



# Friedreich Ataxia: Treatment with Genetic Approach

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## **Author's contribution**

*Author MLN designed the study, performed the literature searches and wrote the first draft of the manuscript. The author read and approved the final manuscript.*

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## **ABSTRACT**

Friedreich ataxia (FA) is a disorder in the nervous system inherited to the Mendel's law. Mutations in the *FXN* gene trigger the FA disorder. The *FXN* gene occupies chromosome 9q21.11 in the chromosome map. Four classes of alleles are in the mutated *FXN* gene. These include normal alleles, changeable normal alleles, complete penetrance alleles, and borderline alleles. Adequate treatments are unavailable for this disorder at present. However, to treat FA, genetic approach can be used. The approach may comprise genetic counseling and use of advanced therapy, gene therapy for instance. In genetic counseling, if both parents are carriers, a child has a 50% carrier and a 25% FA. To detect people with carrier, amniocentesis can be used for instance. To study FA for human needs, DNA banking is needed as used in gene therapy. Gene therapy is a method to correct damaged cells of patients. This technique has attracted attention of researchers to perform research for treatment of various diseases, particularly FA.

*Keywords: Ataxia; Friedreich ataxia; FA; FXN.*

## **1. INTRODUCTION**

FA is a genetic disorder that has an effect on the nervous system. The disorder triggers movement

problems [1]. This disorder is named after Nikolaus Friedreich. He was the man who first described the disorder [2,3], in 1863 [2]. FA disorder is results from the GAA triplet repeats in

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the *FXN* gene [1,4]. It affects about 2 in 100,000 to 4 in 100,000 people [5] and the carrier frequency is 1:60 to 1:100 [5,6]. FA disorder is the most common among people in Europe, the Middle East, North Africa, South Asia [5], and quite rare in the Far East, at least, there are some cases in Japan. It means that FA may also exist in the other regions in the Far East. Both male and female have the same chances of inheriting the disorder.

Neurological symptoms comprise areflexia and extensor plantar responses, dysphagia, dysarthria, hearing problems, nystagmus, progressive gait limb ataxia with associated limb muscle weakness [3,7], and loss of sensory [3,7-9]. Other complications comprise such as insulin intolerance (30%) [5], scoliosis and hypertrophic cardiomyopathy [5,10]. FA patients have cardiomyopathy around 75% [5]. These symptoms typically appear around adolescence. Earlier and later onsets can also occur in this disorder [11]. The life expectancy is around 40-50 years [9].

Currently, there is no effective cure for FA [5,12]. Medical treatments currently focus on antioxidants such as Idebenone, coenzyme Q<sub>10</sub>, and vitamin E. Agents (e.g. erythropoietin) have also been used. These therapies do not have positive results in the neurological aspects of FA [5,13]. Therefore, it still needs another way or technique to help FA patients and their families to reduce or overcome the problems associated with FA.

In this article, the author describes progress in the study of FA. The author focuses on the genetic aspects. These include the *FXN* gene, mutations in the *FXN* gene, and treatment with

genetic approach. The genetic approach comprises genetic counseling and gene therapy.

## 2. GENES IN FA

A gene is the primary physical and functional unit of genetic. Genes serve as instructions to construct protein molecules. Genes make up DNA. Mutations can occur in a gene. A gene mutation is a permanent change in the DNA. Gene mutations cause protein damage. A genetic disorder is a condition caused by mutations in at least one gene [14], such as hemophilia A and FA. Mutations in the *FXN* gene can cause various GAA triplet repeats. These mutations can comprise point mutations, deletion and insertion.

### 2.1 The *FXN* Gene

The formal name of the *FXN* gene is "frataxin". The *FXN* is the gene formal symbol. Other names comprise CyaY, FA, FARR, FRDA, FRDA\_Human, Friedreich ataxia, MGC57199, and X25 [4]. The human *FXN* gene occupies chromosome 9q in the chromosome map [7,12], 9q21.11 [9,15]. This gene encodes a small protein of 210 amino acids connected with the mitochondrial inner membrane, frataxin [15]. *FXN* gene spans about 80 kb [16-18]. The cytogenetic location of *FXN* gene is on the q arm of chromosome 9 at position 21.11 (Fig. 1). The *FXN* gene includes base pair 69,035,259 to base pair 69,100,178 [19].

Baralle et al. [16] showed that the *FXN* gene possesses 7 exons, namely, 1, 2, 3, 4, 5a, 5b, and non-coding exon 6, leading to the transcription of 3 dissimilar mRNAs. The core transcript size is 1.3 kb. It comprises

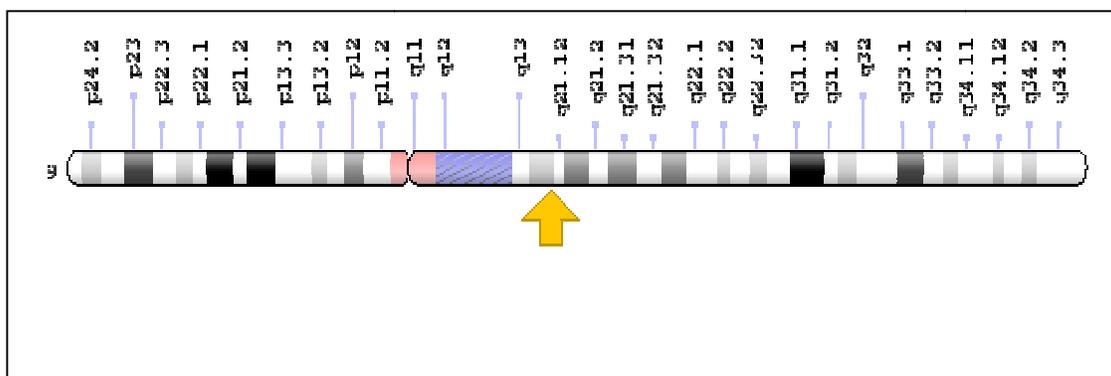


Fig. 1. The *FXN* gene location on chromosome 9 at position 21.11 (from reference [4])

exons 1-5a [18]. The location of GAA triplet repeats is within a primate-specific Alu sequence in the about 11 kb first introns [16].

