018).
3.4. Genetic counseling

Related marriages increase the expression of a genetic disorder incidence. Unrelated marriages can help to hide a genetic disorder. It shows that it is important to plan an unrelated marriage to hide a genetic disorder. This requires contacting a genetic professional, or a counselor in a genetic disorder for getting information in details (Nelwan, 2017a) regarding disorders such as AKU and Friedreich ataxia. A healthy married couple may have questions whether or not they have chances of getting an AKU child. A genetic counselor should have their answers to those questions. Counselors may also answer questions for testing of AKU. It relies on a family whether or not they would like to use genetic counseling suggestions. A genetic counselor can help to provide ideas about how a genetic disorder such as AKU can be avoided its appearance in a family.

AKU may be inherited in an autosomal recessive pattern (Damarla et al., 2017; and Rana et al., 2015). Autosomal dominant inheritance in AKU are uncommon cases (Rana et al., 2015). Damarla et al. suggested that AKU is in either autosomal recessive or autosomal dominant trait (Damarla et al., 2017). At the conception of autosomal recessive, each sib of an affected individual has a 25% chance of being affected (recessive homozygote), a 50% of being asymptomatic carrier (heterozygote), and a 25% of being unaffected and not a carrier (dominant homozygote). Once an at-risk sib is known to be unaffected, the chance of his/her being a carrier is 2/3. At the conception of autosomal dominant, the chance of being affected is 75%. It includes a 25% of dominant homozygote and a 50% of being carrier or heterozygote. The chance of being an unaffected is 25%.

In the autosomal recessive pattern, the incidence of AKU would be lower if the parents are unrelated. If the parents were relative, consanguinity, the incidence of alkaptonuria would be higher. The mathematical equation for a consanguinity marriage is:

\[(q^2 + pqF)/q^2\]

where \(F\) is the coefficient of consanguinity. The equation of \(F\) is \(F = \Sigma (\frac{1}{2})^p + m + 1\) (Kalmes and Huret, 2002; and Nelwan, 2013). If prevalence of AKU is one in 250,000, and if the parents are cousins, the chance of an expression of AKU would be as follows:

\[F = (\frac{1}{2})^{2+2+1} = \frac{1}{16}\]

\[q^2 = 1/250,000 = 0.000004\]

\[q = \sqrt{0.000004} = 0.002\]

\[p = 1 - 0.002 = 0.998\]

If the accurate equation is used, the result is:

\[(q^2 + pqF)/q^2 = 32.1875 = 32.19\]

Thus, the AKU would be about 32.19 times higher if the parents were consanguinity cousins than if the parents were unrelated (Nelwan, 2013).

Carrier detection and prenatal diagnosis are possible if the disease-causing mutations for AKU have existed in the family. The testing can use DNA extracted from chorionic villus sampling at 10-12 weeks gestation. The testing can also use DNA extracted from fetal cells through amniocentesis at 15-18 weeks gestation. Preimplantation genetic diagnosis for at-risk pregnancies is also possible (Introne and Gahl, 2019).

Prenatal testing for AKU, that does not affect intellect and life span, are uncommon. However, most centers would consider decisions for prenatal testing to be the choice of the parents. Discussion of these issues is appropriate (Introne and Gahl, 2019; and Nelwan, 2013).

3.5. Nitisinone

No treatment for AKU is available at present (Nelwan, 2013; and Selvakumar et al., 2018). However, nitisinone is under research for treating AKU. It blocks the HGD enzyme and it should be suggested as an efficient drug for treating AKU (Alajoulin et al., 2015; and Nickavar and Azar, 2018). Patients treated with nitisinone have demonstrated reduction of plasma HGA levels (Azami and Maleki, 2015; Introne and Gahl, 2019; and Selvakumar et al., 2018). Nickavar et al reported the use of nitisinone and low tyrosine diet to treat AKU (Nickavar and Azar, 2018). At present, use of nitisinone in patients with AKU is still in clinical trials. However, the FDA has not yet approved this drug for treating AKU. AKU society estimates that one percent of the AKU population is taking nitisinone off-label (Aku Society, 2019; and Nelwan, 2013). FDA has approved nitisinone for treating tyrosinemia type 1 (Nelwan, 2013; and Phornphutkul et al., 2002), and its permission does not
include AKU. Montagutelli et al. discovered a murine model of AKU. The authors observed that nitisinone reduced HGA in the urine (Montagutelli et al., 1994). However, no long-term studies in animals have been performed to evaluate the carcinogenic potential of nitisinone (Nelwan, 2013). Long-term studies are needed to confirm its helpfulness (Hakim et al., 2018).

