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

YGLAND, EMIL; Taroni, Franco; Gellera, Cinzia; Caldarazzo, Serena; Duno, Morten; Soller, Maria; Puschmann, Andreas

Published in:
Parkinsonism & Related Disorders

DOI:
10.1016/j.parkreldis.2014.04.018

2014

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

Emil Ygland [a], Franco Taroni [b], Cinzia Gellera [b], Serena Caldarazzo [b], Morten Duno [c], Maria Soller [d], Andreas Puschmann [a,*]

[a] Department of Neurology, Skåne University Hospital, and Department for Neurology, Clinical Sciences, Lund University, Lund, Sweden
[b] Unit of Genetics of Neurodegenerative and Metabolic Disease, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[c] Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
[d] Department of Clinical Genetics, Skåne University Hospital, University and Regional Laboratories, Lund University, Lund, Sweden

***Corresponding author:** Dr. Andreas Puschmann, Department of Neurology, Skåne University Hospital, Getingevägen 4, S-221 85 Lund, Sweden. E-mail: Andreas.Puschmann@med.lu.se; telephone: 0046-46-171000; fax: 0046-46-177940

Key words: Friedreich Ataxia, Point Mutation, Disease Progression, Hemochromatosis, Genetic Counseling

Short title: Friedreich ataxia FXN p.R165P point mutation

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. 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 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 test, where the number of times probands can pronounce "pata" within a given time is assessed. 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). One p.R165P patient had been diagnosed with organic delusional syndrome at the age of 25 and one GAA-TRE homozygote reported delusions. Patient 7 had a monozygotic twin brother with very similar disease characteristics regarding symptoms and disease progression. This twin brother died suddenly at the age of 15, due to sudden cardiac death according to information from the family. Original medical records could not be retrieved. Patient 4 had the highest FARS score in this study and comorbid hemochromatosis, whereas his sibling (Patient 3) without hemochromatosis had much milder disease. The siblings had similar age of onset and age at investigation; Patient 4 had slightly shorter GAA-TRE length. p.R165P patients had significant higher mean levels of frataxin protein than GAA-TRE homozygotes. Table 1 and Figure 1 provide detailed results from the clinical examination and frataxin measurements. Table 1 includes data on the two FXN p.R165P patients reported previously [4, 9].

4. Discussion

FXN p.R165P compound heterozygotes showed less disabling FRDA-symptoms than GAA-TRE homozygotes. We found that although initial disease progression to becoming wheelchair dependent was similarly rapid in both groups, long term functional outcome was better in the p.R165P patients (Figure 1A-B). At the time of our examination, these patients had less disabling dysarthria and significantly better upper limb function, which we suggest may explain why they also were more independent in ADL than the patients with classic FRDA caused by GAA-TRE. The difference was noted although the p.R165P patients were examined after longer mean disease duration than GAA-TRE patients, and had developed symptoms at the same age or earlier. GAA-TRE in the shorter or only expanded allele had similar lengths in both groups. The patients reported here had 600-1,100 GAA-TRE, which is representative for FRDA patients reported for larger cohorts [5]. Age at onset was inversely correlated with GGA-TRE length in the homozygous patients included in this study, and was very similar to data reported previously [5].

The FXN p.R165P point mutation has previously been described in two Italian siblings only [4]. It has been suggested to be pathogenic because it was present in the two siblings but absent in 50 healthy individuals [4]. Our independent identification of this mutation in two additional Swedish families provides strong evidence for this mutation's pathogenicity. The c.494G>C variant is absent from the 1000 Genome and ESP databases. The clinical picture of the p.R165P patients in this study is very similar to the previous description of FRDA patients with this mutation (Table 1, ref. [4]). Speech and upper limb function were better preserved in all patients with this mutation as compared to GAA-TRE homozygotes. The previous report found retained reflexes, unusual in FRDA; similarly, all three p.R165P patients in this study had retained upper limb tendon reflexes and one showed marked hyperreflexia when eliciting the patellar reflex. In this study, Patient 9 was diagnosed with organic delusional syndrome.