Frataxin is a nuclear-encoded mitochondrial iron chaperone [20] involved in iron-sulfur biogenesis and heme biosynthesis [16,21], maintenance of anti-oxidant defenses, and iron detoxification [22]. This protein location is in cells throughout the body, with the highest levels in the heart, liver, muscles, pancreas, and spinal cord, and muscles used for voluntary movement [4].

## 2.2 Mutations in *FXN* Gene

FA is most commonly in the form of a GAA triplet repeat in the *FXN* gene [9,11,13,23] in both alleles [24]. Frataxin reduction causes mitochondrial dysfunction. Mitochondrial dysfunction causes cell perniciousness and cell decrease [25]. Mutations occur in the *FXN* gene encoding frataxin on chromosome 9q21.11.

Point mutation, deletion, and/or insertion cause around 2% of cases of FA [16,20]. These mutations are compound heterozygous for GAA triplet repeats [20]. Around 98% of cases of FA are homozygous for GAA triplet repeats in the *FXN* gene [16,20,26]. Lodi et al. mentioned data indicating that 97% of cases of FA are the GAA triplet repeats in the *FXN* gene [7,27,28]. Castaldo et al. concluded that epigenetic alterations in the *FXN* gene might cause or contribute to gene silencing in FA [7].

Four classes of alleles associate with mutations in the *FXN* gene (Table 1). These comprise the following: normal alleles, changeable normal alleles, complete penetrance alleles, and borderline alleles. Normal alleles may include 5-30 GAA triplet repeats [7,8], 5-32 GAA triplet repeats [29], 5-33 GAA triplet repeats [5], 6-34 triplet repeats [30,31], and 6-36 GAA triplet repeats [11,32]. Bidichandani and Delatycki reported that more than 80%-85% of alleles contain fewer than 12 GAA triplet repeats (short normal, SN). Approximately 15% have 12-33 GAA triplet repeats (long normal; LN). Although normal alleles more than 30 GAA triplet repeats

are rare [5], it may also reach up to 40 GAA triplet repeats [33], or up to 43 GAA triplet repeats [34]. It seems that normal individuals may comprise between 5 and 30-43 GAA triplet repeats. It means individuals with more than 30 or 43 GAA triplet repeats belongs to changeable normal alleles.

Changeable normal alleles may comprise 31-69 GAA triplet repeats. It means changeable normal alleles may comprise 31-43 up to 65-69 GAA triplet repeats. Bidichandani and Delatycki [5] showed that although the precise frequency of these alleles has not been officially established, they likely account for less than 1% *FXN* alleles.

Complete penetrance alleles may comprise 44-1,700 GAA triplet repeats [34], 60-1,700 triplet repeats [35], 66 -1,700 GAA triplet repeats [5,30,31,36], 70-1,700 GAA triplet repeat [11,29], 60 to more than 1,300 GAA triplet repeats [20]. Complete penetrance may also comprise 100 to more than 1,500 GAA triplet repeats [33], most commonly 600-900 GAA triplet repeats [29,32,34]. The greater part of expanded alleles contain between 600 and 1,200 GAA triplet repeats [5,36]. On the other hand, the alleles comprise 100-300 GAA triplet repeats are associated with the late-onset FA/very late-onset FA (Bidichandani and Delatycki) [5].

Borderline alleles are alleles located between normal and complete penetrance alleles. These alleles may comprise 32-44 up to 65-69 GAA triplet repeats. Bidichandani and Delatycki [5] showed that it is possible that incomplete penetrance associated with both borderline and enlarged alleles having fewer than 100 GAA triplet repeats. Both a borderline and a complete penetrance allele may produce late-onset FA/very late-onset FA. Sharma *et al* showed that somatic unsteadiness of the borderline allele was important for the clinical expression of the FA phenotype. Then, alleles with fewer than 30 GAA triplet repeats are unlikely to result in FA. Although the precise frequency of borderline alleles has not been officially established, they account for less than 1% of *FXN* alleles.

**Table 1. Allele with mutations in the *FXN* gene**

Kinds	Triplet repeats	References
Normal	5-34 GAA	[5,7,8,11,29,32,33]
Changeable Normal	31-43 to 65-69 GAA	[5]
Compl. Penetrance	44-1700 GAA	[5,11,20,29,30-34,36]
Borderline	32-44 to 65-69 GAA	[5]

### 3. GENETIC COUNSELING

Genetic counseling is the procedure of giving people and families with information on the hereditary disorders. The information can include character, heredity and implication of hereditary disorders. Genetic counseling helps people and their families to make informed medical and personal decisions [5]. The following sections include inheritance of FA, carrier detection and DNA banking.

#### 3.1 Heredity of Friedreich Ataxia

Parents with FA carriers inherit two copies of the defective *FXN* gene to their affected children. These parents typically do not show signs and symptoms of the FA disorder [4]. When both parents are carriers, each of their children has a close approach to a 25% normal, a 50% carrier, and a 25% FA.

Commonly, the incidence of FA would be lower if the parents are unrelated. Romeo *et al* showed that the FA disorder is between 1 in 22,000 and 1 in 25,000 in related family in Italy. The occurrence is between 1 in 25,000 and 1 in 28,000 in southern Italy [7]. Southern Italy is the place where consanguineous marriages are concentrated. It seems that unrelated marriages can help to reduce the occurrence of genetic disorder, particularly FA.

In Japan, Hirayama *et al* estimated that all forms of spinocerebellar degeneration were about 4.53 per 100,000; 2.4% were FA. Silveira *et al.* found Portuguese and Brazilian had a GAA triplet repeat in the *FXN* gene as many as 64% of recessive inheritance cases in unrelated families. Morino *et al.* indicated that there is a low predisposition to the instability of the GAA triplet repeat in Cuba [7]. Mexico has a lower case than average occurrence of FA. The FA has not been documented in Native Americans, Southeast Asians, and Sub-Saharan Africans [5].

Colombo and Carobene estimated that FA disorder has existed at least  $682 \pm 203$  generations ago. Harpending *et al.* stated that, if each generation is around 25 years [7], it was about at least back to 11,500 to 20,500 years B.C. It was a period of the population expansion in the Upper Paleolithic era.

