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1 ***Review Paper***

2 # Friedreich Ataxia: Treatment with Genetic Approach

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4

5 ## ABSTRACT

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

17 **Keywords:** Ataxia, Friedreich ataxia, FA, FXN

18

19 ## 1. INTRODUCTION

20 FA is a genetic disorder that has an effect on the nervous system. The disorder triggers movement
21 problems [1]. This disorder is named after Nikolaus Friedreich. He was the man who first described
22 the disorder [2-3], in 1863 [2]. FA disorder is results from the GAA triplet repeats in the *FXN* gene [1,
23 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
24 [5-6]. FA disorder is the most common among people in Europe, the Middle East, North Africa, South
25 Asia [5], and quite rare in the Far East, at least, there are some cases in Japan. It means that FA may
26 also exist in the other regions in the Far East. Both male and female have the same chances of
27 inheriting the disorder.

28 Neurological symptoms comprise areflexia and extensor plantar responses, dysphagia,
29 dysarthria, hearing problems, nystagmus, progressive gait limb ataxia with associated limb muscle
30 weakness [3, 7], and loss of sensory [3, 7-9]. Other complications comprise such as insulin
31 intolerance (30 %) [5], scoliosis and hypertrophic cardiomyopathy [5, 10]. FA patients have

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32 cardiomyopathy around 75 % [5]. These symptoms typically appear around adolescence. Earlier and
33 later onsets can also occur in this disorder [11]. The life expectancy is around 40-50 years [9].
34 Currently, there is no effective cure for FA [5, 12]. Medical treatments currently focus on
35 antioxidants such as Idebenone, coenzyme Q_{10}, and vitamin E. Agents (e.g. erythropoietin) have also
36 been used. These therapies do not have positive results in the neurological aspects of FA [5, 13].
37 Therefore, it still needs another way or technique to help FA patients and their families to reduce or
38 overcome the problems associated with FA.
39 In this article, the author describes progress in the study of FA. The author focuses on the
40 genetic aspects. These include the *FXN* gene, mutations in the *FXN* gene, and treatment with genetic
41 approach. The genetic approach comprises genetic counseling and gene therapy.

42 **2. Genes in FA**

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

49 **2.1 The FXN Gene**

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

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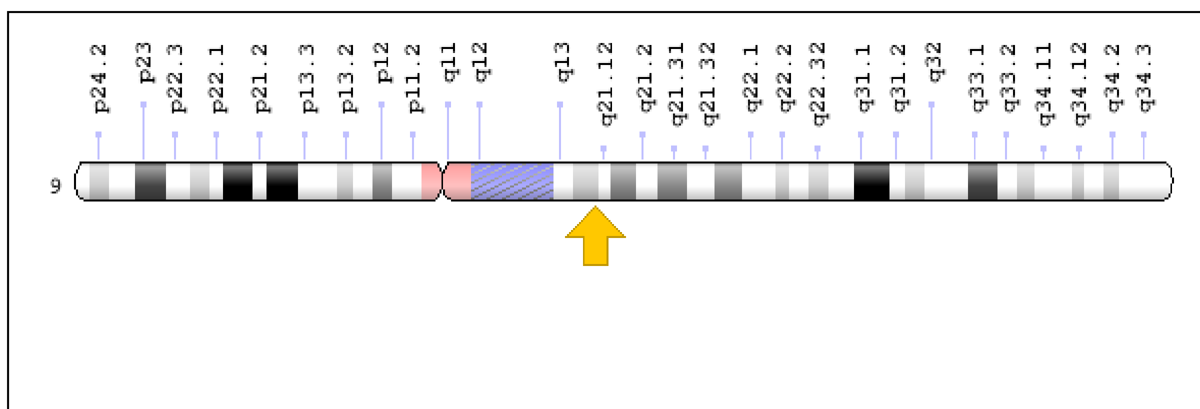

Fig. 1. The *FXN* gene location on chromosome 9 at position 21.11 (from reference [4]).

Baralle *et al* showed that the *FXN* gene possesses 7 exons [16], 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 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

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82 GAA triplet repeats [5], 6-34 triplet repeats [30-31], and 6-36 GAA triplet repeats [11, 32].
83 Bidichandani and Delatycki reported that more than 80%-85% of alleles contain fewer than 12 GAA
84 triplet repeats (short normal, SN). Approximately 15% have 12-33 GAA triplet repeats (long normal;
85 LN). Although normal alleles more than 30 GAA triplet repeats are rare [5], it may also reach up to 40
86 GAA triplet repeats [33], or up to 43 GAA triplet repeats [34]. It seems that normal individuals may
87 comprise between 5 and 30-43 GAA triplet repeats. It means individuals with more than 30 or 43 GAA
88 triplet repeats belongs to changeable normal alleles.