Both p.R165P sisters previously reported had psychosis with delusions and bipolar disorder, however their sibling without FRDA had also bipolar disorder. Psychosis in FRDA patients has otherwise been described only in case reports and thus seems to be rare [10]. We suggest psychosis is more common in p.R165P associated FRDA.

All three p.R165P carriers in the present study had pale optic disks. The Italian p.R165P patients reportedly had no optic atrophy, but may not have been examined by an ophthalmologist. An overrepresentation of optic disc pallor was reported in FRDA patients with other pathogenic *FXN* truncating and missense mutations [2].A mild clinical presentation, similar to p.R165P patients, was also described for FRDA patients with a few of the other known *FXN* missense mutations. Particularly the p.D122Y and p.G130V point mutations have been associated with a disease subtype that starts early with spastic gait disturbance, but then progresses more slowly; cerebellar ataxia and dysarthria may be mild or even absent, tendon reflexes may be partially or fully retained [2]. The milder phenotype in carriers with some of the point mutations in *FXN* might be due to a subsiding rate of disease progression after leg function is affected in the initial stages of the disease. Alternatively, specific frataxin mutations may differentially affect neurons in various locations [4]. Such spatial specificity may cause more damage to neurons involved in lower limb functioning and vision, but relatively milder impairment of the neuronal substrates of speech and upper limb function. In classical FRDA, frataxin levels are very low [1, 9]. p.R165P compound heterozygotes had higher mean frataxin levels than GAA-TRE homozygotes in both the present study and previous analyses of two Italian siblings [9]. Their atypical disease phenotype may be related to altered function of mutated frataxin in these patients, combined with lower total frataxin expression. By a mechanism yet to be explored, this situation might increase the likelihood for psychiatric symptoms.

Patient 4 had the highest FARS score in this study and comorbid hemochromatosis, whereas his sibling, Patient 3, without hemochromatosis had much milder disease. The mean frataxin level from Patient 4 was similar to other GAA-TRE patients. Unfortunately, blood sampling was not possible from Patient 3. Frataxin deficiency leads to intramitochondrial iron accumulation [1]. Additive effects of comorbid FRDA and hemochromatosis on intracellular iron accumulation may explain the more severe phenotype in Patient 4. Two possible mechanisms can be proposed. High cytosolic iron levels in hemochromatosis may inhibit transport of iron out of mitochondria, aggravating mitochondrial iron accumulation. Alternatively, and perhaps more likely, free radical production in hemochromatosis may add oxidative stress affecting mitochondria.

This study has several limitations. The neurologist who performed the clinical examinations was not blinded to the patients' genotypes. However, most of the clinical items collected were of an objective nature why we consider the risk or bias to be minimal. All patients examined had relatively advanced disease and reached high scores in some of the FARS subscores. FARS has been reported to be useful in all stages of the disease, not only in patients with maintained ambulation [11]. However, due to the ceiling effect in some FARS-subscores, total FARS score may not adequately reflect clinical variability. Also, the 9-hole peg test was hard to execute for several patients. Patients had not been followed longitudinally with standardized functional assessments at regular intervals, why we used the age at wheelchair confinement as a milestone to determine the initial progression of lower-body impairment. A main limitation may be the fact that only three point mutation carriers were examined. However, this is a practical problem frequently encountered in the study of rare recessive gene mutations in genes where many pathogenic mutations have been identified in different locations throughout the gene. In such cases, each mutation may be very rare and larger case series cannot be collected [12].

In order to identify point-mutation induced FRDA patients, sequence analysis of *FXN* is indicated in patients who have a clinical picture of FRDA, even if this is milder or atypical, but are only heterozygous carriers of a GAA-TRE. Interestingly, no patients have so far been described who were homozygous for *FXN* point mutations, without GAA-TRE [1].

