Molecular and Clinical Investigation of Iranian Patients with Friedreich Ataxia

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ABSTRACT

Background: Friedreich ataxia (FRDA) is an autosomal recessive disorder caused by guanine-adenine-adenine (GAA) triplet expansions in the *FXN* gene. Its product, frataxin, which severely reduces in FRDA patients, leads to oxidative damage in mitochondria. The purpose of this study was to evaluate the triple nucleotide repeated expansions in Iranian FRDA patients and to elucidate distinguishable FRDA clinical differences in these patients. **Methods**: A number of 22 Iranian patients (8 females and 14 males) from 16 unrelated families were studied. DNA was extracted from the peripheral blood of patients. The frequency and length of $(GAA)_n$ repeats in intron 1 of the *FXN* gene were analyzed using long-range PCR. In this study, the clinical criteria of FRDA in our patients and the variability in their clinical signs were also demonstrated. **Results**: An inverse relationship was observed between GAA repeat size and the age of onset. Although some distinguishable clinical features (such as limb ataxia and lower limb areflexia) were found in our patients, 90-95% of them had extensor plantar response and dysarthria. The results showed only one positive diabetes patient and also different effects on eye movement abnormality among our patients. **Conclusion**: The onset age of symptoms showed a significant inverse correlation with allele size in our patients ($P>0.05$). Based on comparisons of the clinical data of all patients, clinical presentation of FRDA in Iranian patients did not differ significantly from other FRDA patients previously reported. *Iran. Biomed. J. 18 (1): 28-33, 2014*

Keywords: Friedreich ataxia (FRDA), Frataxin, Mitochondria

INTRODUCTION

Friedreich ataxia (FRDA, MIM 229300), first described by the German investigator Nicholaus Friedreich, is an autosomal recessive neuro-degenerative disorder caused by mutations in the *FXN* gene which results in reduced levels of the mitochondrial protein, frataxin. The majority of FRDA patients (96%) are homozygous for an unstable guanine-adenine-adenine (GAA) trinucleotide repeat expansion in intron 1 of the *FXN* gene, located at 9q13-q21.1, but a few patients are heterozygous with point mutations found on the other allele [1].

FRDA encodes for frataxin, a protein of 210 amino acids with an N-terminal leader peptide which directs its subcellular localization to the mitochondria [1]. The *FXN* gene is expressed at various levels in different cells due to differences in cellular mitochondrial numbers. In adult humans, *FXN* mRNA is most abundant in cells of the heart and spinal cord, liver, skeletal muscle, and pancreas [1]. The highest levels of *FXN* mRNA are found in the spinal cord, dorsal root ganglia, proliferating neural cells, cortical plates, and ganglionic eminence. *FXN* mRNA expression is also high in heart, liver, and brown fat [2, 3].

Frataxin deficiency leads to elevated intramito-chondrial iron levels and Fe-S cluster-containing enzymes deficiency, enhanced sensitivity to oxidative stress [4]. The disease is characterized by gait and limb ataxia, dysarthria, absence of tendon reflexes, impairment of positional and vibratory senses, scoliosis, pes cavus, and also cardiomyopathy, which is the predominant cause of death in FRDA patients [5]. Additional features include nystagmus, optic atrophy, deafness, and diabetes mellitus in a minority of patients [6]. The neuropathology of FRDA shows systematic differences in comparison with other hereditary ataxias, with major changes in the spinal cord,

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peripheral nerves, and cerebellum [2]. Degeneration of the corticospinal and pyramidal tracts results in muscle weakness and extensor plantar responses [7].

The prevalence of FRDA is approximately 1 in 50,000 in the white population, with a carrier rate of 1:60 to 1:120 [1, 8, 9]. FRDA is only detected in individuals of European, North African, Middle Eastern or Indian origin and is very rare, or inexistent, in sub-Saharan Africans, Amerindians and Asians [10]. In this study, we aimed to measure the sizes of three nucleotide repeated expansions and clinically compare our FRDA patients with other FRDA patients.

MATERIALS AND METHODS

In this study, 22 patients (8 females and 14 males) from 16 unrelated families that diagnosed with FRDA were clinically analyzed regarding their clinical aspects and PCR test. We basically adopted the clinical criteria of Harding [8] and Geffroy [11]. All of the patients were informed on the aims of the study and gave their informed consent to genetic analysis. The patients were referred to a consultant neurologist in the Medical Genetics Department, Special Medical Center (Tehran, Iran) for assessment.