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

93 Complete penetrance alleles may comprise 44-1,700 GAA triplet repeats [34], 60-1,700 triplet
94 repeats [35], 66 -1,700 GAA triplet repeats [5, 30-31, 36], 70-1,700 GAA triplet repeat [11, 29], 60 to
95 more than 1,300 GAA triplet repeats [20]. Complete penetrance may also comprise 100 to more than
96 1,500 GAA triplet repeats [33], most commonly 600-900 GAA triplet repeats [29, 32-34]. The greater
97 part of expanded alleles contain between 600 and 1,200 GAA triplet repeats [5, 36]. On the other
98 hand, the alleles comprise 100-300 GAA triplet repeats are associated with the late-onset FA/very
99 late-onset FA (Bidichandani and Delatycki) [5].

100 Borderline alleles are alleles located between normal and complete penetrance alleles. These
101 alleles may comprise 32-44 up to 65-69 GAA triplet repeats. Bidichandani and Delatycki [5] showed
102 that it is possible that incomplete penetrance associated with both borderline and enlarged alleles
103 having fewer than 100 GAA triplet repeats. Both a borderline and a complete penetrance allele may
104 produce late-onset FA/very late-onset FA. Sharma *et al* showed that somatic unsteadiness of the
105 borderline allele was important for the clinical expression of the FA phenotype. Then, alleles with
106 fewer than 30 GAA triplet repeats are unlikely to result in FA. Although the precise frequency of
107 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]

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108 **3. Genetic Counseling**

109 Genetic counseling is the procedure of giving people and families with information on the hereditary
110 disorders. The information can include character, heredity and implication of hereditary disorders.
111 Genetic counseling helps people and their families to make informed medical and personal decisions
112 [5]. The following sections include inheritance of FA, carrier detection and DNA banking.

113 **3.1 Heredity of Friedreich Ataxia**

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

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

123 In Japan, Hirayama *et al* estimated that all forms of spinocerebellar degeneration were about
124 4.53 per 100,000; 2.4% were FA. Silveira *et al* found Portuguese and Brazilian had a GAA triplet
125 repeat in the *FXN* gene as many as 64% of recessive inheritance cases in unrelated families. Morino
126 *et al* indicated that there is a low predisposition to the instability of the GAA triplet repeat in Cuba [7].
127 Mexico has a lower case than average occurrence of FA. The FA has not been documented in Native
128 Americans, Southeast Asians, and Sub-Saharan Africans [5].

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

133 **3.2 Carrier Detection**

134 Carrier detection can be made. This technique uses DNA from chorion villus sampling at 10-12 weeks
135 gestation. To detect carriers of FA, amniocentesis can also be used. This technique uses DNA from
136 fetal cells at 15-18 weeks gestation. For at-risk pregnancies, preimplantation genetic diagnosis is also

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137 likely made [37]. Carrier detection for the number of GAA triplet repeat is possible for individuals
138 whose reproductive spouse with FA has been identified. Carriers are not at-risk of developing FA [5].

139 **3.3 DNA Banking**

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

145 **4. Gene therapy**

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

150 **4.1 Gene Shipping Vehicles**

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

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

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

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

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

184 AAV infects both dividing and non-dividing cells. AAV induces a smaller invulnerable
185 response. In the transgene manifestation, AAV keeps on there for a longer time in cells. The AAV
186 vectors are non-integrating vectors [47], safely integrating [48], or low risk of integrating [49] into the
187 host genome. It seems reasonable to suggest that AAV vectors safely integrate into the host genome
188 as shown in Table 1. Rincon *et al* showed that the AAV vectors are single-stranded DNA molecules
189 [46]. These vectors have a genome of 4.7 kb [37, 50]. It is a small gene-shipping vehicle. To lessen
190 the drawbacks of AAV vectors, Choi *et al* established an AAV vector with a 5.2 kb genome [47]. It
191 shows that AAV may be developed to get the bigger genome [47] or even big genome [37] as HSV-1
192 vectors. There are at least 12 vector serotypes, and a number of AAV variants engineered by, e.g.,
193 viral evolution, which show definite transduction sketches. These comprise, among other things,
194 AAV1, AAV2, AAV5, AAV8, AAVrh [49], AAV6, and AAV9 [46]. Strict perniciousness and serologic
195 and cellular invulnerability have been performed for AAV1, AAV2, AAV5, AAV8 and AAVrh10 [49].