In summary, our findings indicate that p.R165P is associated with a less disabling disease. There is a group of FRDA patients with different point mutations who share a similar and milder clinical picture. The sudden death of the twin-brother to Patient 7 suggests that severe cardiac involvement can occur in p.R165P patients, why the clinical follow-up of patients with this mutation should include regular assessments of cardiac function, as has been recommended for all FRDA patients [1]. Comorbid FRDA and hemochromatosis may cause more severe FRDA symptoms through additive effects on iron metabolism.

Acknowledgement

We thank the patients for their participation in this study.

This project was financed by NEURO Sweden, the Greta and Johan Kock Foundation and by governmental funding for clinical research within the Swedish National Health Services (ALF-YF) and by research funds allocated to undergraduate medical research education at Lund University. FT was funded by grants from Telethon-Italia (GGP09301), the Italian Ministry of Health (RF-2009-1539841) and ERA-Net E-Rare-2 JTC2011 (Euro-SCAR).

Authors' Roles

Emil Ygland: Conception, organization and execution of the research project, writing of the first manuscript draft and review and critique of subsequent manuscript versions.

Franco Taroni: Execution of the research project (frataxin analyses), review and critique of the manuscript.

Cinzia Gellera: Execution of the research project (frataxin analyses).

Serena Caldarazzo: Execution of the research project (frataxin analyses).

Morten Duno: Execution of the research project (genetic analyses), review and critique of the manuscript.

Maria Soller: Execution of the research project (genetic analyses).

Andreas Puschmann: Conception, organization and execution of the research project, review and critique of the manuscript

Relevant conflicts of interest/financial disclosures:

None.

Full Financial Disclosures of all Authors for the Past Year:

Emil Ygland is employed by the Region Skåne hospital trust, Sweden and has received research funds allocated to undergraduate medical research education at Lund University.

Franco Taroni is employed (full staff member) by the Fondazione IRCCS Istituto Neurologico Carlo Besta (Milan, Italy) and has received research funds from Fondazione IRCCS Istituto Neurologico Carlo Besta, Telethon-Italia, Italian Ministry of Health, and EU (through the E-Rare project program)

Cinzia Gellera is employed (full staff member) by the Fondazione IRCCS Istituto Neurologico Carlo Besta (Milan, Italy) and has received research funds from Fondazione IRCCS Istituto Neurologico Carlo Besta and ARISLA Italia

Serena Caldarazzo is employed (postdoctoral research contract) by the Fondazione IRCCS Istituto Neurologico Carlo Besta (Milan, Italy)

Morten Duno: None.

Maria Soller: None.

Andreas Puschmann is employed by the Region Skåne hospital trust, Sweden, and receives Governmental funding of clinical research within the Swedish National Health Services (ALF-YF), and research funding from Greta and Johan Kocks Stiftelser and NEURO Sweden, and has received research support from The Swedish Parkinson Foundation (Parkinsonfonden) as well as The Swedish Parkinson Academy.

References

1. Pandolfo M. Friedreich ataxia. Handb Clin Neurol. 2012;103:275-94.
2. Cossee M, Durr A, Schmitt M, Dahl N, Trouillas P, Allinson P, et al. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. Ann Neurol. 1999;45:200-6.
3. Gellera C, Castellotti B, Mariotti C, Mineri R, Seveso V, Didonato S, et al. Frataxin gene point mutations in Italian Friedreich ataxia patients. Neurogenetics. 2007;8:289-99.
4. De Michele G, Filla A, Cavalcanti F, Tammaro A, Monticelli A, Pianese L, et al. Atypical Friedreich ataxia phenotype associated with a novel missense mutation in the X25 gene. Neurology. 2000;54:496-9.
5. Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, et al. The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet. 1996;59:554-60.
6. Koeppen AH. Friedreich's ataxia: pathology, pathogenesis, and molecular genetics. J Neurol Sci. 2011;303:1-12.
7. Subramony SH, May W, Lynch D, Gomez C, Fischbeck K, Hallett M, et al. Measuring Friedreich ataxia: Interrater reliability of a neurologic rating scale. Neurology. 2005;64:1261-2.
8. Ciotti P, Di Maria E, Bellone E, Ajmar F, Mandich P. Triplet repeat primed PCR (TP PCR) in molecular diagnostic testing for Friedreich ataxia. J Mol Diagn. 2004;6:285-9.
9. Sacca F, Puorro G, Antenora A, Marsili A, Denaro A, Piro R, et al. A combined nucleic acid and protein analysis in Friedreich ataxia: implications for diagnosis, pathogenesis and clinical trial design. PLoS One. 2011;6:e17627.

