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Original Article

# Novel Mutation in FRDA Gene among Iranian Patients with Friedreich's Ataxia

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## Abstract

**Introduction:** Friedrich Ataxia's diagnosis is typically based on clinical symptoms and extended GAA repeats. However, in some rare cases the disease is caused as a result of the mutation in the exons of the FRDA (Friedreich's ataxia) gene. The current study aimed to examine point mutations in exon 1 of the FRDA gene with the goal of finding a better way for diagnosing people suspected of this disease.

**Materials and Methods:** In this study, 30 suspected patients of Friedrich Ataxia underwent PCR molecular test. Subsequently, sequencing and long PCR were utilized to assess exon 1 in five patients with extended repeats.

**Results:** In total, 25 participants who had extended repeats were diagnosed with Friedrich Ataxia. In one out of the five patients, the nucleotide change from G to T was observed in the nucleotide number 815324.

**Conclusion:** Since the change had a heterozygous nature, it did not cause any deficiency in Frataxin protein. Given that family marriages are prevalent in Iran, there is a possibility of homozygosity with this mutation or other mutations. It is thus recommended that gene sequencing should be performed for individuals with suspected Friedrich Ataxia.

**Keywords**: Ataxia Friedrich, Mutation; FRDA, Iranian Patients, Homozygosity

## 1. Introduction

Friedrich Ataxia (FA) is the most common type of Ataxia which is inherited as autosomal recessive. Reduction of Frataxin, which is a mitochondrial protein, leads to the clinical and pathological manifestation of this disease [1-3].

Normally, 1 in 5000 people suffers from this disease. In some societies with a lot of family marriages, the prevalence of this disease is even higher. Whites are more vulnerable to FA, while males and females are equally susceptible to it. The first symptoms of this disease are usually manifested between 5 to 15 years of age, with some early and late manifestations being respectively reported at ages of 18 months and 30 years. Through progressive degeneration of the sensory nerves, the disease eventually leads to weakness and disabilities. The most common cause of death among FA patients is heart failure [4, 5].

In most cases, the molecular cause of FA is the extension of GAA repeats (more than 30 repeats) in intron 1 of both alleles of FXN. The extended repeats hinder gene transcription, hence causing deficiency in Frataxin. The gene defining FRDA or FXN

Frataxin is located in chromosome 9q13, with a length of 80 kbp and 7 exons [6, 7]. As already mentioned, in most of the cases, FA is caused by mutation in the extension of homozygous tri nucleotide repeats. However, in some patients, FA is caused by the extension of triple repeats in a single allele and point mutation in the other one [8, 9]. The absence of homozygous genotypes for point mutations may be due to the low occurrence of these mutations [10,11]. Considering molecular diagnosis, there are around 33 repeats in the PCR product band of normal people. In patients, however, the band length goes up, with the extended bands being confirmed only by Long PCR. Regarding people with 500 bp bands and FA symptoms, two scenarios are possible: first, such people are not infected by FA, meaning that they should undergo other diagnostic tests that are used for patients with diseases that have phenotypic similarity to this disease. The second possibility is that such people have an extended GAA allele and a point mutation in the second allele. That is, they are compound heterozygote. Given that the majority of reported mutations have occurred in exon 1 and that family marriages are common in Iran, the current study examined exon 1 among people with FA symptoms and 500 bp bands to investigate point mutations.

## 2. Materials and Methods

### 2.1 Study Design and Patients

Thirty people (18 males and 12 females) suspected of FA, who had referred to Tehran Special Diseases Center, were selected for the study. These participants had FA phenotypic features (including muscle weakness, imbalance, and speech impairment). Written informed consent was obtained from all the patients.

### 2.2 DNA Extraction

QIAGEN kit was used to extract DNA from the blood of the 30 patients who were suspected of FA.