In this report, 22 clinically diagnosed cases (median age 16.4 years, range 4-29) were described as having FRDA. Peripheral blood samples were obtained using EDTA and DNA was purified after lysing the white blood cells using a DNA extraction kit (DNAfast Kit, Genfanavaran, Tehran, Iran). The quality of DNA extracted from blood samples is shown in Figure 1. A special PCR technique was used to identify FRDA patients. A Flexigene blood DNA kit (DNA fast,
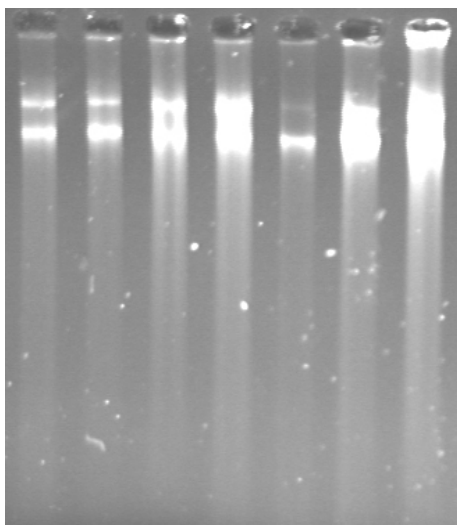

Fig. 1. Quality of extracted DNA from several different patients.

QIAGEN, Cat. No. 51204) was used for the isolation of DNA from the blood samples. Long-range PCR was set up according to the protocol of Expand Long Template PCR System kit (Roche, Mannheim, Germany). The primers 5200Eco (5'-GGG CTG GCA GAT TCC TCC AG-3') and 5200Not (5'-TAA GTA TCC GCG CCG GGA AC-3') generated a 1.5-kb normal fragment. The GAA repeat length was calculated according to the size of the PCR product (457 + 3n bp, where; n shows the number of GAA triplets) [1]. The PCR conditions were conducted with initial denaturation at 94°C for 2 min, then 10 cycles of denaturation at 92°C for 10 s, annealing at 64°C for 30 s and extension at 68°C for 12 min, followed by 25 cycles in which the length of the 64°C step was increased by 20 s/cycle. This was followed by a final extension at 68°C for 7 min.

Statistical analysis. Nonparametric statistical method with Spearman's rank correlation coefficient was used to assess the relationship between allele sizes and age of onset of clinical symptoms, where *P*<0.05 was regarded as statistically significant. Statistical analyses were performed using the GraphPad Prism software.

RESULTS AND DISCUSSION

The native nationalities and geographical origins of our patients were different. All patients had gait and limb ataxia, abnormalities in tendon reflexes, impairment of position, and vibratory sense. Foot deformity and dysarthria approximately were present in all patients. Ten patients were wheelchair bound. The expansion size inversely correlated with wheelchair use [12]. Marked variability of FRDA symptoms between individuals and within families is related to intergenerational instability of the GAA expansion [13].

In our patients, the onset of symptoms occurred between 2 and 23 years, in which two patients had a very low and rare age of onset. However, in a previous study, the age range of patients was 6–31 years [14]. Age of diagnosis varied from 4 to 29 years. Campuzano and his colleagues [1] reported that the age of FRDA onset varied from 2 to over 51 years. The gender, age of onset and diagnosis, number of GAA repeats, and the most important clinical findings of our patients are summarized in Table 1.

Parkinson and his colleagues [13] have found frequencies of clinical features in selected case series in FRDA patients. We compared clinical manifestation of our patients with these results (Table 2).

Molecular analysis of the *FXN* gene in the patients showed that they were homozygous for a large number of GAA repeats. The (GAA)n repeats in FRDA