Phornputkul et al. reported that urinary HGA excretion decreases from 0.9 to 0.13 g per day after a 10-day treatment of nitisinone in a 51-year old woman. The same reduction also occurred after a 9-day treatment of nitisinone in a 59-year old woman. Plasma tyrosine levels in these AKU patients increased with no clinical signs or symptoms. The authors suggested the long-term safety and efficacy of this nitisinone treatment requires further evaluation (Phornputkul et al., 2002).

Ranganath et al. suggested that nitisinone reduced HGA in AKU patients (Table 4). In their study, the authors found that the most efficacious dose was 8 mg/day. In this dose, the reduction of HGA was 98% (Ranganath et al., 2016).

### Table 4: Use of nitisinone in AKU patients

| Group | Dose per day | u-HGA24 |
|-------|--------------|---------|
| 1     | control      | 0.15 mmol |
| 2     | 1 mg         | 0.57 mmol |
| 3     | 2 mg         | 1.44 mmol |
| 4     | 4 mg         | 3.26 mmol |
| 5     | 8 mg         | 31.53 mmol |

**Note:** u-HGA24 = once daily urinary excretion; mg = milligram; mmol = millimoles; and group 5 is the best.

Treatment with nitisinone is unlikely to cause depression in patients with AKU. Nitisinone does not have direct effect on monoamine neurotransmitter metabolism in the central nervous system (CNS) of mice with AKU. It is unlikely to result altered mood or cognition in humans with AKU disorder (Davison et al., 2019).

#### 3.6. Genetic manipulation techniques

Nelwan introduced the idea that genetic manipulation techniques may be beneficial to treat monogenic recessive disorders (Nelwan, 2018a; Nelwan, 2017a; and Nelwan 2017c). Genetic manipulation techniques may include AAV, CRISPR/Cas9 system, EJHTs (Nelwan, 2017) and iPSCs (Nelwan, 2017b). Monogenic recessive disorders may include AKU, Friedreich ataxia, and oculocutaneous albinism. These techniques may help to fix incorrect sequences in a genetic disorder such as AKU. With this correction, it may help the HGD gene to work normally and increase the enzyme deficiency, for example.

Genetic manipulation using virus delivery tools may include AAV, adenoviruses, herpes simplex virus type 1 (HSV-1), retrovirus, and Sendai virus. AAV vectors are non-integrating vectors, safely integrating, or low risk of integrating into the host genome. These vectors have a genome of 4.7 kb. However, Nelwan(2017a) have created an AAV vector with a 5.2 kb genome, suggesting that AAV vectors may be developed for a bigger genome or even big genome as HSV-1 vectors. At least 12 vector serotypes have been available. These include such as AAV1, AAVrh, and AAV9 (Nelwan, 2017a). Adenoviruses are non-enveloped and non-integrating vectors that penetrate the cells. These vectors have the capacity of cargo up to 30 kb (Nelwan, 2017a; and Nelwan, 2017b). Lentiviruses belong to retroviruses family. Retroviruses are single-stranded RNA viruses. These viruses integrate into the host cells. Retroviruses have a genome of 7-10 kb. Sendai virus has a genome of about 15.4 kb. This vector is a non-integrating vector. An HSV-1 vector is a possible option to accommodate a large DNA molecule and is a non-integrating vector. HSV-1 vectors have a genome of 152 kb (Nelwan, 2017a).

Gene delivery vectors such as AAV and HSV-1 may deliver gene-editing tools. Gene-editing tools include meganucleases (MNs), ZFNs, TALENs, and CRISPR/Cas9 system. MNs as gene editing tool has not been widely used. Both ZFNs and TALENs have the same techniques and use different DNA binding arrays: zinc finger arrays and TAL effectors repeats. Finally, the CRISPR/Cas9 system uses sgRNA to produce site-specific gene editing aim cells with great frequency (Nelwan, 2017b).
CRISPR/Cas9 systems NHEJ-mediated DSB repair leads to the introduction of small insertions or deletions at the targeted site. It results in the knockout of gene function through frameshift mutations (Shankar et al., 2018). CRISPR/Cas9 system is the most popular method for editing mutated genes in various researches at present. However, Anuar et al. used TALENs to impair genetically the function of all three H2A.B3 genes that used only one pair rather than multiple pairs. It could reduce the likelihood of mosaicism. Importantly, it reduces the possibility of off-target mutations (Anuar et al., 2019). However, the CRISPR/Cas9 system and EJHTs may allow correct insertion in a large fragment.