### 2.3 PCR for Intron 1 of FRDA Gene

After extracting DNA, for intron1 of FRDA gene amplification, PCR was carried out using DNA thermal cyclers and GAA-F and GAA-R primers in 25 µl of a solution containing 2.5 µl of buffer, 0.5 µl of MgCl2, 0.5 µl of dNTP, 0.5µl of each primer, 2 µl of DNA and 0.5µl Taq DNA polymerase (Ependorf Master Cycler Gradiant). The PCR reaction was performed for 30 cycles composed of the following steps: 95 ºC for 5 min, 94 ºC for 45″, 64 ºC for 30″, 72 ºC for 2 min and 72 ºC for 10 min. In order to assess test reliability, both negative control (without the disease) and positive control (with the disease) samples were used. The oligonucleotides used as specific primers for gene were as follows:
GAA-F:
gggattggttgccagtgcttaaaagttag.GAA-R:
gatctaaggaccatcatggccacacttgcc.

### 2.4 PCR for Exon 1 of FRDA Gene

For exon 1 of FRDA gene amplification, PCR was carried out, using DNA thermal cyclers and F and R primers in 25 µl of a solution containing 2.5 µl of buffer, 0.5 µl of MgCl2, 0.5 µl of dNTP, 0.5µl of each primer, 2 µl of DNA 5 µl of DMSO, and 0.5µl Taq DNA polymerase. (Eppendorf Master Cycler Gradiant). The PCR reaction was performed for 30 cycles composed of the following steps: 94 ºC for 5 min, 94 ºC for 50″, 68 ºC for 50″, 72 ºC for 35 ″ and 72 ºC for 1 min. The oligonucleotides used as specific primers for gene were as follows:
F: agcacccagcgctggagg 67.4°C, R: ccgcggctgttcccgg 69.7°C.

## 2.5 Long PCR

While studying the sequence of FXN exons for exon 1, heterozygous mutation was observed in one of the patients. Based on the patient's phenotype and mutation observed in the gene, it was hypothesized that the patient was an abnormal case of FA; that is, he had point mutation in one allele and extended GAA repeats in the other, which could not be detected by PCR; therefore, Long PCR was done. For this PCR using DNA thermal cyclers Bam r and 2500f primers and two solutions that solution 1 containing 7.9 µl of Dd H2O, 0.5 µl of dNTP Mix 10mM, 0.8 µl of each primer, 2 µl of DNA and solution 2 containing 8 µl of Dd H2O, 2.5 µl of Expand Buffer without MgCl2, 1.6 µl of MgCl2 25mM and 0.4 µl of Expand Enzyme (1 and 2 kit solutions of expand high fidelity PCR system of Roche). The PCR reaction was performed for 20 cycles composed of the following steps: 94 ºC for 5 min, 94 ºC for 20″, 68 ºC for 2.5 min and 17 cycles composed of the following steps: 94 ºC for 20″, 68 ºC for 2.5 min MWG Biotech Inc Primus 25 Thermal Cycler, 110 VAC). The oligonucleotides used as specific primers for gene were as follows:
T.
F: caatccaggacagtcagggcttt. R: ggmagggatccgtctgggcaaagg.

## 2.6 Electrophoresis

In order to make sure about the duplication of the specific components of each gene and the absence of non-specific products and dimmer primer, 5 µl of the PCR product was loaded on 1.5% agarose gel and electrophoresed for 30 minutes. Electrophoresis was carried out at 140 V for 30 min.

## 2.7 Sequencing

Upon sequencing PCR samples (Kosar Kavosh Fanavaran Company, ABI 3700), Finch TV was used for reading the sequence. Then, NCBI website was consulted to compare the results for the obtained sequence with normal ones.

## 3. Results

No band was obtained for 25 patients. Thus, they were diagnosed with FA. The results of Long PCR for this patient indicated that the other allele of this gene had a band of 1380 bp. In one of the five heterozygous patients with 500 bp band, nucleotide G has changed to nucleotide