Table 1. Clinical features of FRDA patients

FRDA	Gender	Consanguineous marriages	HFX (History of family)	Age of onset	Age of Diagnosis	Limb Ataxia	Lower-Limb Areflexia	Decreased Vibration Sense	Extensor Plantar Response	Axonal Neuropathy	Dysarthria	Eye Movement Abnormality*	Optic Atrophy	Food Deformity	Diabetes or Glucose Intolerance	GAA repeat
01	F	None	4	13	17	+	+	+	+	+	+	N	_	+	_	384
02	M	First	1	11	13	+	+	+	+	+	+	N	_	+	_	329
03	M	First	1	14	16	+	+	+	+	+	+	N	_	+	_	426
04	M	None	7	12	25	+	+	+	+	_	+	ND	_	_	_	566
05	M	None	7	17	21	+	+	+	+	+	+	ND	_	+	_	269
06	M	None	7	7	19	+	+	_	+	_	+	N	_	_	_	890
07	M	None	1	5	21	+	+	_	+	_	+	NS	_	_	_	947
08	M	None	1	6	19	+	+	_	None	_	+		_	_	_	878
09	F	First	None	12	16	+	+	_	+	_	+	NS	+	_	_	498
10	M	None	1	23	29	+	+	_	+	+	+	ND	_	+	_	247
11	M	None	1	15	19	+	+	_	None	_	+		_	_	_	584
12	M	None	1	15	15	+	+	+	+	_	_		_	_	_	618
13	F	First	None	6	25	+	+	+	+	+	+	N	_	+	_	908
14	F	None	3	13	28	+	+	_	+	+	+	N	_	+	_	405
15	F	None	1	2	4	+	+	+	+	+	+	N	_	+	_	981
16	M	First	1	12	26	+	+	+	+	+	+	N	_	+	_	432
17	F	None	None	3	8	+	+	_	+	_	+	ND	+	_	+	812
18	F	First	2	13	16	+	+	+	+	+	+	ND	_	+	_	479
19	M	First	2	9	10	+	+	+	+	+	+	N	_	+	_	554
20	M	First	1	15	17	+	+	+	+	_	+	N	_	_	_	360
21	M	None	1	8	10	+	+	+	+	_	+	NS	_	_	_	714
22	F	None	None	6	9	+	+	+	+	_	+		_	+	_	792

* N:Nystagmus; ND: Nystagmus Dysmetric Saccades; NS: Nystagmus square wave jerks

Table 2. Frequencies of clinical features of FRDA patients

| Clinical feature	No. of patients	Harding [8]	Ackroyd *et al.* [15]	Filla *et al.* [16]	Dürr *et al.* [17]	Schöls *et al.* [18]	Lamont *et al.* [19]	Delatycki *et al.* [20]	McCabe *et al.* [21]	Our study
No. of patients	94	115	12	80	140	38	56	51	58	22
Male : female	1 : 2	1 : 1.2	1 : 1.0	1 : 0.7	1 : 1.1	1 : 1.4	-	1 : 0.8	-	1 : 0.6
Age at onset (years)	7.97	10.52 (1.7-27)	5.8 (3-9)	11.6 (2-23)	15.5 (2-51)	14.15 (5-36)	(3-30)	10.5 (1-26)	-	10.77 (2-23)
Wheelchair-bound (%)	-	72	-	43	-	78	-	55	-	45
Gait ataxia (%)	100	100	100	100	100	100	-	100	100	100
Limb ataxia (%)	-	99	100	94	99	100	100	100	-	100
Dysarthria (%)	100	97	75	84	91	100	91	95	93	95
Lower limb areflexia (%)	100	99	100	100	87	84	87	98	86	100
Loss of vibration sense (%)	-	73	67	91	78	83	87	88	89	63
Extensor plantar responses (%)	94	89	42	75	79	95	96	74	93	90
Foot deformity (%)	89	55	83	90	55	82	-	74	79	54
Nystagmus (%)	42	20	25	29	40	39	-	-	40	45
Diabetes (%)	19	10	-	14	32	6	-	8	7	4.5

patients were observed in both alleles, ranging from 247 to 981 GAA motifs. Comparison of the age of onset with the number of GAA repeats demonstrated a statistically significant inverse correlation ($r = -0.86$, $n = 22$, Fig. 2).

In our study, the size of GAA expansions (mean = 594 GAA units) reflects the instability of this expansion during transmission. We observed a strong inverse correlation ($r = -0.86$) between GAA expansion size and age of onset in our patients, which confirms the previous finding [13].

It is important to know which clinical features are likely to be found in early cases of FRDA. As disease duration is such an important factor in determining the presence of certain clinical features in FRDA, Harding [22] proposed analyzing the frequency of various physical signs according to the duration of symptoms. The phenotype associated with the FRDA mutation is much wider than that defined by clinical criteria [23].

The first symptoms of FRDA patients usually appear around puberty, but the age of onset can vary from infancy (2–3 years) to adulthood (after 25 years old). Gait instability is a typical presenting symptom. Scoliosis, first diagnosed as idiopathic, is often already present when neurological symptoms appear [24]. Limb ataxia causes increasing difficulty in activities requiring dexterity and precision, such as writing, dressing and handling utensils. Central limb weakness appears to worsen with FRDA progression, where patients lose the ability to walk, stand and eventually sit without support 10 to 15 years after onset, requiring wheelchairs to carry out everyday activities [25, 17]. Male to female distribution is equal [12].