Five methods are available for EJHTs: NHEJ, microhomology-mediated end-joining (MMEJ), HR, homology-mediated end-joining (HMEJ), and homology-independent targeted integration (HITI). HITI depends on NHEJ repair mechanism. NHEJ-based method presented random directions in integration and various types of indels at the junctions. The NHEJ is active in the entire cell cycle. The MMEJ-based method display low efficiency in the cultured cell. The MMEJ is active in the early S/G1 phase. The HR-mediated method allows correct insertion in a large fragment (Nelwan, 2018a). HR is active during S/G2 phase (Nelwan, 2018a; Chu et al., 2019; and Yoshino et al., 2019), using the sister chromatid as recombination template. Finally, the HMEJ-based method achieves transgenic integration in mouse and monkey embryos, as well as in hepatocytes and neurons in vivo. All methods may be useful for generating animal models and for targeted gene therapies (Nelwan, 2018a). Programmable nucleases assisted EJHTs are a feasible approach to generate knock-in non-human primate models of human diseases.

The iPSC is a genetic engineering technique for obtaining the same stage as embryonic growth phase and embryonic properties through reprogramming factors. These reprogramming factors include c-Myc, Klf4, Oct4, Sox2 Lin28, and Nanog. The iPSCs, which are free of virus, can be I-Myc, c-Myc, and Lin28. The iPSCs can produce large numbers of diseased cells for drug screening and can differentiate into various target cells in appropriate culture cell conditions (Nelwan, 2017b; and Nelwan, 2018a).

The author did not find any reference relating to genetic manipulation tools for treating AKU. However, Nelwan and Pan et al. suggested that genetic manipulation techniques such as CRISPR/Cas9 system (Nelwan, 2017b; and Pan et al., 2016) and iPSCs might be useful for treating monogenic recessive disorders (Nelwan, 2017b) such as AKU and PKU.

4. Discussion

Stenn et al. discovered that the Egyptian mummy Harwa, dating from 1500 B.C, was AKU. Harwa was the first known patient with AKU. Phornphutkul et al. (2002) provided a review of the natural history of AKU. The author suggested that Garrod described AKU as the first disorder in humans inherited according to the Law of Segregation (MIM #203500) (Stenn et al., 1977).

To slow down AKU development, once it is suspected, both medical history and diagnosis are relevant. For patients who plan to have a child and come from a family or community with a high frequency of AKU, they require genetic counseling (Cieszyński et al., 2016). Genetic counselors may provide information regarding the diagnosis and treatment of AKU.

Several strategies are available to diagnose AKU. These include CT, genetic tests, GCMS, LC-MS/MS, X-rays (Table 3), and physical examination. In addition, CT can be used for diagnosing cervical and lumbar spine; calcification, osteophytosis, and vacuum phenomenon. X-rays could be beneficial for diagnosing such as renal stones, arthritis, and ochronosis in the conjunctiva and cornea. Use of X-rays may be beneficial for diagnosing calcification of the ear cartilage. Physical examination may find ochronosis signs: purple or black discoloration in on the skin of the hands (Introne and Gahl, 2019). In this study, the author only has two children with AKU (Table 2). It indicates the need for an important diagnostic tool for diagnosing AKU for children. It may be genetic tests: single-gene testing and genome sequencing. Use of genetic tests in children may be useful for treating AKU as early as possible. Treatments may include the use of vitamin C and nitisinone. In addition, genetic manipulation techniques may be useful to treat AKU in the future.

