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Short communication

Atypical Friedreich ataxia in patients with FXN p.R165P point mutation or comorbid hemochromatosis

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ABSTRACT

Background: Compound heterozygosity for a trinucleotide repeat expansion and a point mutation in the *FXN* gene is a rare cause of Friedreich ataxia (FRDA).
Methods: We identified three Swedish FRDA patients with an FXN p.R165P missense mutation and compared their clinical features with six homozygote trinucleotide repeat expansion carriers. Patients were assessed clinically. Trinucleotide expansion length was determined and lymphocyte frataxin levels measured.
Results: p.R165P mutation carriers became wheelchair bound early, but had retained reflexes, better arm function, milder dysarthria, and were more independent in activities of daily living. One p.R165P mutation carrier developed psychosis. Frataxin levels were higher than in homozygous trinucleotide expansion patients. One patient with homozygous trinucleotide repeat expansions and comorbid hemochromatosis had more severe FRDA symptoms than his sibling without hemochromatosis.
Conclusion: p.R165P patients progress to a less disabling disease state than typical FRDA. Comorbid hemochromatosis may worsen FRDA symptoms through additive effects on iron metabolism.

1. Introduction

Friedreich ataxia (FRDA) is a hereditary disorder with progressive neuropathy, postural ataxia, dysarthria, muscle weakness and possible complications including cardiomyopathy, diabetes mellitus and loss of hearing or vision. Lack or malfunction of the frataxin protein due to mutations in the *FXN* gene is the known cause of the disease [1]. Most FRDA patients are homozygous for GAA trinucleotide repeat expansions in *FXN* (GAA-TRE). Rarely, FRDA may be caused by heterozygosity for GAA-TRE and an *FXN* point mutation [2–4]. The severity of clinical disease varies widely between patients. In GAA-TRE homozygotes, the number of repeats in the shorter GAA-TRE allele is inversely correlated with age of onset, likelihood for milder, late-onset FRDA as well as age of death, and directly correlated with symptom severity and risk for developing cardiomyopathy [5,6]. As pathogenic point mutations in *FXN* are rare, it has been difficult to associate a particular clinical phenotype with such mutations. Milder disease has been reported with the p.D122Y and p.G130V point mutations, but classical FRDA with other mutations [2]. *FXN* c.494G>C (p.R165P) missense mutations have previously been described in two Italian siblings with atypical but not milder disease [4]. We identified this mutation in three FRDA patients from two Swedish families and compared their clinical phenotype with GAA-TRE homozygotes.

2. Methods

All nine patients diagnosed with FRDA known at the Department of Neurology at Skåne University Hospital, Sweden, or personally to the authors, were included in this study. The study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. Ethical approval was waived.

During one study visit, patients were interviewed, examined clinically and assessed with FRDA rating scale (FARS) [7], including staging of global functioning, questions on activities of daily living (ADL), neurological examination as well as the 9-hole peg test and the PATA speech test where the number of times probands can pronounce “pata” within a given time is assessed. All neurological examinations for this study were performed by one neurologist specialized in movement disorders. Hearing impairment was classified as mild (+) or severe (++). Visual acuity was assessed with low letter visual acuity test with the Monoyer vision chart or finger

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Table 1
Clinical features, genetic testing and frataxin measurement

