RESEARCH ARTICLE

Calcitriol Treatment Is Safe and Increases Frataxin Levels in Friedreich Ataxia Patients

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ABSTRACT: Background: Calcitriol, the active form of vitamin D (also known as 1,25-dihydroxycholecalciferol), improves the phenotype and increases frataxin levels in cell models of Friedreich ataxia (FRDA).
Objectives: Based on these results, we aimed measuring the effects of a calcitriol dose of 0.25 mcg/24h in the neurological function and frataxin levels when administered to FRDA patients for a year.
Methods: 20 FRDA patients where recluted and 15 patients completed the treatment for a year. Evaluations of neurological function changes (SARA scale, 9-HPT, 8-MWT, PATA test) and quality of life (Barthel Scale and Short Form (36) Health Survey [SF-36] quality of life questionnaire) were performed. Frataxin amounts were measured in isolated platelets obtained from these FRDA patients, from heterozygous FRDA carriers (relatives of the FA patients) and from non-heterozygous sex and age matched controls.
Results: Although the patients did not experience any observable neurological improvement, there was a statistically significant increase in frataxin levels from initial values, 5.5 to 7.0 pg/μg after 12 months. Differences in frataxin levels referred to total protein levels were observed among sex- and age-matched controls (18.1 pg/μg), relative controls (10.1 pg/μg), and FRDA patients (5.7 pg/μg). The treatment was well tolerated by most patients, and only some of them experienced minor adverse effects at the beginning of the trial.
Conclusions: Calcitriol dosage used (0.25 mcg/24 h) is safe for FRDA patients, and it increases frataxin levels. We cannot rule out that higher doses administered longer could yield neurological benefits. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: Friedreich ataxia; calcitriol; ataxia; vitamin D; frataxin

Friedreich ataxia (FRDA) is the most common autosomal recessive ataxia caused by homozygous expansion of a GAA trinucleotide repeat in *FXN* gene[1,2] or, less frequently, by a heterozygous compound mutation of GAA repeats and a point mutation in the same gene.[3,4] Clinical manifestations are progressive limb and gait ataxia due to cerebellar atrophy, axonal polyneuropathy, and dorsal cord syndrome.

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Additional features could include scoliosis, diabetes, and hypertrophic cardiomyopathy, the latter being the main cause of death in FRDA patients.[5,6] The symptoms usually start in childhood or adolescence, but they can appear later.[7,8] The mutation results in low frataxin levels, being the main cause of neurodegeneration. Although the exact function of frataxin is still a matter of debate, its deficiency has been associated with abnormal iron–sulfur cluster enzyme activities, mitochondrial iron accumulation, and impaired mitochondrial function.[9-11] Calcitriol, which is the active form of vitamin D, is synthesized inside the mitochondria by cytochrome CYP27B1, a heme-containing hydroxylase acting on the precursor form, 25-OH-vitamin D3 (or calcidiol), and rendering 1,25-(OH) 2-vitamin D3.[12] CYP27B1 function relies on the activities of ferredoxin 1 (FDX1), an Fe/S cluster-containing protein, which transfers electrons from ferredoxin reductase to the cytochrome.[13-15] Calcitriol maintains cellular redox balance and calcium signaling pathways as it contributes to regulating the expression of Nrf2 and Klotho.[16-18] Besides these functions, calcitriol exerts, to cite some examples, protective effects against glutamate-induced excitotoxicity in hippocampal cells[19] and delays amyotrophic lateral sclerosis progression by inducing axonal regeneration.[20] Moreover, this compound has been approved for the treatment of renal chronic insufficiency, hypoparathyroidism, and vitamin D–dependent or resistant rickets.[21]

A recent study performed in primary cultures of frataxin-deficient dorsal-root ganglia neurons found that FDX1 protein level was reduced by 50% of the control levels; interestingly, lymphoblastoid cell lines obtained from FRDA patients showed a 60% reduction in FDX1 levels compared to cells obtained from healthy donors.[22] Of note, supplementing dorsal-root ganglia neuronal cultures with 20 nM calcitriol restored altered parameters such as mitochondrial membrane potential, calcium homeostasis, and improved neuronal survival. Moreover, calcitriol was able to significantly increase frataxin levels in both frataxin-deficient dorsal-root ganglia neurons and lymphoblastoid cell lines.[22] Based on the already-well-known properties of calcitriol and the recent findings mentioned earlier, we performed a pilot clinical trial in which a low dose of calcitriol was administered to 15 FRDA patients for a year. In these patients we measured whether frataxin levels in platelets increased and the effect and safety of low doses of calcitriol on neurological function and quality of life.