Azami and Maleki showed that use of vitamin C and nitisinone in an AKU patient reduced complaint of mild back pain (Azami and Maleki, 2015). It suggests that vitamin C along with nitisinone is beneficial to help patients with AKU (Phornphutkul et al., 2002). However, Rathore et al. stated that there are concerns about the side effects using nitisinone. These side effects can be ameliorated by reducing the dietary intake of tyrosine (Rathore et al., 2016). Nitisinone reduces urinary HGA excretion by nearly 70% (Cieszyński et al., 2016) to 95%.
Use of nitisinone requires dietary restriction as its side effect is hypertyrosinemia. To delay the onset and progression of AKU, a combination of protein restriction diet, vitamin C, and nitisinone are required. Vitamin C in doses of 1 g/day represents a rational treatment in older children and adults. However, benefit of vitamin C in AKU patients is doubtful. Vitamin C serves as a cofactor for 4-hydroxyphenylpyruvat dioxygenase (HPPD). HPPD results in increase of HGA production (Alajoulin et al., 2015). The use of vitamin C is not recommended (India AKU Society). Long-term safety and efficacy of nitisinone treatment are unknown (Cieszyński et al., 2016).

Introne and Gahl reported that nitisinone reduced urinary HGA excretion by at least 69% in two individuals. However, it elevated plasma tyrosine concentrations, triggering photophobia. The other side effect is cornea crystals. Theoretically, neurologic complications associated with tyrosinemia type III may develop. Additionally, low-dose nitisinone reduced urinary HGA by up to 95% in nine individuals with AKU. In the same study, the author informed the use of nitisinone in seven individuals for up to 15 weeks. These patients received 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. Liver transaminase levels returned to normal after stopping nitisinone. Introne introduced the idea that in a three-year therapeutically trial, 2 mg of nitisinone reduced urine and plasma HGA by 95%. Plasma tyrosine averaged 800 µM without dietary restriction. Side effects were minimal. One affected individual developed corneal crystals that required discontinuation of nitisinone (Introne and Gahl, 1994).

Introne and Gahl reported that nitisinone reduced urinary HGA excretion by at least 69% in two individuals. However, it elevated plasma tyrosine concentrations, triggering photophobia. The other side effect is cornea crystals. Theoretically, neurologic complications associated with tyrosinemia type III may develop. Additionally, low-dose nitisinone reduced urinary HGA by up to 95% in nine individuals with AKU. In the same study, the author informed the use of nitisinone in seven individuals for up to 15 weeks. These patients received 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. Liver transaminase levels returned to normal after stopping nitisinone. Introne introduced the idea that in a three-year therapeutically trial, 2 mg of nitisinone reduced urine and plasma HGA by 95%. Plasma tyrosine averaged 800 µM without dietary restriction. Side effects were minimal. One affected individual developed corneal crystals that required discontinuation of nitisinone (Introne and Gahl, 1994).

Use of nitisinone requires dietary restriction as its side effect is hypertyrosinemia. To delay the onset and progression of AKU, a combination of protein restriction diet, vitamin C, and nitisinone are required. Vitamin C in doses of 1 g/day represents a rational treatment in older children and adults. However, benefit of vitamin C in AKU patients is doubtful. Vitamin C serves as a cofactor for 4-hydroxyphenylpyruvat dioxygenase (HPPD). HPPD results in increase of HGA production (Alajoulin et al., 2015). The use of vitamin C is not recommended (India AKU Society). Long-term safety and efficacy of nitisinone treatment are unknown (Cieszyński et al., 2016).

Introne and Gahl reported that nitisinone reduced urinary HGA excretion by at least 69% in two individuals. However, it elevated plasma tyrosine concentrations, triggering photophobia. The other side effect is cornea crystals. Theoretically, neurologic complications associated with tyrosinemia type III may develop. Additionally, low-dose nitisinone reduced urinary HGA by up to 95% in nine individuals with AKU. In the same study, the author informed the use of nitisinone in seven individuals for up to 15 weeks. These patients received 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. Liver transaminase levels returned to normal after stopping nitisinone. Introne introduced the idea that in a three-year therapeutically trial, 2 mg of nitisinone reduced urine and plasma HGA by 95%. Plasma tyrosine averaged 800 µM without dietary restriction. Side effects were minimal. One affected individual developed corneal crystals that required discontinuation of nitisinone (Introne and Gahl, 1994).