	Pt-1	Pt-2	Pt-3*	Pt-4*	Pt-5	Pt-6	Pt-7	Pt-8°	Pt-9°	*p*-Value	Italian1+ (Ref. [4])	Italian2+ (Ref. [4])
Genotype												
FXN GAA-TRE genotype	Hom	Hom	Hom	Hom	Hom	Hom	Het	Het	Het		Het	Het
GAA repeats allele 1	700	1000	1000	800	600	1000	1000	1000	900	*0.396*	940	1100
GAA repeats allele 2	900	1100	1000	1000	800	1000	Normal	Normal	Normal		Normal	Normal
FXN Point mutation	N/A	N/A	N/A	N/A	N/A	N/A	p.R165P	p.R165P	p.R165P		p.R165P	p.R165P
Hemochromatosis			No	Yes▲								
Clinical features												
Age at onset (years)	12	10	5	4	13	5	5	2	5		8	3
Age at examination (years)	44	39	30	31	39	23	62	37	34		32	23
Duration of disease	32	29	25	27	26	18	57	35	29	***0.052***	24	20
Age when wheelchair bound	27	30	13	12	27	9	13	12	8	*0.191*	19	12
Duration when wheelchair bound	15	20	8	8	14	4	8	10	3	*0.294*	11	9
Dysarthria	+++	++(+)	+	+++	++(+)	+	+	(+)	+		0	+
Hearing impairment	+	N/A	+	++	+	+	+	0	0		N/D	N/D
Visual acuity	CF4	0.2	0.7	CF4	0.9	0.05	CF0.5	N/A	CF0.3		N/D	N/D
Optic disc pallor	N/A	+	N/A	+#	0	++	++#	+	++#		0	0
Cardiomyopathy	Yes	Yes	Yes	Yes	No	No	No§	N/A	Yes		No	No
Spasticity in lower limb	0	+	+	+++	N/A	+++	0	N/A	++		0	++
Upper limb tendon reflex	0	0	0	0	0	0	Normal	Normal	Brisk		Brisk	Brisk
Lower limb tendon reflex	0	0	0	0	0	0	0	Brisk	0		0	Brisk
Decreased position sense	+++	+	+	+++	++	+++	+	(+)	+		+	++
Friedreich Ataxia Rating Scale												
I Functional staging	5	6	5	6	5.5	6	6	5	5	*0.901*	N/D	N/D
II Activities of daily living	27.5	28.5	21.5	31.5	24.5	26	28	17	16	*0.197*	N/D	N/D
III Neurological examination	74.5	92.5	69	110.5	78.5	98	75	68	77.5	*0.197*	N/D	N/D
A Bulbar subscore	*2.5*	*3.5*	*1*	*7.5*	*2.5*	*2*	*3*	*2*	*2.5*	*0.895*	N/D	N/D
B Upper limb subscore	*13*	*25*	*11*	*36*	*12*	*28*	*9*	*6.5*	*11*	***0.028***	N/D	N/D
C Lower limb subscore	*16*	*16*	*16*	*16*	*16*	*16*	*16*	*16*	*16*	*1.000*	N/D	N/D
D PNS subscore	*16*	*21*	*14*	*23*	*22*	*25*	*20*	*18.5*	*20*	*0.437*	N/D	N/D
E Upright stability subscore	*27*	*27*	*27*	*28*	*26*	*27*	*27*	*25*	*28*	*0.887*	N/D	N/D
Total	107	127	95.5	148	108.5	130	109	90	98.5	*0.197*	N/D	N/D
Performance tests												
9-hole PEG test right/left (min)	3.0/2.0	N/A	1.5/3.5	N/A	N/A**	N/A	N/A	0.5/0.5	N/A		N/D	N/D
Mean nr of "PATA"s per 10 s	16.5	8.5	14.5	N/A	7.5	13.5	23.5	15.5	17	***0.053***	N/D	N/D
Frataxin protein level (ng/mg protein)												
Lymphycytes/lymphoblasts	1.36	1.00	N/A	1.33	2.18	0.46	3.89	4.22	4.85	***0.036***	Higher than in GAA-TRE (Ref. [9])	

*, ° and +, sibling pairs; ▲, confirmed as *HFE* c.845G>A (p.C282Y) homozygote; #, optic atrophy diagnosed by ophthalmologist; §, probable sudden cardiac death in affected twin brother (see main text); ** due to visual impairment. CF, maximal distance (m) to correctly counting fingers; Het, heterozygous; Hom, homozygous; N/A, not assessed; N/D, no data. *p*-Values were calculated comparing patients 1–6 with patients 7–9. Italian1 and 2; data on the two Italian siblings with FXN p.R165P previously reported. Bold represents *p*-values with (near) statistical significance.

counting. The presence and length of GAA-TRE were assessed essentially as described elsewhere [5,8]; for the present study, all patients' GAA-TRE were reassessed and directly compared in one assay. In patients heterozygous for GAA-TRE, the entire coding and exon flanking sequences of *FXN* were sequenced using standard procedures. Six patients (Patients 1–6) were homozygous for GAA-TRE whereas three patients (Patients 7–9) were compound heterozygous for GAA-TRE and the c.494G>C (p.R165P) mutation.

Levels of frataxin protein in blood lymphocytes and lymphoblasts were determined in all patients but one, using a "Rapid Microplate Assay Kit for Frataxin Quantity" (MitoSciences, Eugene, Oregon; principle of a solid-phase ELISA). Homogenized samples were suspended in PBS buffer and proteases inhibitors to 5.5 mg protein/ml. Protein content was determined by Bradford assay (Bio-rad Laboratories, Hercules, CA) and adjusted to the final concentration for the test of 5 mg protein/ml. Measurements were performed in duplicate to quadruplicate; age-matched healthy subjects served as controls. Mann–Whitney *U*-test was used for statistical analyses.

We compared our findings with the published data on the only two patients with FXN p.R165P mutation previously reported in the literature [4,9].

3. Results

There were no significant differences between the GAA-TRE homozygotes and the p.R165P patients regarding GAA-TRE length. Age at symptom onset was 8.2 ± 4.0 (mean ± SD) years for GAA-TRE homozygotes and 4.0 ± 1.7 years for p.R165P compound heterozygotes. GAA-TRE homozygote patients were confined to a wheelchair after 11.5 ± 5.9, p.R165P patients after 7.0 ± 3.6 years, and were examined within this study after 26.2 ± 4.7 years disease duration, and 40.3 ± 14.7 years. GAA-TRE homozygotes had total FARS score results of 119.3 ± 19.1 points whereas p.R165P patients had 99.1 ± 9.5.

p.R165P carriers had significantly better preserved upper limb function. Upper limb tendon reflexes were absent in all GAA-TRE homozygotes examined, but retained in all three p.R165P patients; Patient 8 also had a brisk knee jerk reflex with crossed reaction whereas ankle and knee jerks were absent in all other patients. p.R165P patients had milder dysarthria than GAA-TRE homozygotes, with close to statistically significant difference in the PATA speech test. p.R165P patients remained more independent in ADL, despite longer disease duration. We found no difference between GAA-TRE homozygotes and p.R165P heterozygotes regarding lower limb function or presence of pes cavus, scoliosis, diabetes mellitus, sensation of light touch, pinprick and temperature (Table 1).

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