#### 3.2 Carrier Detection

Carrier detection can be made. This technique uses DNA from chorion villus sampling at 10-12

weeks gestation. To detect carriers of FA, amniocentesis can also be used. This technique uses DNA from fetal cells at 15-18 weeks gestation. For at-risk pregnancies, preimplantation genetic diagnosis is also likely made [37]. Carrier detection for the number of GAA triplet repeat is possible for individuals whose reproductive spouse with FA has been identified. Carriers are not at-risk of developing FA [5].

#### 3.3 DNA Banking

DNA banking is the storage of an individual hereditary material for likely future use. DNA banking derived from blood DNA, saliva and other tissues. Bidichandani and Delatycki stated that testing technique and our knowledge of genes, allelic variants, and diseases will rise in the future with DNA banking [5], including DNA banking from FA people. To predict the FA disorder, FA DNA from DNA banking can be used. In gene therapy, DNA banking is needed.

### 4. GENE THERAPY

To treat several hereditary disorders such as FA and hemophilia A, hereditary augmentation therapy (HAT) has been used. Nelwan, and Strachan and Reap stated that HAT target is clinical reversible disorders. It also assists to have no precise for manifestation levels of the inserted gene and a clinical reaction at low demonstration levels [37-39].

#### 4.1 Gene Shipping Vehicles

The patient's cells genetically manipulated; that is, the corrected genes, gene segments or oligonucleotides should be introduced into the DNA molecule; e.g. bacterial plasmids. This plasmid contains edited sequences required for gene manifestation in target cells. To facilitate the adequate cellular uptake of molecules, the corrected materials should be packaged within suitable gene shipping vehicles [40].

One of the oldest vectors is pBR322. The pBR322 plasmid has 4,362 bp. This plasmid is resistant to ampicillin and tetracycline. *E. coli* was bacteria used to construct pBR322 plasmid. *E. coli* cells are normally vulnerable to these two antibiotics. *E. coli* cannot grow when ampicillin or tetracycline is present [37,41]. To date, there are available many plasmids such as pSVAV2 and pTRP56FPFVIII-BDD [42].

Viral vectors have become the preferred gene shipping vehicles in the field of gene therapy. These vectors have enormously high effectiveness of gene relocation in somatic cells [40]. There are available several types of vectors in gene therapy field. These can comprise the following: retrovirus, adenoviruses, adeno-associated viruses (AAV), herpes simplex virus type 1 (HSV-1), and Sendai virus, Table 2. To treat FA in the animal models, these vectors such as AAV and HSV-1 have been used.

Lentiviruses belong to retrovirus family. The viruses are able to transduce both cells proliferation and cells nonproliferation. It helps to increase the scope of aim cells [40]. Retroviruses are single-stranded RNA viruses. These viruses can produce double-stranded DNA versions by using an enzyme called reverse transcriptase. Retroviruses integrate into the host cells. There are seven genera of retroviruses. These comprise the following: *Alpha*, *Beta*, *Gamma*, *Delta*, *Epsilon*, *Lentivirus* and *Spumavirus* [43]. Retroviruses have a genome of 7-10 kb [44]. These viruses occupy yeast, insect, animal, and plant kingdoms [45]. Although slowly silencing of gene manifestation can occur, retroviruses are one of the favorite genes shipping vehicles in gene therapy [40]. The typical lentivirus vectors originated from the HIV type 1 [42].

Adenoviruses are double-stranded DNA molecules [40,46] with 36 kb genome [40]. The viruses are non-enveloped and non-integrating vectors that penetrate the cells [40,46]. Adenoviruses genome occupies as an episome for several cell divisions shortly after release into the nucleus of the host cell [40]. The early adenovirus vectors trigger T-cell-interceded immune reactions that remove the gene-modified cells. The latest generation adenovirus vectors display T-cell immune reaction reduced by removing the remaining viral genes. These vectors have the capacity of cargo up to 30 kb. However, both the early generation and later generation of adenovirus vector particles trigger quickly the innate invulnerable system supplying to significant dose-limiting toxicity [46].

AAV infects both dividing and non-dividing cells. AAV induces a smaller invulnerable response. In the transgene manifestation, AAV keeps on there for a longer time in cells. The AAV vectors are non-integrating vectors [47], safely integrating [48], or low risk of integrating [49] into the host genome. It seems reasonable to suggest that AAV vectors safely integrate into the host

genome as shown in Table 1. Rincon *et al* showed that the AAV vectors are single-stranded DNA molecules [46]. These vectors have a genome of 4.7 kb [37,44]. It is a small gene-shipping vehicle. To lessen the drawbacks of AAV vectors, Choi *et al.* [47] established an AAV vector with a 5.2 kb genome. It shows that AAV may be developed to get the bigger genome [47] or even big genome [37] as HSV-1 vectors. There are at least 12 vector serotypes, and a number of AAV variants engineered by, e.g., viral evolution, which show definite transduction sketches. These comprise, among other things, AAV1, AAV2, AAV5, AAV8, AAVrh [49], AAV6, and AAV9 [46]. Strict perniciousness and serologic and cellular invulnerability have been performed for AAV1, AAV2, AAV5, AAV8 and AAVrh10 [49].

Viral vectors, such as retrovirus and AAV, have a limited packaging. Those vectors cannot accommodate large DNA molecules. A HSV-1 vector is a possible option to accommodate large DNA molecules [40,50]. HSV-1 vectors are non-integrating vectors [50]. These vectors have a genome of 152 kb. Goss *et al.* [50] showed that HSV-1 vectors are linear double-stranded DNA molecules. There are at least 75 gene products in HSV-1 [51]. Thus, the vectors based on HSV-1 have an especially bigger capacity to accommodate the DNA than other virus vectors, namely, retroviruses, adenoviruses, AAV, and Sendai virus.

In the HSV-1 amplicon vectors, the capacity of transgene insert is up to 150 kb. This capacity allows for the insertion of medical genes such as dystrophin (full length cDNA of 17.3 kb) and frataxin (encoded by the 135 kb *FXN* gene) for the medicinal of Friedreich ataxia, for example [52]. It is impossible with other vector system.