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196 Viral vectors, such as retrovirus and AAV, have a limited packaging. Those vectors cannot
197 accommodate large DNA molecules. A HSV-1 vector is a possible option to accommodate large DNA
198 molecules [40, 51]. HSV-1 vectors are non-integrating vectors [51]. These vectors have a genome of
199 152 kb. Goss *et al* showed that HSV-1 vectors are linear double-stranded DNA molecules. There are
200 at least 75 gene products in HSV-1 [52]. Thus, the vectors based on HSV-1 have an especially bigger
201 capacity to accommodate the DNA than other virus vectors, namely, retroviruses, adenoviruses, AAV,
202 and Sendai virus.

203 In the HSV-1 amplicon vectors, the capacity of transgene insert is up to 150 kb. This capacity
204 allows for the insertion of medical genes such as dystrophin (full length cDNA of 17.3 kb) and frataxin
205 (encoded by the 135 kb *FXN* gene) for the medicinal of Friedreich ataxia, for example [53]. It is
206 impossible with other vector system.

207 Sendai virus efficiently transduces the respiratory tract cells of mice and humans. The virus is
208 a vital respiratory pathogen of rats and mice [54]. Sendai virus has a genome of about 15.4 kb [55-
209 56]. The virus replicates in the cytoplasm without integrating into the host genome. Sendai virus is an
210 RNA virus Paramyxoviridae [57]. These virus vectors have been used for clinical studies of gene
211 therapy for AIDS vaccines, for example.

212 Artificial chromosomes, both yeast (YACs) and bacteria (BACs), can also be used as gene
213 shipping vehicles. Studies have highlighted the benefits of big fragments. These studies were made in
214 transgenic mice. The experience obtained from using YACs and BACs has shown that big fragments
215 control particular tissue manifestation at endogenous flats. For example, transgenic mice carrying the
216 completely human cystic fibrosis transmembrane regulator gene (*CFTR*) conveys the *CFTR* in a
217 suitable tissue and complement the *cftr* error in null mice. In addition, the use of YAC or BAC carrying
218 the entire frataxin has saved frataxin knock-out mice from embryonic lethality. However, Perez-Luz
219 and Diaz-Nido showed that to cleanse supercoiled DNA with BAC shipping vector or another large
220 vector is quite complicated. To get higher effectiveness of shipping, tests to optimize the whole
221 system should be done [40]. Virmouni *et al* have estabilished 4 human *FXN* YAC transgenic mouse
222 models: Y47R, YG8R, YG8sR and YG22R. These mice originated from crossbreeding transgenic
223 mice containing entire human genomic YAC *FXN* gene and rose with knock-out *Fxn* heterozygous
224 mice with a GAA triplet repeat [58]. The mouse models are useful for studies towards a therapy
225 using, such as advanced therapy; that is, gene therapy or cell therapy method to treat FA disease.

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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, 50]
HSV-1 (iBAC)	135 kb	[18, 40]
Sendai virus	15.4 kb	[55-56]
YG8sR	Big	[64]

226

227 **4.2 Therapy for FA**

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

233 To fight FA, both lentiveral and AAV vectors have been used. Both have resulted in the
234 fractional editing of their sensitivity to oxidant pressure [40, 51]. Palomo *et al* [59] described a
235 neuronal cell model for FA. The authors used lentiviral in their study. These vectors carry minigenes
236 encoding frataxin-specific shRNAs. These vectors silence the manifestation of this gene. Palomo *et al*
237 showed that abnormal frataxin manifestation driven by lentiviral vector transduction could save the
238 death of frataxin-deficient neuron-like cells. These vectors diminish the manifestation of frataxin to 8 to
239 30%. Carletti *et al* introduced a neuronal model displaying some major biochemical and morphological
240 features of the FA. This model can silence the mouse NSC34 motor neurons for the frataxin gene with
241 shRNA lentiviral vectors. RT-PCR analysis showed a 30% - 60% decrease of the frataxin mRNA
242 when compared to the mock control. In this study, Carletti *et al* established 70% shRNA 37 and 40%
243 shRNA 38 with 70% and 40% residual amounts of frataxin, respectively. The immunocytochemistry
244 demonstrated that the differentiation marker Neurofilament Heavy Chain was highly manifested in the
245 cells similar to neurons with abnormal frataxin. However, the morphometric analysis showed that
246 shRNA 40% silenced cells were almost devoid of neuritis, whereas the shRNA 70% neurons still
247 displayed short processes. In addition, the blue native gel electrophoresis (BNGE) and western
248 blotting analysis showed that about 45% reduction of mitochondrial Complex 1 (CI) reached the
249 statistical analysis ($p < 0.05$), when compared to the mock control. No lower molecular weight