10. Lishman W. Organic psychiatry: the psychological consequences of cerebral disorders. Oxford: Blackwell Science; 1998.
11. Friedman LS, Farmer JM, Perlman S, Wilmot G, Gomez CM, Bushara KO, et al. Measuring the rate of progression in Friedreich ataxia: implications for clinical trial design. Mov Disord. 2010;25:426-32.
12. Puschmann A. Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. Parkinsonism Relat Disord. 2013;19:407-15.

Table and figure legends

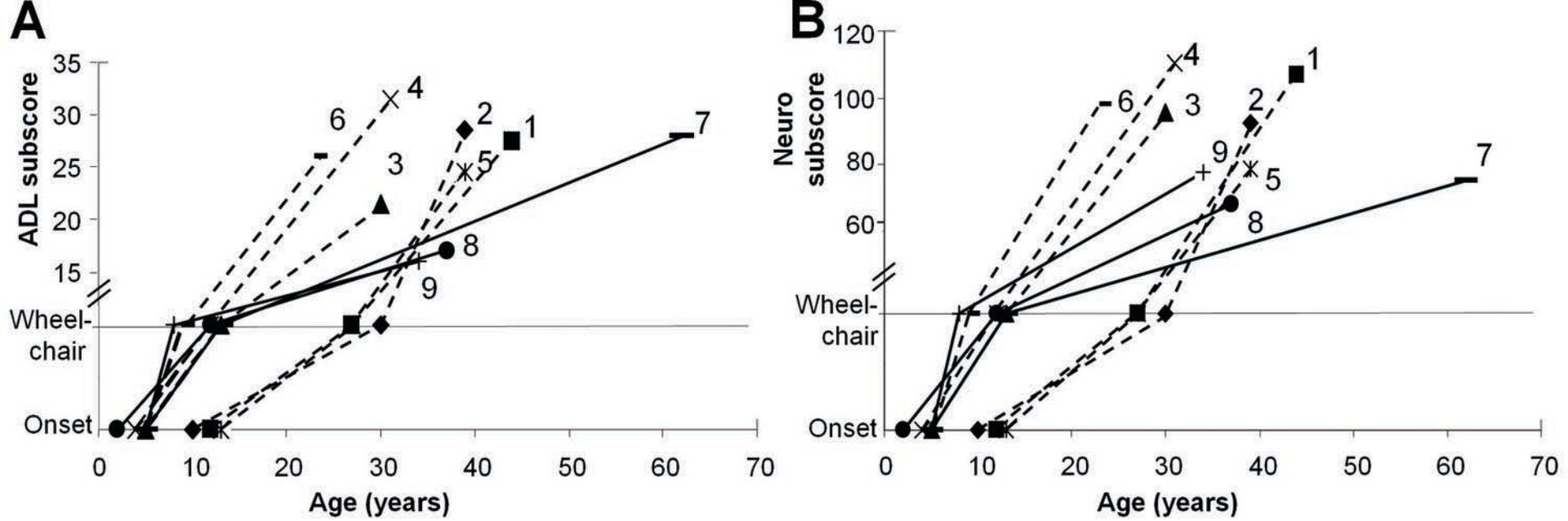
Table 1: Clinical features, genetic testing and frataxin measurement

*, ° 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 .