Sendai virus efficiently transduces the respiratory tract cells of mice and humans. The virus is a vital respiratory pathogen of rats and mice [53]. Sendai virus has a genome of about 15.4 kb [54,55]. The virus replicates in the cytoplasm without integrating into the host genome. Sendai virus is an RNA virus Paramyxoviridae [56]. These virus vectors have been used for clinical studies of gene therapy for AIDS vaccines, for example.

Artificial chromosomes, both yeast (YACs) and bacteria (BACs), can also be used as gene shipping vehicles. Studies have highlighted the benefits of big fragments. These studies were

made in transgenic mice. The experience obtained from using YACs and BACs has shown that big fragments control particular tissue manifestation at endogenous flats. For example, transgenic mice carrying the completely human cystic fibrosis transmembrane regulator gene (*CFTR*) conveys the *CFTR* in a suitable tissue and complement the *CFTR* error in null mice. In addition, the use of YAC or BAC carrying the entire frataxin has saved frataxin knock-out mice from embryonic lethality. However, Perez-Luz and Diaz-Nido showed that to cleanse supercoiled DNA with BAC shipping vector or another large vector is quite complicated. To get higher effectiveness of shipping, tests to optimize the whole system should be done [40]. Virmouni et al. [57] have established 4 human *FXN* YAC transgenic mouse models: Y47R, YG8R, YG8sR and YG22R. These mice originated from crossbreeding transgenic mice containing entire human genomic YAC *FXN* gene and rose with knock-out *Fxn* heterozygous mice with a GAA triplet repeat. The mouse models are useful for studies towards a therapy using, such as advanced therapy; that is, gene therapy or cell therapy method to treat FA disease.

**Table 2. Potential gene shipping vehicles for FA gene therapy**

Vehicles	Cargo capacity	References
Retroviruses	7-10 kb	[44]
Adenoviruses	36 kb	[40]
AAV	4.7 kb	[37,44]
HSV-1 (iBAC)	135 kb	[18,40]
Sendai virus	15.4 kb	[54,55]
YG8sR	Big	[63]

#### 4.2 Therapy for FA

FA is a good candidate for a treatment with gene therapy since FA is an autosomal recessive disorder. A rise in frataxin could significantly improve FA patient's health. Gene therapy can help to correct frataxin levels, suggesting that gene therapy is useful for FA patients (Table 3). Generally, to conduct a gene therapy research, it needs methods such as cell culture, real-time PCR (RT-PCR) and statistical analysis.

To fight FA, both lentiviral and AAV vectors have been used. Both have resulted in the fractional editing of their sensitivity to oxidant pressure [40,50]. Palomo et al. [58] described a neuronal cell model for FA. The authors used lentiviral in their study. These vectors carry minigenes encoding frataxin-specific shRNAs. These

vectors silence the manifestation of this gene. Palomo et al. [58] showed that abnormal frataxin manifestation driven by lentiviral vector transduction could save the death of frataxin-deficient neuron-like cells. These vectors diminish the manifestation of frataxin to 8 to 30%. Carletti et al. [59] introduced a neuronal model displaying some major biochemical and morphological features of the FA. This model can silence the mouse NSC34 motor neurons for the frataxin gene with shRNA lentiviral vectors. RT-PCR analysis showed a 30% - 60% decrease of the frataxin mRNA when compared to the mock control. In this study, Carletti et al. [59] established 70% shRNA 37 and 40% shRNA 38 with 70% and 40% residual amounts of frataxin, respectively. The immunocytochemistry demonstrated that the differentiation marker Neurofilament Heavy Chain was highly manifested in the cells similar to neurons with abnormal frataxin. However, the morphometric analysis showed that shRNA 40% silenced cells were almost devoid of neuritis, whereas the shRNA 70% neurons still displayed short processes. In addition, the blue native gel electrophoresis (BNGE) and western blotting analysis showed that about 45% reduction of mitochondrial Complex 1 (CI) reached the statistical analysis ( $p < 0.05$ ), when compared to the mock control. No lower molecular weight complexes were evident in the shRNA 40%, indicating that a reduced level of frataxin affected the stability of CI [59]. This study result can be a suitable model to study the effect of frataxin insufficiency in neurons and a potential beneficial healing target for FA.

Gérard et al. [60] showed that the administration of  $6 \times 10^{11}$  v.p of AAV9-hFA (AAV9-hFA) coding for the human frataxin to the neuron-specific enolase Cre (NSE-cre) and to the muscle creatine kinase Cre (MCK-cre) improved the manifestation of the frataxin protein in the FA mouse models. In addition, the administration clearly diminished their heart hypertrophy and increased their heart function [60]. It seems that an AAV9-hFA has a good possibility for treating FA disorders in humans. Gérard et al. [60] used PCR to detect virus in their study. In addition, they used RT-PCR following the treatment with AAV9-hFA into NSE-cre and MCK-cre mice. Dipstick method was also used. Both NSE-cre and MCK-cre had the AAV9-hFA in all tissues examined. It comprised brain, heart, kidney, liver, and muscle. The AAV9-hFA improved the survival of the NSE-cre and MCK-cre mice. This AAV9-hFA administration has disadvantages. It

was unavailable in the brain in the MCK-cre mice. The NSE-cre mice developed nervous system symptoms and it increased rapidly.

HSV-1 amplicon vectors can save the neurodegeneration triggered by the frataxin absence in both cultivated neurons and *in vivo* [18,40,51]. HSV-1 amplicon vectors are plasmids or contagious BAC (iBAC-FA) [40]. Gomez-Sebastian et al. [18] established HSV-1 amplicon vectors containing iBAC-FA of 135 kb holding the total 80 kb genomic locus [40]. These vectors can restore the normal point of frataxin in fibroblast from patients. HSV-1 amplicon vectors can contaminate the cells in which other vectors are difficult to contaminate them [40]. Perez-Luz et al showed how iBAC-FA manifestation from the 135 kb human *FXN* genomic locus produces the 3 frataxin isoforms in either cultivated neuron cells or *in vivo*. This genomic locus produces the right manifestation of frataxin isoforms in patient-originated cells after the transport of the iBAC-FA [61]. It shows that large vectors such as HSV-1 are useful tools for treating FA disease in humans.