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250 complexes were evident in the shRNA 40%, indicating that a reduced level of frataxin affected the
251 stability of CI [60]. This study result can be a suitable model to study the effect of frataxin insufficiency
252 in neurons and a potential beneficial healing target for FA.

253 Gérard *et al* showed that the administration of 6 x 10^{11} v.p of AAV9-hFA (AAV9-hFA) coding
254 for the human frataxin to the neuron-specific enolase Cre (NSE-cre) and to the muscle creatine
255 kinase Cre (MCK-cre) improved the manifestation of the frataxin protein in the FA mouse models. In
256 addition, the administration clearly diminished their heart hypertrophy and increased their heart
257 function [61]. It seems that an AAV9-hFA has a good possibility for treating FA disorders in humans.
258 Gérard *et al* used PCR to detect virus in their study. In addition, they used RT-PCR following the
259 treatment with AAV9-hFA into NSE-cre and MCK-cre mice. Dipstick method was also used. Both
260 NSE-cre and MCK-cre had the AAV9-hFA in all tissues examined. It comprised brain, heart, kidney,
261 liver, and muscle. The AAV9-hFA improved the survival of the NSE-cre and MCK-cre mice. This
262 AAV9-hFA administration has disadvantages. It was unavailable in the brain in the MCK-cre mice.
263 The NSE-cre mice developed nervous system symptoms and it increased rapidly.

264 HSV-1 amplicon vectors can save the neurodegeration triggered by the frataxin absence in
265 both cultivated neurons and *in vivo* [18, 40, 52]. HSV-1 amplicon vectors are plasmids or contagious
266 BAC (iBAC-*FA*) [40]. Gomez-Sebastian *et al* established HSV-1 amplicon vectors containing iBAC-
267 *FA* of 135 kb holding the total 80 kb genomic locus [18, 40]. These vectors can restore the normal
268 point of frataxin in fibroblast from patients. HSV-1 amplicon vectors can contaminate the cells in which
269 other vectors are difficult to contaminate them [40]. Perez-Luz *et al* showed how iBAC-*FA*
270 manifestation from the 135 kb human *FXN* genomic locus produces the 3 frataxin isoforms in either
271 cultivated neuron cells or *in vivo*. This genomic locus produces the right manifestation of frataxin
272 isoforms in patient-originated cells after the transport of the iBAC-*FA* [62]. It shows that large vectors
273 such as HSV-1 are useful tools for treating FA disease in humans.

274 YG8R transgenic mice will be particularly useful for any FA medicinal strategies using
275 compounds that aim the mutated human *FXN* gene sequence to raise frataxin manifestation [58].
276 Disadvantages of YG8R mice are neurogeneration that guides to the damage of neurons. It also
277 shows pancreatic senescence [63]. To repair the disadvantages of YG8R mice, Virmouni *et al*
278 established the YG8sR mice. These mice originated from YG8R breeding. PCR genotyping analysis
279 and DNA sequencing showed that YG8sR manage a single GAA triplet repeat mutation. This GAA

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280 triplet repeat has both intergenerational and somatic unsteadiness. Both exist in FA patients. [64]. In
281 the YG8sR mice, the authors showed behavior deficits, glucose intolerance and supersensitive to
282 insulin. It did not exist in Y47R and wild type (WT) mice. These YG8sR mice also have GAA triplet
283 repeat unsteadiness in the brain and cerebellum, the manifestation decline of *FA*, *FAST-1* and
284 frataxin, and presence of pathological vacuoles within neurons of the dorsal root ganglia. The YG8sR
285 model could be one of the excellent methods for the study of FA disease mechanisms and treatment
286 using advanced therapy as cell therapy or gene therapy.