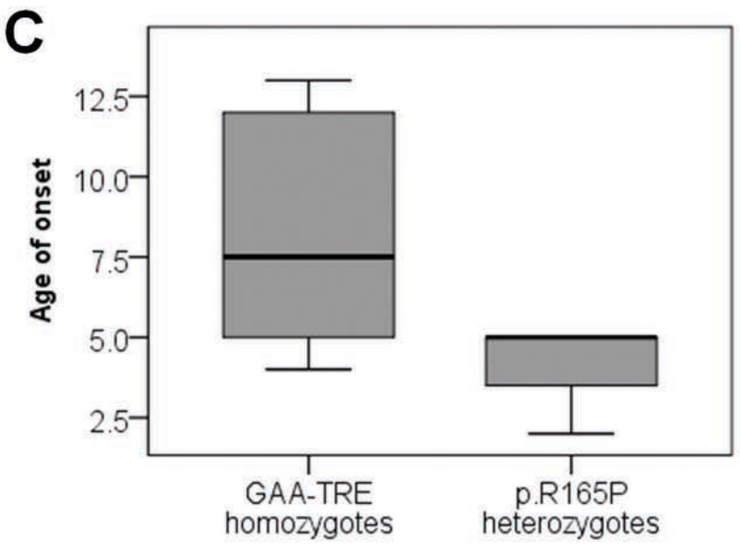
Figure 1: Clinical severity and frataxin levels correlated to genotype

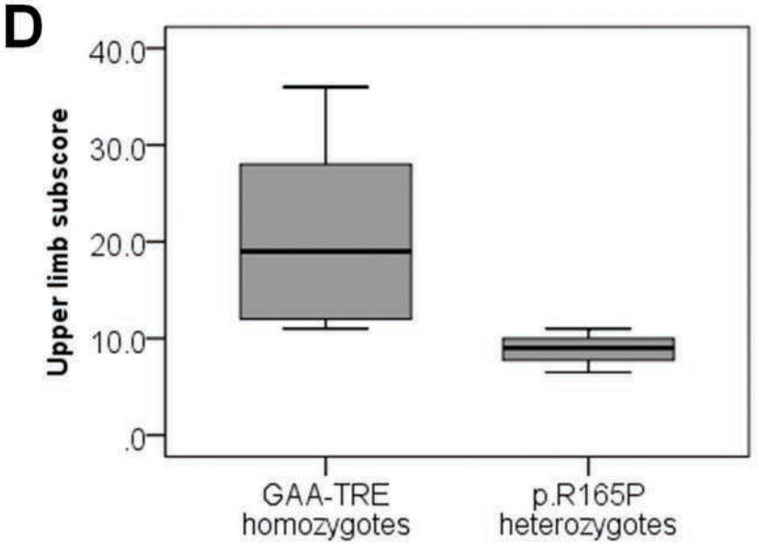
A and **B**: Visualization of initial disease progression from symptom onset to the age at wheelchair confinement. Dotted lines: GAA-TRE homozygotes. Solid lines: FXN p.R165P point mutation carriers. **A:** disease progression to FARS ADL subscore at study examination. **B:** disease progression to FARS neurological subscore at study examination. **C-E:** Comparison of clinical variables and of mean frataxin levels between GAA-TRE homozygotes and p.R165P point mutation carriers. Bold lines indicate median, blocks delineate interquartile range and thin lines entire range. Nd: not determined.