YG8R transgenic mice will be particularly useful for any FA medicinal strategies using compounds that aim the mutated human *FXN* gene sequence to raise frataxin manifestation [58]. Disadvantages of YG8R mice are neurogeneration that guides to the damage of neurons. It also shows pancreatic senescence [62]. To repair the disadvantages of YG8R mice, Virmouni et al. [63] established the YG8sR mice. These mice originated from YG8R breeding. PCR genotyping analysis and DNA sequencing showed that YG8sR manage a single GAA triplet repeat mutation. This GAA triplet repeat has both intergenerational and somatic unsteadiness. Both exist in FA patients [63]. In the YG8sR mice, the authors showed behavior deficits, glucose intolerance and supersensitive to insulin. It did not exist in Y47R and wild type mice. These YG8sR mice also have GAA triplet repeat unsteadiness in the brain and cerebellum, the manifestation decline of *FA*, *FAST-1* and frataxin, and presence of pathological vacuoles within neurons of the dorsal root ganglia. The YG8sR model could be one of the excellent methods for the study of FA disease mechanisms and treatment using advanced therapy as cell therapy or gene therapy.

Khonsari et al. [64] reported that lentivirus gene therapy could drive flats of DNA twin thread fractures in FA patient and YG8sR cells.

Quantitative RT-PCR at 2, 8 and 12 weeks in YG8sR and normal mice showed different manifestation in these two mice cures. *FXN* manifestation in the human and YG8sR cells reached to 96- and 210-fold, respectively, following the cures contrasted with uncontaminated FA fibroblasts and 0.5- and 0.2-fold, respectively, contrasted with natural fibroblasts. Over time, this manifestation fell about 50%. This study showed that the manifestation continued larger than in uncontaminated FA fibroblasts over 12-week cure following therapeutic gene shipping. Lateral flow immunoassay showed that the frataxin protein flats of the contaminated cells raised 42- and 17-fold, respectively, contrasted with uncured FA cells 0.48- and 0.2- fold, respectively, of natural fibroblasts. These frataxin protein flats diminished in human and mouse fibroblasts over time; however, these flats continued higher than in uncured FA fibroblasts. In addition,  $\gamma$ -irradiation of FA patient and YG8sR cells showed incorrect DNA correction on the *FXN* gene relocation. This study shows that the flats of DNA twin thread fractures are higher than in the natural fibroblasts [64]. It seems that the lentivirus gene therapy reverses *FXN* gene segments unsteadiness in FA patient and YG8sR mouse fibroblasts.

Virmouni et al. [57] showed that both YG8R and YG22R mice, along with intergenerational GAA triplet repeat, have rather late-onset effects. These animal models would be beneficial to develop further GAA repeat-based FA transgenic mice with a single-copy large GAA triplet repeat expansion mutation. These transgenic mice may produce more severe early-onset effects [57]. It shows that GAA triplet repeats in FA from late-onset to early-onset phenotype can be manipulated. It means that the late-onset phenotype can also be manipulated to obtain normal transgenic mice.

Li et al. [65] established double-stranded RNAs (dsRNA) paired to the GAA triplet repeat. These RNAs can tie to aim RNAs. However, they cannot engage the cleavage role of Ago2. The dsRNA improved manifestation of FA mRNA in the patient-originated FA cells. It also improved protein in those cells. These improvements were the same as the wild type levels. After adding of dsRNA, the protein improvement can reach as long as 15 days. The authors examined the intronic transcription and RNA: Ago2 using RNA immunoprecipitation (RIP) with an against-Ago2 antibody. There is dissimilar in quantities of

recruitment of RNA establishment by against-GAA (anti-FA) occurs at the point of RNA production. It does not occur at the point of RNAs tie. Finally, Li et al. [65] observed that both anti-FA and single-stranded locked nucleic acid (LNA) improved levels of FXN protein and RNA manifestation. It shows that dsRNA and LNA oligonucleotides activate manifestation of FA RNA and protein. The anti-FA, either dsRNA or LANA, can guide compounds to increase of agents for repairing medical levels of FA protein. It seems that anti-FA and LANA can be very helpful to treat the FA disorder.

Ouellet et al. [66] used the YG8sR with the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (Cas9, CRISPR associated 9) system to edit the *F8* gene *in vitro* and *in vivo*. This combination can eliminate the irregular GAA triplet repeats. It repairs the *FXN* gene transcriptional activity and protein stage [66]. Vannocci et al. [67] developed the transcription activator-like effectors nucleases (TALENs) system to control a cell line. The author's primary established an exogenous inducible shape of the gene. Then, the authors knocked down the endogenous *FXN* gene with TALENs. The immunofluorescence and western blot analyses disclosed that this technique facilitates switching on/off the *FXN* gene in the cells in a time-controlled method. It is partly copying what occurs in the FA disorder. The authors showed that genome-editing techniques with induced pluripotent stem cells can be useful to fight FA [67].

**Table 3. Vehicles in gene therapy research for FA disorder**

Vehicles	Fratxin repair	References
Lentivirus	30-60 %	[59]
AAV9-hhFA	Improvement	[60]
iBAC-FA	Up to normal	[40,61]
YG8sR	Improvement	[64]
RNAs/LANs	Improvement	[65]
CRISPR/Cas9	Improvement	[66]
TALENs	Study	[67]

## 5. CONCLUSION

Friedreich ataxia associates with a GAA triplet repeat in the *FXN* gene. FA patients inherit the disorder from one generation to the next according to the Mendel's first principle, the monohybrid. It is an autosomal recessive disorder. Genetic counseling can help to direct patients with FA disorders and their families about how to face the disorder. There are no

efficient drugs to treat FA patients at present. Animal models for the study of disease mechanisms and treatment are already available. To cure the disorder, gene therapy has showed crucial progresses in slowing down FA disorder in the animal models. This technique improved the frataxin level in FA disorder in the animal models. Various genetic correction methods are available to correct erroneous segments in the *FXN* gene. It comprises such as lentiviral vectors, use of RNAs and LNAs, and genome-editing with iPSCs. For example, lentivirus gene therapy corrects erroneous segments in the *FXN* gene in FA patient and YG8sR mouse fibroblasts. It seems that gene therapy would be very valuable for treating FA.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