287 Khonsari *et al* reported that lentivirus gene therapy could drive flats of DNA twin thread
288 fractures in FA patient and YG8sR cells. Quantitative RT-PCR at 2, 8 and 12 weeks in YG8sR and
289 normal mice showed different manifestation in these two mice cures. *FXN* manifestation in the human
290 and YG8sR cells reached to 96- and 210-fold, respectively, following the cures contrasted with
291 uncontaminated FA fibroblasts and 0.5- and 0.2-fold, respectively, contrasted with natural fibroblasts.
292 Over time, this manifestation fell about 50%. This study showed that the manifestation continued
293 larger than in uncontaminated FA fibroblasts over 12-week cure following therapeutic gene shipping.
294 Lateral flow immunoassay showed that the frataxin protein flats of the contaminated cells raised 42-
295 and 17-fold, respectively, contrasted with uncured FA cells 0.48- and 0.2- fold, respectively, of natural
296 fibroblasts. These frataxin protein flats diminished in human and mouse fibroblasts over time;
297 however, these flats continued higher than in uncured FA fibroblasts. In addition, y-irradiation of FA
298 patient and YG8sR cells showed incorrect DNA correction on the *FXN* gene relocation. This study
299 shows that the flats of DNA twin thread fractures are higher than in the natural fibroblasts [65]. It
300 seems that the lentivirus gene therapy reverses *FXN* gene segments unsteadiness in FA patient and
301 YG8sR mouse fibroblasts.

302 Virmouni *et al* showed that both YG8R and YG22R mice, along with intergenerational GAA
303 triplet repeat, have rather late-onset effects. These animal models would be beneficial to develop
304 further GAA repeat-based FA transgenic mice with a single-copy large GAA triplet repeat expansion
305 mutation. These transgenic mice may produce more severe early-onset effects [58]. It shows that
306 GAA triplet repeats in FA from late-onset to early-onset phenotype can be manipulated. It means that
307 the late-onset phenotype can also be manipulated to obtain normal transgenic mice.

308 Li *et al* established double-stranded RNAs (dsRNA) paired to the GAA triplet repeat. These
309 RNAs can tie to aim RNAs. However, they cannot engage the cleavage role of Ago2. The dsRNA

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310 improved manifestation of FA mRNA in the patient-originated FA cells. It also improved protein in
311 those cells. These improvements were the same as the WT levels. After adding of dsRNA, the protein
312 improvement can reach as long as 15 days. The authors examined the intronic transcription and RNA:
313 Ago2 using RNA immunoprecipitation (RIP) with an against-Ago2 antibody. There is dissimilar in
314 quantities of recruitment of RNA establishment by against-GAA (anti-FA) occurs at the point of RNA
315 production. It does not occur at the point of RNAs tie. Finally, Li *et al* observed that both anti-FA and
316 single-stranded locked nucleic acid (LNA) improved levels of FXN protein and RNA manifestation [66].
317 It shows that dsRNA and LNA oligonucleotides activate manifestation of *FA* RNA and protein. The
318 anti-FA, either dsRNA or LANs, can guide compounds to increase of agents for repairing medical
319 levels of FA protein. It seems that anti-FA and LANs can be very helpful to treat the FA disorder.

Table 3. Vehicles in gene therapy research for FA disorder

Vehicles	Fratxin repair	References
Lentivirus	30-60 %	[60]
AAV9-hFA	Improvement	[61]
iBAC-FA	Up to normal	[40, 62]
YG8sR	Improvement	[65]
RNAs/LANs	Improvement	[66]

320

321 ## 5. CONCLUSION

322 Friedreich ataxia associates with a GAA triplet repeat in the *FXN* gene. FA patients inherit the
323 disorder from one generation to the next according to the Mendel's first principle, the monohibryd. It is
324 an autosomal recessive disorder. Genetic counseling can help to direct patients with FA disorders and
325 their families about how to face the disorder. There are no efficient drugs to treat FA patients at
326 present. Animal models for the study of disease mechanisms and treatment are already available. To
327 cure the disorder, gene therapy has showed crucial progresses in slowing down FA disorder in the
328 animal models. This technique improved the frataxin level in FA disorder in the animal models.
329 Various genetic correction methods are available to correct erroneous segments in the *FXN* gene. It
330 comprises such as lentiviral vectors, AAV vectors, HSV-1 amplicon vector, and use of RNAs and
331 LNAs. For example, lentivirus gene therapy corrects erroneous segments in the *FXN* gene in FA
332 patient and YG8sR mouse fibroblasts. It seems that gene therapy would be very valuable for treating
333 FA.

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335 **COMPETING INTERESTS**

336 The author has declared that no competing interests exist.

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