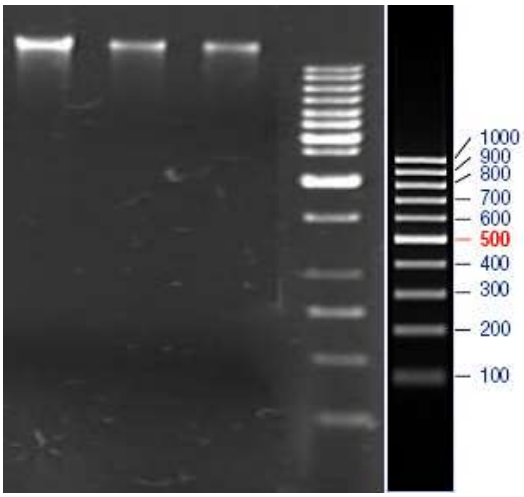


**Figure 1.** DNA bands extracted by QIAGEN kits, Ladder 100.

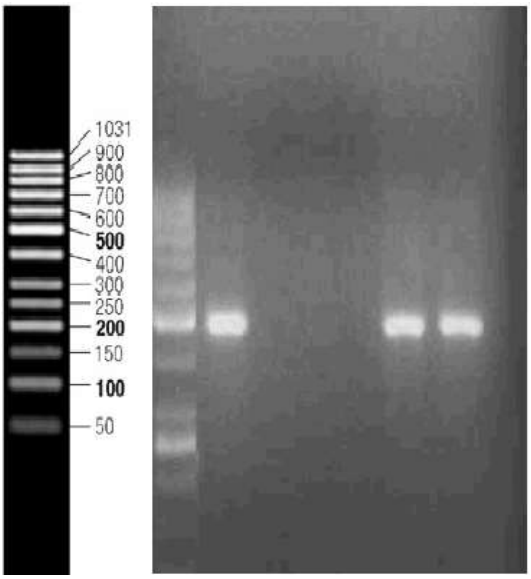


**Figure 2.** From left to right: 1. Ladder 50, PCR Product of intron 1: 2. 500 bp band, 3. Positive control, 4. No band was obtained (patients with extended sequence), 5. Negative band, 6. 500 bp band, 7. No band was obtained (patients with extended sequence).