## REFERENCES

- Home Page: National Library of Medicine (US). Genetics home reference [Internet]. Bethesda (MD): The Library; 2017. Available:<http://ghr.nlm.nih.gov/condition/friedreich-ataxia>
- National Ataxia Foundation. Friedreich's Ataxia (FRDA). NAF FAQ Sheet—Friedreich's Ataxia—10; 2013. Available:<http://www.ataxia.org>
- Schöls L, Amoiridis G, Przuntek H, Frank G, Epplen JG, Epplen C. Friedreich's ataxia revision of the phenotype according to molecular genetics. *Brain*. 1997;120: 2131-2140.
- Home Page: National Library of Medicine (US). Genetics home reference [Internet]. Bethesda (MD): The Library; 2017. Available:<http://ghr.nlm.nih.gov/gene/FXN>
- Bidichandani SI, Delatycki MB. Friedreich Ataxia. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. *GeneReviews™* [Internet]. Seattle (WA): University of Washington, Seattle (WA); 1993-2015; 1998. Available:<https://www.ncbi.nlm.nih.gov/books/NBK1281/> [Updated 2017 June 1]
- Sandi C, Sandi M, Jassal H, Ezzatizadeh V, Anjomani-Virmouni S, et al. Generation and characterization of Friedreich Ataxia YG8R mouse fibroblast and neural stem cell models. *PLoS ONE*. 2014;9(2):e89488. DOI: 10.1371/journal.pone.0089488

7. Online Mendelian Inheritance in Man. Available:<http://omim.org/entry/229300>
8. Al-Mahdawi S, Pinto RM, Varshney D, Lawrence L, Lowrie MB, Hughes S, et al. GAA repeat expansion mouse models of Friedreich ataxia exhibit oxidative stress leading to progressive neural and cardiac pathology. *Genomics*. 2006;580-590. DOI: 10.1016/j.ygeno.2006.06.015
9. Giunti P, Greenfield J, Stevenson AJ, Parkinson MA, Hartman JL, Sandtmann R, et al. Impact of Friedreich's ataxia on health-care resource utilization in the United Kingdom and Germany. *Orphaned of Rare Diseases*. 2013;8:38. DOI: 10.1186/1750-1172-8-38
10. White, et al. Exploration of transitional life events in individuals with Friedreich ataxia: Implications for genetic counseling. *Behavioral and Brain Functions*. 2010;6:65. DOI: 10.1186/1744-9081-6-65
11. Bandiera S, Cartault F, Jannot AS, Hatem E, Girard M, Rivai L, et al. Genetic variations creating MicroRNA target sites in the *FXN* 3'-UTR affect frataxin expression in Friedreich ataxia. *PLoS ONE*. 2013;8:e54791. DOI: 10.1371/journal.pone.005454791
12. Soragni E, Xu C, Plasterer HL, Jacques V, Rusche JS, Gottesfeld JM. Rational for the development of 2-Aminobenzamide Histone Deacetylase inhibitors as therapeutics for Friedreich ataxia. *J Child Neural*. 2012;27(9):1164-1173. DOI: 10.1177/0883073812448533
13. Jones J, Estirado A, Redondo C, Martinez, S. Stem cells from wildtype and Friedreich's ataxia mice present similar neuroprotective properties in dorsal root ganglia cells. *PLoS ONE*. 2013;8(5):e62807. DOI: 10.1371/journal.pone.0062807
14. Home Page: National Library of Medicine (US). Genetics home reference [Internet]. Bethesda (MD): The Library; 2017. Available:<http://ghr.nlm.nih.gov/handbook>
15. Bolinches-Amoros A, Molla B, Pla-Martin D, Palau F, Gonzalez-Cabo P. Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism. *Frontiers in Cellular Neuroscience*. 2014;8(1):124. DOI: 10.3389/fncel.2014.00124
16. Online Mendelian Inheritance in Man. Available:<http://omim.org/entry/606829>
17. Baralle M, Pastor T, Bussani E, Pagani F. Influence of Friedreich ataxia GAA noncoding repeat expansions on pre-mRNA processing. *Am J Hum Genet*. 2008;83:77-88.
18. Gomez-Sebastian S, Gimenez-Cassina A, Diaz-Nido J, Lim F, White-Martins R. Infectious delivery and expression of a 135 kb human *FRDA* genomic DNA locus components Friedreich's ataxia gene transfer using an HSV-1 amplicon vector. *Molecular Therapy*. 2006;15(2):248-254. DOI: 10.1038/sj.mt.6300021
19. GeneCards. Weizmann Institute of Science, Rehovot; 2015. Available:<http://www.genecards.org/cgi-bin/carddisp.pl?gene=FXN>
20. Li L, Voullaire L, Sandi C, Pook MA, Ioannou PA, Delatycki MB, et al. Pharmacological screening using an *FXN-EGFP* cellular genomic reporter assay for the therapy of Friedreich ataxia. *PLoS ONE*. 2013;8(2):e55940. DOI: 10.1371/journal.pone.0055940
21. Schmucker S, Argentini M, Carelle-Calmes N, Martelli A, Puccio H. The *in vivo* mitochondrial two-step maturation of human frataxin. *Hum Molec Genet*. 2008;17:3521-3531.
22. Kemp K, Mallam E, Hares K, Witherick J, Scolding N, Wilkins A. Mesenchymal stem cells restore frataxin expression and increase hydrogen peroxide scavenging enzymes in Friedreich ataxia. *PLoS ONE*. 2011;6(10):e26098. DOI: 10.1371/journal.pone.0026098
23. Hick A, Wattenhofer-Donzé M, Chintawar S, Tropel P, Simard JP, Vaucamps N, et al. Neurons and cardiomyocytes derived from induced pluripotent stem cells as a model for mitochondrial defects in Friedreich's ataxia. *Disease Models & Mechanisms*. 2013;6:608-621. DOI: 10.1242/dmm.010900
24. Plasterer HL, Deutsch EC, Belmonte M, Egan E, Lynch DR, Rusche JR. Development of Frataxin gene expression measures for the evaluation of experimental treatments in Friedreich's ataxia. *PLoS ONE*. 2013;8(5):e63958. DOI: 10.1371/journal.pone.0063958
25. Bird MJ, Needham K, Frazier AN, van Rooijen J, Leung J, Hough S, et al. Functional characterization of Friedreich ataxia iPS-derived neural progenitors and