Patients and Methods

Study Design

The study was conducted for over 12 months. It was an open, non-placebo clinical trial, and it was conducted in the Hospital Josep Trueta/Hospital Santa Caterina in Girona, Spain, between September 2021 and September 2022.

The inclusion criteria were that patients must (1) be between 16 and 65 years, (2) have genetically confirmed FRDA (two pathological GAA triplet repeats in the *FXN* gene or one pathological GAA triplet repeat and one point mutation in the *FXN* gene), and (3) have retained the ability to walk with any kind of external aid (scoring Scale for the Assessment and Rating of Ataxia [SARA] gate item ≤6). Women were asked to use an effective contraceptive method during the trial. The exclusion criteria included severe visual or auditory loss, cognitive decline, serious psychiatric illness or substance abuse, cardiac disease with heart failure or symptomatic valvular or coronary artery disease, or any neurological or any other kind of disease that could interfere with the trial. The patients could not participate in other clinical trials or be under treatment on drugs that could increase calcium concentration levels (ie, digoxin, thiazide diuretics, cholestyramine, corticoids, laxatives with magnesium, barbiturates, and antiepileptic drugs). Women should not have a positive pregnancy test or be breastfeeding. Another withdrawal criterion was being unable to follow the treatment indications and the appointments. Having hypercalcemia or elevated serum creatinine prior to the beginning of calcitriol treatment was also an exclusion criterion. Also, patients developing hypercalcemia during the trial were excluded from the study. This clinical trial included FRDA patients with different mobility degrees, and the hypercalcemia risk is higher in low-mobility subjects. The normal calcium level in our laboratory ranged from 8.6 to 10.1 mg/dL. The hypercalcemia threshold was established at the minimum hypercalcemia level (≥10 2 mg/dL) to guarantee the security of the FRDA patients who were included in the study.

Every patient was given six appointments to assess the neurological function and to control the hypercalcemia risk: at the start of the study (M0), at 15 days (M0.5), in the 4th month (M4), in the 6th month (M6), in the 8th month (M8), and in the 12th month (M12). The neurological scales used in the study were the SARA, the 9-Hole Peg Test (9-HPT), the 8 Meters Walking Test (8-MWT), and the PATA velocity test. These tests were performed at the M0, M6, and M12 appointments. The patient's quality of life and daily-life activities were also examined using the Barthel scale and the Short Form (36) Health Survey (SF-36) questionnaire at the M0 and M12 appointments.

An electrocardiogram and a blood analysis control were performed in all the appointments, except for the sixth-month appointment. Frataxin levels were tested at M0, M0.5, M4, and M12. Frataxin levels were also measured in FRDA carriers (either a heterozygous sibling or one of both parents) and age- and sex-matched

controls with no known relatives affected by FRDA. The controls must not have any neurological disease or a condition related with calcium metabolism, and must not be under treatment on calcium or vitamin D.

The trial was approved by the AEMPS (Spanish Agency for Medication and Healthcare Products) (EudraCT number: 2020-001092-32) and also the hospital Local Committee (CEIM Girona). The trial was registered in Clinicaltrial.gov (identifier NCT04801303).

Primary and Secondary Endpoints

The main objective of the trial was to evaluate the effects of calcitriol on the neurological symptoms of patients with FRDA. The SARA, 9-HPT, 8-8-MWT, and PATA velocity test (PATA test) were used to assess the changes in neurological functions.

There were three secondary objectives. The first was to assess the treatment safety and the hypercalcemia risk with low doses of calcitriol (0.25 mcg of calcitriol every 24 hours) by performing basal and control electrocardiograms and blood tests with calcium and renal function. The second was to control the effects of calcitriol on daily-life activities and the life quality of FRDA patients, which were assessed using the Barthel Index for Activities of Daily Living (Barthel scale) and the quality-of-life test (SF-36 questionnaire). The third was to measure whether there was an increase in frataxin levels during calcitriol treatment as it occurred in the cell cultures. To validate the frataxin level measurement, a blood analysis was also done in two kinds of controls for every FRDA patient: a heterozygous FRDA control and a healthy age- and sex-matched control.