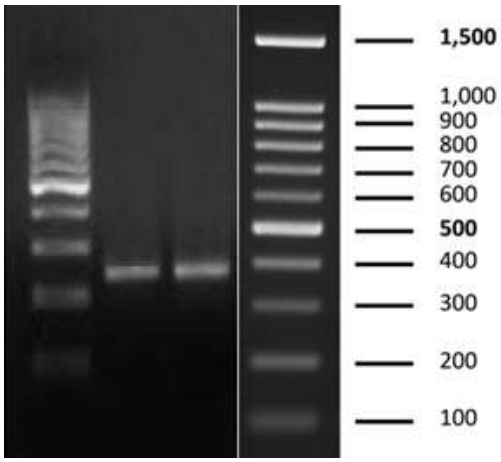


**Figure 3**. PCR Product of exon 1(240 bp ), Ladder100 bp.

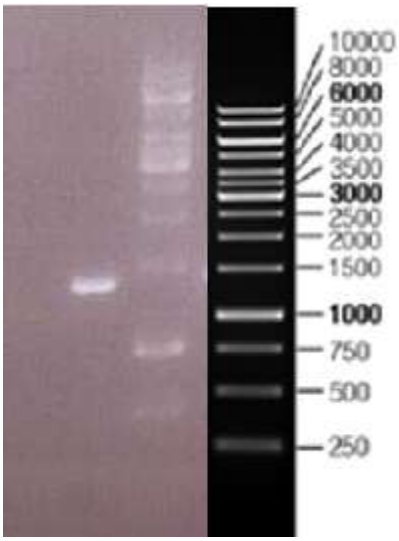


**Figure 4.** From left to right: Ladder 1kb, Long PCR Product of intron 1(1380 bp).

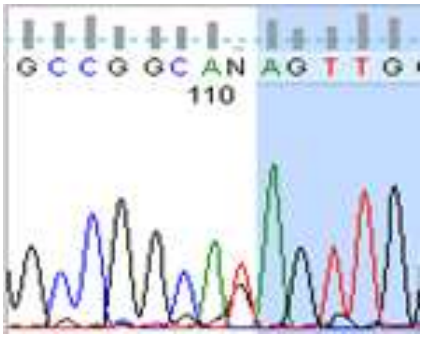


**Figure 5.** The nucleotide change from G to T was observed in the nucleotide number 815324.

## 4. Discussion

It is important to conduct molecular investigation of FA, given that phenotypic features are common in most of the neurological diseases. It is therefore difficult to diagnose FA solely based on clinical features [12]. The most common type of mutation which is observed in over 98% of patients with FRDA is the extension of three repeats GAA in the intron of FXN. About 5% of people who suffer from FRDA are compound heterozygous, consisting of disease-making GAA extension in one allele and disabling mutation of FXN in the other [13]. In molecular diagnosis of this disease, if normal band is obtained from PCR product, diagnosis tests that are used for similar diseases will be exploited for the patients based on their phenotypic symptoms. Nevertheless, it is likely that a particular patient suffers from an abnormal case of FRDA (which happens in 5% of the cases). In such cases, due to the extension of GAA repeats, band length increase in one allele, no band is observed in the normal PCR, and the normal band has point mutation (500 bp band). In this study, in order to diagnose patients, intron 1 was reproduced using GAA-F and GAA-R primers. All in all, 25 patients were diagnosed with FA because they had extended repeats. In some patients (5%), FA is caused by factors such as deletion of few nucleotides [13,14] point mutations [14, 15,16] deletion-insertion mutations [17], deletion of complete exons [15, 18], and complete deletion of FRDA [19]. In such cases, most of the reported mutations occur in exon 1. Thus, in the 5 patients with no observed extended band, exon 1 was sequenced to study the relationship between point mutations and infection with FA. Similar attempts have already been made. For example, guanine nucleotide has changed to thymine in exon 1, which causes deficiency in protein translation; guanine nucleotide has been deleted in exon 1, a change that causes deficiency at the end of Frataxin translation (100delG); cytosine nucleotide has been deleted in exon 1, which leads to early termination of Frataxin translation (104delC) [20]; adenine nucleotide has changed to guanine in intron 1, which causes deficiency in termination of Frataxin translation (Splice Donor) [21]; adenine nucleotide has changed to cytosine and thymine nucleotide has changed to cytosine in exon 1, leading to deficiency in starting the translation; cytosine nucleotide has been deleted in exon 1, which leads to deficiency at the end of Frataxin translation (158delC) [22]; guanine nucleotide has changed to adenine in exon 1, which leads to early termination of Frataxin translation (M1I) [23]; cytosine nucleotide has been deleted in exon 1, which causes deficiency at the termination of Frataxin translation (118delC) [24]; guanine nucleotide has been converted to cytosine in intron 1, which causes deficiency in termination of Frataxin translation (Splice Donor) [25]. Likewise, three point mutations found that c.241T>G in exon 2 , c.574A>T in exon 5 and c.367T>G in exon 3 in patients heterozygous for the GAA repeat expansion [26]. In another study, exons 1, 2, 3, 4, 5b,

and 5a were investigated among patients. Several polymorphisms were observed; in exon 1, homozygote nucleotide A changed to nucleotide G number 815284. That is, CCA codon was converted to CCG codon. Both of these codons have to do with proline amino acid. In the intron region of exon 2, homozygote nucleotide C changed to G number 825954. In the intron region of exon 3, nucleotide T (which was in the form of homozygote in some participants and heterozygote in others) changed to C number 832729. In exon 4, no heterozygote or homozygote mutation was observed. Among the patients, nucleotide G number 852225 was added in exon 5a. No heterozygote or homozygote mutation was observed in exon 5b [27, 28]. In the current study, in exon 1 in one of the patients, a heterozygote mutation was observed, in that, nucleotide G changed to nucleotide T. In other words, GAG codon was converted to TAG codon, in homozygote mutation the codon that is related to glutaric acid is converted to stop codon.

## 5. Conclusion

Given that heterozygote mutation was observed, this type of change does not lead to Frataxin deficiency. Based on the phenotypic symptoms of the FA patients, it is hypothesized that mutation has occurred in areas other than the studied ones (e.g. the promoter region of this gene). Since family marriages are dominant in Iran, it is highly likely that homozygote children with this mutation or other mutations are born. It is therefore necessary to conduct gene sequencing in diagnosing patients who are suspected of FA.

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## Conflict of interest

The authors declare no conflict of interest.

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