- their integration in the adult brain. *PLoS ONE*. 2014;9(7):e101718.  
DOI: 10.1371/journal.pone.0101718
26. Deutsch EC, Seyer LA, Periman SL, Yu J, Lynch DR. Clinical monitoring in a patient with Friedreich ataxia and osteogenic sarcoma. *J Child Neurol*. 2012;27(9):1159-1163.  
DOI: 10.1177/0883073812448460
27. Friedman LS, Farmer JM, Perlman S, Wilmot G, Gomez C, Bushara KO, et al. Measuring the rate of progression in Friedreich ataxia: Implications for clinical trial design. *Mov Disord*. 2010;25(4):426-432.  
DOI: 10.1002/mds.22912
28. Lynch DR, Deutch EC, Wilson RB, Tannekoon G. Unanswered questions in Friedreich ataxia. *J Child Neurol*. 2012;27(9):1223-1229.  
DOI: 10.1177/0883073812453498
29. Ezzatizadeh V, Sandi C, Sandi M, Anjomani-Virmouni S, Al-Mahdawi S, Pook MA. MutL $\alpha$  heterodimers modify the molecular phenotype of Friedreich ataxia. *PLoS ONE*. 2014;9(6):e100523.  
DOI: 10.1371/journal.pone.0100523
30. Du J, Campau E, Soragni E, Soragni E, Ku S, Pucket JW, et al. Role of mismatch repair enzyme in GAA•TTC Triplet-repeat expansion in Friedreich ataxia induced pluripotent stem cells. *J Biol Chem*. 2012;287:29861-29872.  
DOI: 10.1074/jbc.M112.391961.
31. Ku S, Soragni E, Campau E, Thomas EA, Altun G, Laurent LC, et al. Friedreich's ataxia induced pluripotent stem cells model intergenerational GAA•TCC triplet repeat instability. *Cell Stem Cell*. 2010;7(5): 631-637.  
DOI: 10.1016/j.stem.2010.09.014
32. Ditch S, Sammarco MC, Banerje A, Grabczyk E. Progressive GAA•TTC repeat expansion in human cell lines. *PLoS Genet*. 2009;5(10):e1000704.  
DOI: 10.1371/journal.pgen.1000704
33. Martelli A, Puccio H. Dysregulation of cellular iron metabolism in Friedreich ataxia: From primary iron-sulfur cluster deficit to mitochondrial iron accumulation. *Frontiers in Pharmacology*. 2014;5(1):130.  
DOI: 10.3389/fphar.2014.00130
34. Sandi C, Sandi M, Virmouni SA, Al-Mahdawi S, Pook MA. Epigenetic-based therapies for Friedreich ataxia. *Frontiers in Genetics*. 2014;5(1):165.  
DOI: 10.3389/fgene.2014.00165
35. Rai M, Soragni E, Jenssen K, Burnett R, Herman D, Coppola G, et al. HDAC inhibitors correct Frataxin deficiency in a Friedreich ataxia mouse model. *PLoS ONE*. 2008;3(4):e1958.  
DOI: 10.1371/journal.pone.0001958
36. De Biase I, Rasmussen A, Monticelli A, Al-Mahdawi S, Pook M, Cocozza S, et al. Somatic instability of the expanded GAA triplet-repeat sequence in Friedreich ataxia progresses throughout life. *Genomics*. 2007;90:1-5.  
DOI: 10.1016/j.ygeno.2007.04.001.
37. Nelwan ML. Overcome Alkaptonuria. *Journal of Biology, Agriculture and Healthcare*. 2013;3(10):101-107.
38. Nelwan ML. The tyrosinemia type 1. *Advances in Life Science and Technology*. 2013;14:7-19.
39. Strachan T, Read AP. Human molecular genetics. 2<sup>nd</sup> Edition, Wiley-Liss, Chapter 22, Gene Therapy and Other Molecular Genetic-based Therapeutic Approaches; 1999.
40. Perez-Luz S, Diaz-Nido J. Prospect for the use of artificial chromosomes and minichromosome-like episomes in gene therapy. *Journal of Biomedicine and Biotechnology*. 2010;16.  
DOI: 10.1155/2010/642804
41. Brown TA. *Genomes*. Wiley-Liss, Oxford; 2002.
42. Liu X, Ping H, Zhang C. Rapid establishment of a HEK 239 cell line expressing FVIII-BDD using AAV site-specific integration plasmids. *BMC Research Notes*. 2014;7:626.  
DOI: 10.1186/1756-0500-7-626
43. Zhang W, Cao S, Martin JL, Mueller JD, Mansky LM. Morphology and ultrastructure of retrovirus particles. *AIMS Biophys*. 2015;2(3):33-369.  
DOI: 10.3934/biophys.2015.3.343
44. Nelwan ML. Hemophilia A and induced pluripotent stem cells. *Journal of Advances in Biology & Biotechnology*. 2017;14(3):1-11.  
DOI: 10.9734/JABB/2017/35111
45. Grandgenet DP, Pandey KK, Bera S, Alhara H. Multifunctional facts of retrovirus integrase. *World Journal of Biological Chemistry*. 2015;6(3):83-94.  
DOI: 10.4331/wbjc.v6.i3.83
46. Rincon MY, VandenDriessche T, Chuah MK. Gene therapy for cardiovascular disease: Advances in vector development, targeting, and delivery for clinical