Introne and Gahl reported that nitisinone reduced urinary HGA excretion by at least 69% in two individuals. However, it elevated plasma tyrosine concentrations, triggering photophobia. The other side effect is cornea crystals. Theoretically, neurologic complications associated with tyrosinemia type III may develop. Additionally, low-dose nitisinone reduced urinary HGA by up to 95% in nine individuals with AKU. In the same study, the author informed the use of nitisinone in seven individuals for up to 15 weeks. These patients received 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. Liver transaminase levels returned to normal after stopping nitisinone. Introne introduced the idea that in a three-year therapeutically trial, 2 mg of nitisinone reduced urine and plasma HGA by 95%. Plasma tyrosine averaged 800 µM without dietary restriction. Side effects were minimal. One affected individual developed corneal crystals that required discontinuation of nitisinone (Introne and Gahl, 1994).

Use of nitisinone requires dietary restriction as its side effect is hypertyrosinemia. To delay the onset and progression of AKU, a combination of protein restriction diet, vitamin C, and nitisinone are required. Vitamin C in doses of 1 g/day represents a rational treatment in older children and adults. However, benefit of vitamin C in AKU patients is doubtful. Vitamin C serves as a cofactor for 4-hydroxyphenylpyruvat dioxygenase (HPPD). HPPD results in increase of HGA production (Alajoulin et al., 2015). The use of vitamin C is not recommended (India AKU Society). Long-term safety and efficacy of nitisinone treatment are unknown (Cieszyński et al., 2016).

Many strategies are available for treating AKU; genetic manipulation techniques. These include such as AAV, FokI-dCas9, iPSCs, EJHTs. The EJHTs may include MMEJ-mediated technique and HMEJ-mediated technique. Manipulation genetic techniques may be very effective for treating both AKU with autosomal recessive and AKU with autosomal dominant.

In animal models, programmable nucleases and EJHTs may be useful tools for treating recessive disorders such as hemophilia A and AKU. Nelwan et al. developed a modified CRISPR system: FokI-dCas9. FokI-dCas9 corrects p.Arg408Trp in the PAH gene. The p.Arg408Trp is the most common variant in phenylketonuria (PKU). PKU as AKU is a rare autosomal recessive disorder inherited accordance with the law of segregation. However, the autosomal dominant pattern occur quietly rare and uncommon cases in AKU families. To control AKU, the diagnosis has a significant role. Diagnosis techniques include CT, genetic tests, GCMS, LC-MS/MS, and X-rays. Genetics tests include single-gene testing.
and genome sequencing. No efficient drugs are available at present. Use of vitamin C does not have a positive result to elevate patients with AKU. Vitamin C is doubtful for its beneficial for AKU patients. It is not recommended for AKU patients. Genetic counseling may help to direct patients with AKU and their families about how to face or slow down this disorder. Drug candidate to control AKU is nitisinone. Nitisinone has shown remarkable advantage as a drug for treating patients with AKU. Nitisinone does not trigger depression in patients with AKU. Genetic manipulation techniques are potential tools and useful tools to control AKU in the future. These tools may include AAV-mediated RNAi, FokI-dCas9, EJHTs, and iPSCs.

Acknowledgment
Exclusively M. Nelwan performed research and manuscript development.

Funding
This study received no funding from any funding agency.

Conflicts of interest
The author has indicated that he has no conflicts of interests regarding the content of this article.