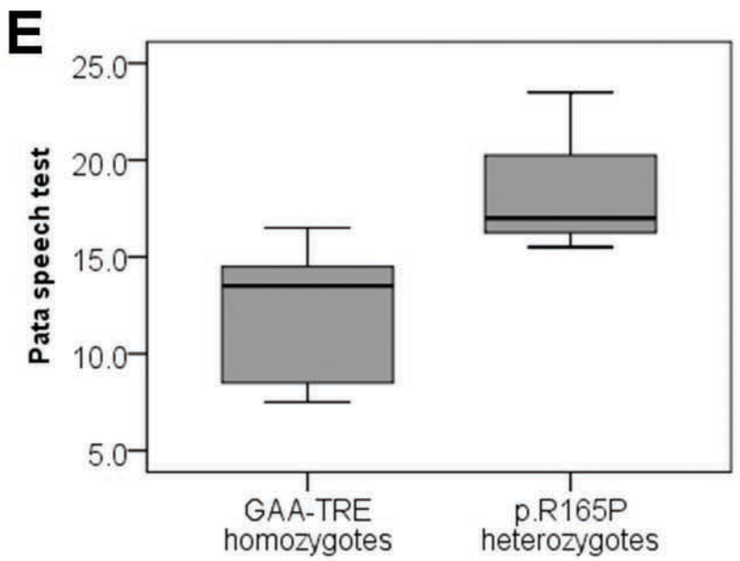
	Pt-1	Pt-2	Pt-3*	Pt-4*	Pt-5	Pt-6	Pt-7	Pt-8°	Pt-9°	*p-value*	Italian1[+] (ref. [4])	Italian2[+] (ref. [4])
***FXN* GAA-TRE Genotype**	Hom	Hom	Hom	Hom	Hom	Hom	**Het**	**Het**	**Het**		**Het**	**Het**
GAA repeats allele 1	700	1000	1000	800	600	1000	1000	1000	900	*0,396*	940	1100
GAA repeats allele 2	900	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▲**								
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 10s	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])	

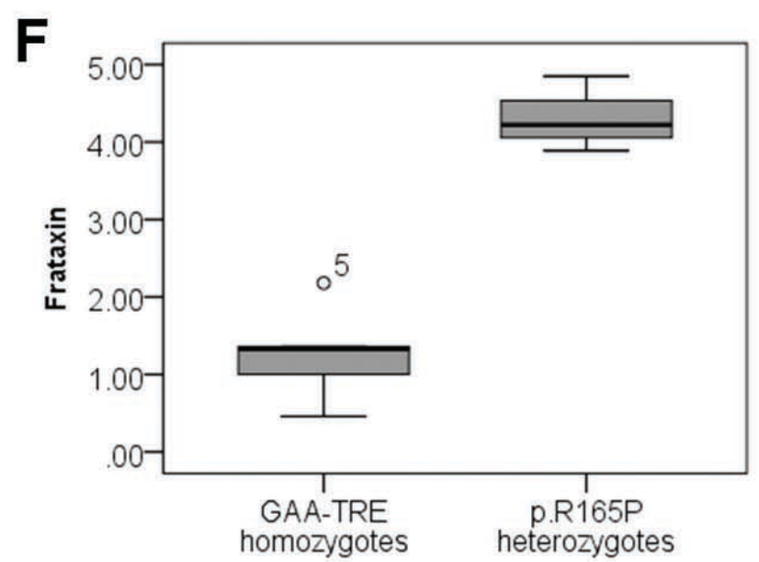

A
ADL subscore
35
30
25
20
15
Wheel-
chair
Onset
0
10
20
30
40
50
60
70
Age (years)
1
2
3
4
5
6
7
8
9

B
Neuro
subscore
120
100
80
60
Wheel-
chair
Onset
0
10
20
30
40
50
60
70
Age (years)
1
2
3
4
5
6
7
8
9


C
Age of onset
12.5
10.0
7.5
5.0
2.5
GAA-TRE
homozygotes
p.R165P
heterozygotes


D
Upper limb subscore
40.0
30.0
20.0
10.0
.0
GAA-TRE
homozygotes
p.R165P
heterozygotes


E
Pata speech test
25.0
20.0
15.0
10.0
5.0
GAA-TRE
homozygotes
p.R165P
heterozygotes


F
Frataxin
5.00
4.00
3.00
2.00
1.00
.00
5
GAA-TRE
homozygotes
p.R165P
heterozygotes