Cardiomyopathy and diabetes are two major complications of the disease [26], and some patients develop skeletal deformation (such as scoliosis and pes cavus), ocular abnormalities, and hearing loss [27]. FRDA is associated with frequent, subclinical optic neuropathy [28]. About 20% of patients with FRDA develop carbohydrate intolerance. Diabetes usually develops at the late stage of the disease, approximately after 15 years [29, 30]. We have only 1 positive diabetic and 21 nondiabetics (less than 5%). Variations in clinical signs and disease progression observed in FRDA cases are very extensive and include age of onset, rate of progression, severity, and duration of the disease [31, 32]. There is a significant influence of disease
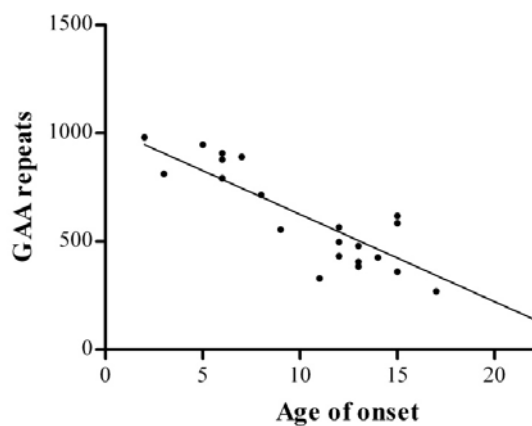
Fig. 2. Association between the age of onset and the number of GAA repeats in patients. Age of onset (years) and number of GAA repeats is significantly inversely correlated ($r = -0.86$, $n = 22$).

duration on the frequency of presentation of dysarthria, becoming wheelchair bound, lower limb weakness, amyotrophy, and decreased vibration sense. No correlation could be established with the development of cardiomyopathy, diabetes and extensor plantar responses, lower limb areflexia and dysarthria, and the size of GAA repeats with the duration of the disease [12].

In FRDA-affected individuals with GAA expansions in both homologues of chromosome 9, repeats ranged from ~70 to more than 1000 triplets, most commonly 600–900 [3]. The size of expansion is highly variable from one generation to the next [33]. To date, all patients with FRDA found to carry such mutations were compound heterozygotes for the GAA repeat expansion [13]. The GAA expansion allows structurally and functionally normal fraxatin synthesis, in whichthe length of the expansion and the cell type influences fraxatin levels. In peripheral blood leukocytes, frataxin levels range from about 5% to 30% of normal. Because the expansion size determines the level of residual frataxin expression, it has an influence on the severity of the phenotype [32].

The size of GAA repeats is directly correlated to an earlier age of onset, more rapid rate of disease progression, cardiomyopathy, and the presence of nonuniversal disease manifestations, such as diabetes, which are indicative of more widespread degeneration [16]. Distinct clinical manifestations in FRDA patients such as dysarthria, skeletal deformities, optic atrophy, and hearing loss show a direct correlation with GAA expansion size [1, 32]. GAA repeat expansion determines the clinical phenotype of FRDA by affecting gene transcription [13].

The size of gene expansion has great impact on the clinical characterization and outcome of the disease. The length of the GAA repeat expansion is not only directly correlated with the severity of the disease but also has an inverse relationship with the age of onset and the level of FRDA gene expression [33]. Age of onset is strongly dependent on the size of GGA expansion and thus depends on complex I activity and intracellular ATP content [34]. A lower-than-normal rate of mitochondrial ATP production and decreased oxidation activity are seen in patients with FRDA, and show a strong negative correlation with the number of GAA repeats in those alleles [35]. Mitochondrial oxidative stress is involved in the pathogenesis of several neurodegenerative diseases, including FRDA [36]. Mutations in the genes encoding NADH dehydrogenase subunits were more frequent in patients than in controls. The number of mutations was negatively correlated with the age of disease onset [34].

Phenotypic diversity of FRDA is increasingly expanding. The structure of GAA repeat expansion together with age of disease onset are important in determining the clinical features and the differential diagnosis of FRDA. This study has contributed to a better understanding of patient condition and its clinical presentations in Iranian FRDA patients.

The onset age of symptoms showed a significant inverse correlation with allele size in our patients (*P*>0.05). Patients with late onset have significantly shorter GAA trinucleotide repeat expansions on both alleles *(P*>0.05). In our data, we showed some differences between the clinical criteria in our FRDA patients and in other studied FRDA patients that were reported before. We showed some variation in the age of onset and rate of clinical symptom occurrence as well as the percentage of clinical manifestation, but we did not observe any unexpected clinical features. In summary, comparison of all patient sets of clinical data indicated that the Iranian FRDA patients not only differ significantly from people of European, North African, Middle Eastern, nor Indian origin but also are significantly different much less than the Middle Eastern ones.

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