References
AKU Society (2019). http://www.rarediseasesindia.org/aku
Alajouline, O.A., Alsouh, M.S., Ja’afred, S.O. and Kalbouneh, H.M. (2015). Spontaneous achilles tendon rupture in alkaptonuria. Saudi Med J., 36(12), 1486-1489. doi:10.15537/smj.2015.12.12834
Alaly, Z. and Mzabi, A. (2017). An unusual cause of chronic low back pain: Ochronosis. Pan African Medical Journal; 26, 81. doi: 10.11174/pamj.2017.26.81.11754
Anuar, N.D., Kurscheid, S., Field, M., Zhang, L., Rebar, E. Gregory, P. et al. (2019). Gene editing of the multi-copy H2A.B gene and its importance for fertility. Genome Biology., 20, 23. doi: 10.1186/s13059-019-1633-3
Atalay, A., Goçen, U., Basturk, Y., Kozanoglu, E. and Yaliniz, H. (2015). Ochronotic involvement of the aortic and mitral valves. Tex Heart Inst J., 42(1), 84-86. doi:10.14503/THIJ-14-4138
Azami, A. and Maleki, N. (2015). Alkaptonuric ochronosis. J Res Med Sci., 20(10), 1018-1019. doi:10.4103/1735-1995.172800
Beltran-Valero de Bernabe, D., Granidino, B., Chiarelli, I., Porfirio, B., Mayatepek, E., Aquaron R. et al. (1998). Mutation and polymorphism analysis of the human homogentisate 1, 2-dioxygenase gene in alkaptonuria patients. Am J Hum Genet., 62, 776-784. doi: 10.1086/301805
Bhattar, P.A., Zawar, V.P., Godse, K.V., Patil, S.P., Nadkarni, N.J. and Gautam, M.M. (2015). Exogenous ochronosis. Indian J Dermatol., 60(6), 537-543. doi:10.4103/0019-5154.169122
Biler, E.D., Yılmaz, S.G., Palamar, M., Hamrah, P. and Sahin, F. (2015). In vivo confocal microscopy and anterior segment optic coherence tomography finding in ocular ochronosis. Case Reports in Ophthalmological Medicine, 2015(6), doi:10.1155/2015/592847
Carrier, D.A. and Harris, C.M. (1990). Bilateral hip and bilateral knee arthroplasties in a patient with ochronotic arthropathy. Orthop. Rev., 19, 1005-1009.
Chatzis, A.C., Kanakis, M.A., Sofianidou, J. and Tsoutsinos, A.J. (2016). Operating the blues. Clinical Case Reports, 4(12), 1201-1202. doi:10.1002/ccr3.710
Chu, C., Yang, Z., Yang, J., Yan, L., Si, C., Kang, Y., et al. (2019). Homologous recombination-mediated targeted integration in monkey embryos using TALEN nucleases. BM C Biotechnology; 19, 7. doi:10.1186/s12896-018-0494-2
Cieszyński, K., Podgórny, J., Mostowska, A., Jagodziński, P. P. and Grzegorzekwska, A. J. (2016). Alkaptonuria: A disease with dark brown urine. Pol Arch Med Wewn., 126(4), 284-285. doi:10.20452/pamw.3355
Couto, A., Rodriguez, A. S., Oliveira, P. and Seara, M. (2018). Ochronotic arthropathy — a rare clinical case. Oxford Medical Case Reports; 9, 302-305. doi:10.1093/omcr/omy069
Cunningham, T. J., Roux, E., Lagier, R. and Fallet, G. H. (1989). Rapidly progressive hip osteoarthrosis: an unusual presentation of ochronosis. Clin. Exp. Rheum., 7, 315-318.
Damarla, N., Linga, P., Goval, M., Tadisina, S. R., Reddy, G.S. and Bommisetti, H. (2017). Alkaptonuria: A case report. Indian Journal of Ophthalmology. 65(6), 518-521.

Davison, A.S., Strittmatter, N., Sutherland, H., Hughes, A.T., Bou-Gharios, G., Milan, A.M., et al. (2019). Assessing the effect of nitisinone induced hypertyrosinaemia on monoamine neurotransmitters in brain tissue from a murine model of alkaptonuria using mass spectrometry imaging. Metabolomics, 15, 68. doi:10.1007/s11306-019-1531-4

Elcioglu, N.H., Aytug, A.F., Muller, C. R., Gurbuz, O., Ergun, T., Cotiloglu, E., et al. (2003). Alkaptonuria caused by compound heterozygote mutations. Genet. Counsel., 14, 207-2013.

Genetics Home Reference (2019a). Alkaptonuria, https://ghr.nlm.nih.gov/condition/alkaptonuria

Genetics Home Reference (2019b). HGD gene, https://ghr.nlm.nih.gov/gene/HGD

Groz M.B. (2014). Personal communication. Baltimore, Md.

Gupta, P.R., Acharya, A., Sabat, D. and Mourya, A. (2017). Arthroscopic diagnosis and treatment of shoulder ochronotic arthropathy – A case report. Journal of Clinical Orthopaedics and Trauma; 8, 580-583. doi:10.1016/j.jcot.2016.11.009

Hakim, R., Rozen, N., Zatkova, A., Krausz, J., Elmalah, I. and Spiegel, R. (2018). Degenerative osteoarthritis with multiple joint arthropathies due to ochronosis: A rare inborn error of tyrosine metabolism. IM AJ, 20, 260-261.