- translation. *Cardiovascular Research*. 2015;108:420.  
DOI: 10.1093/cvr/cvv205
47. Choi JH, Yu NK, Baek GC, Bakes J, Seo D, Nam HJ, et al. Optimization of AAV expression cassettes to improve packaging capacity and transgene expression in neurons. *Molecular Brain*. 2014;7:17.  
DOI: 10.1186/1756-6606-7-17
48. Focosi D, Amabile G, Di Ruscio A, Quaranta P, Tenen DG, Pistello M. Induced pluripotent stem cells in hematology: Current and future applications. *Blood Cancer Journal*. 2014;4:e211.  
DOI: 10.1038/bcj.2014.30
49. Hoyng SA, de Winter F, Tannemaat MR, Blits B, Malessy MJA, Verhaagen J. Gene therapy and peripheral nerve repair: A perspective. *Front Mol Neurosci*. 2015;8:32.  
DOI: 10.3389/fnmol.2015.00032
50. Goss JR, Krisky D, Wechuck J, Wolfe D. Herpes simplex virus-based nerve targeting gene therapy in pain management. *Journal of Pain Research*. 2014;7:71-79.  
DOI: 10.2147/JPR.536619
51. Lim F, Palomo GM, Mauritz C, Giménez-Cassina A, Illana B, Wandosell F, et al. Functional recovery in a Friedreich's ataxia mouse model by frataxin gene transfer using an HSV-1 amplicon vector. *The American Society of Gene Therapy*. 2007;15(6):1072-1078.  
DOI: 10.1038/sj.mt.6300143
52. Ho I, Chan KYW, Miao L, Shim WSN, Guo CM, Cheang P, et al. HSV-1 amplicon viral vector-mediated gene transfer to human bone marrow-derived mesenchymal stem cells. *Cancer Gene Therapy*. 2008;15:553-562.  
DOI: 10.1038/cgt.2008.27
53. Ono M, Hamada Y, Horiuchi Y, Matsuo-Takahashi M, Imoto Y, et al. Generation of induced pluripotent stem cells from human nasal epithelial cells using a Sendai virus vector. *PLoS ONE*. 2012;7(8):e42855.  
DOI: 10.1371/journal.pone.0042855
54. Nakanishi M, Otsu M. Development of Sendai virus vectors and their potential applications in gene therapy and regenerative medicine. *Current Gene Therapy*. 2012;12:410-416.  
DOI: 10.2174/156652312802762518
55. Bernal JA. RNA-based tools for nuclear reprogramming and lineage-conversion: Towards clinical applications. *Journal of Cardiovascular Translational Research*. 2013;6(6):856-968.  
DOI: 10.1007/s12265-013-9494-8
56. Kishino Y, Seki T, Fujita J, Yuasa S, Tohyama S, Kunitomi A, et al. Derivation of transgene-free of human induced pluripotent stem cells from human peripheral T cells in defined culture conditions. *PLoS ONE*. 2014;9(5):e97397.  
DOI: 10.1371/journal.pone.0097397
57. Virmouni SA, Sandi C, Al-Mahdawi S, Pook MA. Cellular, molecular and functional characterization of YAC transgenic mouse models of Friedreich ataxia. *PLoS ONE*. 2014;9(9):e107416.  
DOI: 10.1371/journal.pone.0107416
58. Palomo GM, Cerrato T, Cargini R, Diaz-Nido J. Silencing of frataxin gene expression triggers p53-dependent apoptosis in human neuron-like cells. *Human Molecular Genetics*. 2011;20(14):2807-2822.  
DOI: 10.1093/hmg/ddr187
59. Carletti B, Piermarini E, Tozzi G, Travaglini R, Torraco A, Pastore A, et al. Frataxin silencing inactivates mitochondrial complex I in NSC34 motoneuronal cells and alters glutathione homeostasis. *Int J Mol Sci*. 2014;15:5789-5806.  
DOI: 10.3390/ijms15045789
60. Gérard C, Xiao X, Filali M, Coulombe Z, Arsenault M, Couet J, et al. An AAV9 coding for frataxin clearly improved the symptoms and prolonged the life of Friedreich ataxia mouse models. *Methods & Clinical Development*. 2014;1:14044.  
DOI: 10.1038/mtm.2014.44
61. Perez-Luz S, Gimenez-Cassina A, Frenandez-Frias I, Wade-Martins R, Diaz-Nido J. Delivery of the 135 kb human frataxin genomic DNA locus gives rise to different frataxin isoforms. *Genomic*. 2016;106(2):76-82.
62. Mollá B, Riveiro F, Bolinches-Amoros A, Muñoz DC, Palau F, González-Cabo P. Two different pathogenic mechanisms, dying-back axonal neuropathy and pancreatic senescence, are present in the YG8R mouse model of Friedreich ataxia. *Disease Models and Mechanism*. 2016;9:647-657.  
DOI: 10.1242/dmm.024273

63. Virmouni SA, Ezzatisadeh V, Sandi C, Sandi M, Al-Mahdawi S, Chutake Y, et al. A novel GAA-repeat-expansion-based mouse model of Friedreich's ataxia. *Disease Models and Mechanisms*. 2015;8:225-235. DOI: 10.1242/dmm.018952
64. Khonsari H, Schneider M, Al-Mahdawi S, Chianea YG, Themis M, Parris C, et al. Lentivirus-mediated frataxin gene delivery reverses genome instability in Friedreich ataxia patient and mouse model fibroblasts. *Gene Therapy*. 2016;23:846-856. DOI: 10.1038/gt.2016.61
65. Li L, Matsui M, Corey DR. Activating frataxin expression by repeat-targeted nucleic acids. *Nature Communications*. 2016;7:10606. DOI: 10.1038/ncomms10606
66. Ouellet DL, Cherif K, Rousseau J, Tremblay JP. Deletion of the GAA repeats from the human frataxin gene using CRISPR-Cas9 system in YG8sR-derived cells and mouse models of Friedreich ataxia. *Gene Ther*. 2017;24(5):265-274. DOI: 10.1038/gt.2016.89
67. Vannocci T, Faggianelli N, Zaccagnino S, della Rosa I, Adinolfi S, Pastore A. A new cellular model to follow Friedreich's ataxia development in time-resolved way. *Disease Models & Mechanisms*. 2015;8:711-719. DOI: 10.1242/dmm.020545

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