Harun, M., Hayrettin, Y., Serhat, M., Cuneyt, M., Firat, F. and Ufuk, O. (2014). A rare case of arthropathy: An ochronotic patient with black joints. International Journal of Surgery Reviews; 5, 554-557. doi:10.1016/j.ijscr.2014.06.015

Introne, W.J. and Gahl, W.A. (2003). Alkaptonuria, May 9 [Updated 2016 May 12]. In Adam, M. P., Ardinger, H. H., Pagon, R. A. et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle, 1993-2019.

Isa, Y., Nihei, S., Irifukuhama, Y., Ikeda, T., Matsumoto, H., Nagata, K. et al. (2014). A rare case of acquired mathemoglobinemia associated with alkaptonuria. Intern Med.; 53, 1797-1800. doi:10.2169/internalmedicine.53.1938

Kalmes, R. and Huret, J.L. (2002). Consanguinity. Atlas Genet Cytogenet Oncol Haematol. http://AtlasGeneticsOncology.org/Educ/ConsangID30039ES.html

Karaoǧlu, S., Karaslan, F. and Mermerkaya, M. U. (2016). Long-term result of arthroplasty in the treatment of a case of ochronotic arthropathy. Acta orthopaedica et Traumatologica Turcica; 50, 584-586. doi:10.1016/j.aott.2016.08.018

Kartasasmita, A., Fujiki, K., Iskandar, F., Sovani, I., Fujimaki, T. and Murakami. A. (2011). A novel nonsense mutation in rhodopsin gene in two Indonesian families with autosomal recessive retinitis pigmentosa. Ophthalmic Genet., 32(1), 57-63. doi:10.3109/13816810.2010.535892.

Li, N., Tian, W., Yuan, Q. and He, D. (2016). Cervical spondylotic myelopathy due to the ochronotic arthropathy of the cervical spine. J Korean Neurosurg Soc., 59(1), 65-68. doi:10.3340/jkns.2016.59.1.65

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J. P.A. et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analysis of studies that evaluate healthcare interventions: Explanation and evaluation. PLoS Medicine., 6(7), e1000100. doi:101371/journal.pmed.1000100

Malerba, A., Klein, P., Bachtarzi, H., Harmin, S.A., Cordova, G., Ferry, A. et al. (2017). PABPN1 gene therapy for oculopharyngeal muscular dystrophy. Nature Communications; 8, 14848. doi:10.1038/ncomms14848

Masoud, H.M.F., Alhawai, H.M., Aryanlat, N. T., Murshidi, M.M., Murshidi, M.M. (2017). A rare presentation of alkaptonuria: Extensive prostatic calculi with highlight of stones found in a unique paraprostatic
urethral diverticulum. International Journal of Surgery Case Reports; 38, 192-195. doi:10.1016/j.ijscr.2017.07.041

Mayatepek, E., Kallas, K., Anninos, A. and Muller, E. (1998). Effects of ascorbic acid and low-protein diet in alkaptonuria. Europ. J. Pediat., 157, 867-868.

Mazoochy, H. and Razi, M. (2018). Knee and hip joint replacement surgery in a patient with ochronotic arthropathy: Surgical tips. Arch Bone Jt Surg., 6(6), 577-581.

Milan, A.M., Hughes, A.T., Davison, A.S., Khedr, M., Rovensky, J., Psarelyi, E.E. et al. (2019). Quantification of the flux of tyrosine pathway metabolites during nitisinone treatment of Alkaptonuria. Scientific Reports, 9, 10024. doi:10.1038/s41598-019-46033-x

Montagutelli, X., Lalouette, A., Coude, M., Kamoun, P., Forest, M. and Guenet, J.L. (1994). AKU, a mutation of the mouse homologous to human alkaptonuria, maps to chromosome 16. Genomics, 19(1), 9-11. doi:10.1016/geno.1994.1004

NCBI ClinVar. https://www.ncbi.nlm.nih.gov/clinvar?term=HGDSgene5D

NCBI Gene. https://www.ncbi.nlm.nih.gov/gene/3081

Nelwan, M.L. (2019). Schistosomiasis: Life cycle, diagnosis, and control. Current Therapeutic Research. June, 91, 5-9. doi:10.1016/j.curtheres.2019.06.001

Nelwan, M.L. (2013). Overcome alkaptonuria. Journal of Biology, Agriculture and Healthcare, 3(10), 101-107.

Nelwan, M.L. (2017a). Friedreich ataxia: Treatment with genetic approach. Journal of Advances in Biology & Biotechnology; 14(4), 1-2. doi:10.9734/JABB/2017/36113

Nelwan, M.L. (2017b). Hemophilia A and induced pluripotent stem cells. Journal of Advances in Biology & Biotechnology; 14(3), 1-11. doi:10.9734/JABB/2017/35111

Nelwan, M.L. (2017c). Treat ocucullotaneous albinism with gene therapy. Journal of Advances in Biology & Biotechnology, 16(3), 1-12. doi:10.9734/JABB/2017/38504

Nelwan, M.L. (2018a). Eradication of rabies with mass parental vaccination, post-exposure prophylaxis, and gene therapy: a systematic review. Asian Journal of Research in Medical and Pharmaceutical Sciences, 4(3), 1-12. doi:10.9734/AJRIMPS/2018/43202

Nickavar, A. and Azar, M.R. (2018). Alkaptonuria a new association of distal renal tubular acidosis. Saudi J Kidney Dis Transpl., 29(4), 997-999.

Pan, Y., Shen, N., Jung-Klawitter, S., Betzen, C., Hoffmann, G., Hoheisel, J.D., et al. (2016). CRISPR RNA-guided FokI nucleases repair a PAH variant in a phenylketonuria model. Scientific Reports, 6, 35794. doi:10.1038/srep35794

Phornphutkul, C., Introne, W.J., Perry, M.B., Bernardini, I., Murphy, M.D., Fitzpatrick, D.L., et al. (2002). Natural history of alkaptonuria. New Eng J Med., 347, 2111-2121. doi:10.1056/NEJMoa021736

Rana, A. O., Saeed, U. and Abdullah, I. (2015). Alkaptonuria more than just a mere disease. Journal of Neurosciences in Rural Practice; 6(2), 257-260. doi:10.4103/0976-3147.150312

Ranganath, L.R., Milan, A.M., Hughes, A.T., Dutton, J.J., Fitzgerald, R., Briggs, M.C. et al. (2016). Suitability of nitisinone in alkaptonuria 1 (SONIA 1): an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone in 24-h urinary homogentisic acid excretion inpatients with alkaptonuria after 4 weeks of treatment. Ann Rheum Dis.; 75(2), 362-367. doi:10.1136/annrheumdis-2014-206033

Rathore, F.A., Ayaz, S.B. and Mansoor, S.N. (2016). Ochronotic arthropathy: Two case reports from a developing country. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders; 9, 15-20. doi:10.4137/CMAMD.S31560

Shankar, S., Sreekumar, A., Prasad, D., Das, A.V. and Pillae, M.R. (2018). Genome editing oncogenes with ZFNs and TALENs: caveats in nuclease design. Cancer Cell Int., 18, 169. doi:10.1186/s12935-018-0666-0
Selvakumar, D., Sian, K., Sigito, S. and Singh, T. (2018). Ochronosis of the aortic valve. J Thorac Dis., 10(5), E332-E334. doi:10.21037/jtd.2018.05.16

Stenn, F.F., Milgram, J.W., Lee, S.L., Weigand, R.J. and Veis, A. (1977). Biochemical identification of homogentisic acid pigment in an ochronosis Egyptian mummy. Science, 197, 566-568.

Tekgöz, E., Akincioğlu, E., Çinar, M. and Yılmaz, S. (2018). A case of exogenous ochronosis associated with hydroxychloroquine. Eur J Rheumatol., 5(3), 206-208. doi:10.5152/eurjrheum.2018.17190

Vaithinathan, R., Berson, E. L. and Dryja, T. (1994). Further screening of the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. Genomics, 21(12), 461-463. doi:10.1006/geno.1994.1301

Yoshino, Y., Endo, S., Chen, Z., Qi, H., Watanabe, G. and Chiba, N. (2019). Evaluation of site-specific homologous recombination activity of BRCA1 by direct quantitation of gene editing efficiency. Scientific Reports, 9, 1644. doi:10.1038/s41598-018-38311-x

Cite this article as: Martin L. Nelwan (2021). Control of alkaptonuria with nitisinone and gene therapy: A systematic review. African Journal of Biological Sciences. 3(1), 19-33. doi: 10.33472/AFJBS.3.1.2021.19-33.