Frataxin Level Measurements

Frataxin levels were measured in platelets obtained from patients, carriers, and healthy donors. Platelet samples had been previously used to evaluate frataxin expression in FRDA patients due to their easy accessibility, active mitochondria contain, and low half-life.[23-25]

Venous blood from donors (patients, carriers or controls) was drawn into two 8.5 mL ACD (Citric acid; Na-Citrate; Dextrose) Vacutainer tubes (Becton Dickinson BD #366645, Franklin Lakes, New Jersey, USA). Platelets were obtained using published procedures.[23] Briefly, tubes were centrifuged at 200*g* for 13 min at room temperature (with no brake applied). After the spin, two-thirds of the top layer (the platelet rich plasma or PRP) was transferred into a new tube using a wide orifice transfer pipette and centrifuged at 800*g* for 10 min (at room temperature with no brake applied). Supernatant was discarded and 1 ml of PBS containing 1 µg/ml Prostaglandin E1 (Sigma-Aldrich #P5515, Saint Louis, USA) was added to the pellet, without resuspending it. This preparation was centrifuged at 900*g* for 6 min, at room temperature with no brake applied. Supernatant was discarded and the pellet was resuspended in 250 µl of lysis buffer composed by 0.1 mM Tris (#1.08382), pH 6.5, 3% sodium dodecyl sulfate [SDS] (#L3771), 6.8 mM ethylenediaminetetraacetic acid [EDTA] (#E9884; all reagents from Sigma-Aldrich, St. Louis, Missouri, USA). This platelet lysate was transferred to a screw-capped 1.5 ml polypropylene tube (Corning-Falcon #352059, Corning, New York, USA). Tubes were shipped to the analytical laboratory in dry ice. Once there, they were heated at 95 °C for five minutes, sonicated for five minutes in an ultrasonic bath at room temperature and centrifuged at 10000*g* for five minutes to discard any insoluble material. Protein concentration in the supernatant was calculated on the basis of the absorbance at 280 and 260 nm (measured in an Implen NanoPhotometer N60, Munich, Germany). Samples were aliquoted and stored at −80 °C until their analysis by western blot. For this analysis, 11 µg of protein was mixed with 230 pg of pure recombinant His-tagged frataxin (#ab95502, Abcam, Cambridge, UK) used as an internal standard, 3% beta-mercaptoethanol (#M3148, Sigma-Aldrich, St. Louis, Missouri, USA) and loaded onto 12.5% polyacrylamide SDS-PAGE gels. After electrophoresis, proteins were transferred to nitrocellulose membranes (#10600093, Amersham-Cytiva, Marlborough, US) and frataxin was detected using FXN-specific (#ab219414, Abcam, Cambridge, UK) and a CF™ 640R conjugate secondary antibodies (Sigma-Aldrich, #SAB4600399, Saint Louis, USA). Images were acquired using a ChemiDoc MP system and analysed with ImageLab software (Bio-Rad, Hercules, California, USA). Frataxin was measured in samples obtained from healthy donors, carriers and patients at basal (before starting calcitriol treatment) and at 15 days, 4 months and 12 months of treatment.

Statistical Analysis

The planned number of subjects of this pilot trial was 20. A formal calculation of the sample size was not required due to the kind of study design. The general rules to determine the appropriate sample size for a pilot study have been described in the published scientific literature.[24-27] About 24 to 40 patients are recommended for a pilot study with two intervention groups. For a pilot study with only one group, the calculation can be adapted with an adjustment factor of 0.5. Therefore, in our study a minimum of 15 to 25 subjects were estimated to be needed, assuming a loss ratio of 20%.

Categorical variables were expressed as absolute and relative frequencies and continuous variables as means and standard deviations (SD), or as medians and interquartile range if the distribution was not normal. The

normality of the distribution of the variables involved was checked using the Shapiro–Wilk test.

To assess the effect of calcitriol treatment on neurological function and the evolution of frataxin levels, the Student's *t* test for pairs (normal distribution) or the Wilcoxon test (nonnormal distribution) was used. To compare frataxin levels among the different groups (healthy controls, heterozygous controls, and patients), a one-way analysis of variance (ANOVA) or Kruskal–Wallis test was performed, based on the normality of the variables. We performed a preliminary analysis to test the hypotheses of normality and homoscedasticity. The Student's *t* test or the Mann-Whitney test was used for post hoc pairwise comparisons. For multiple

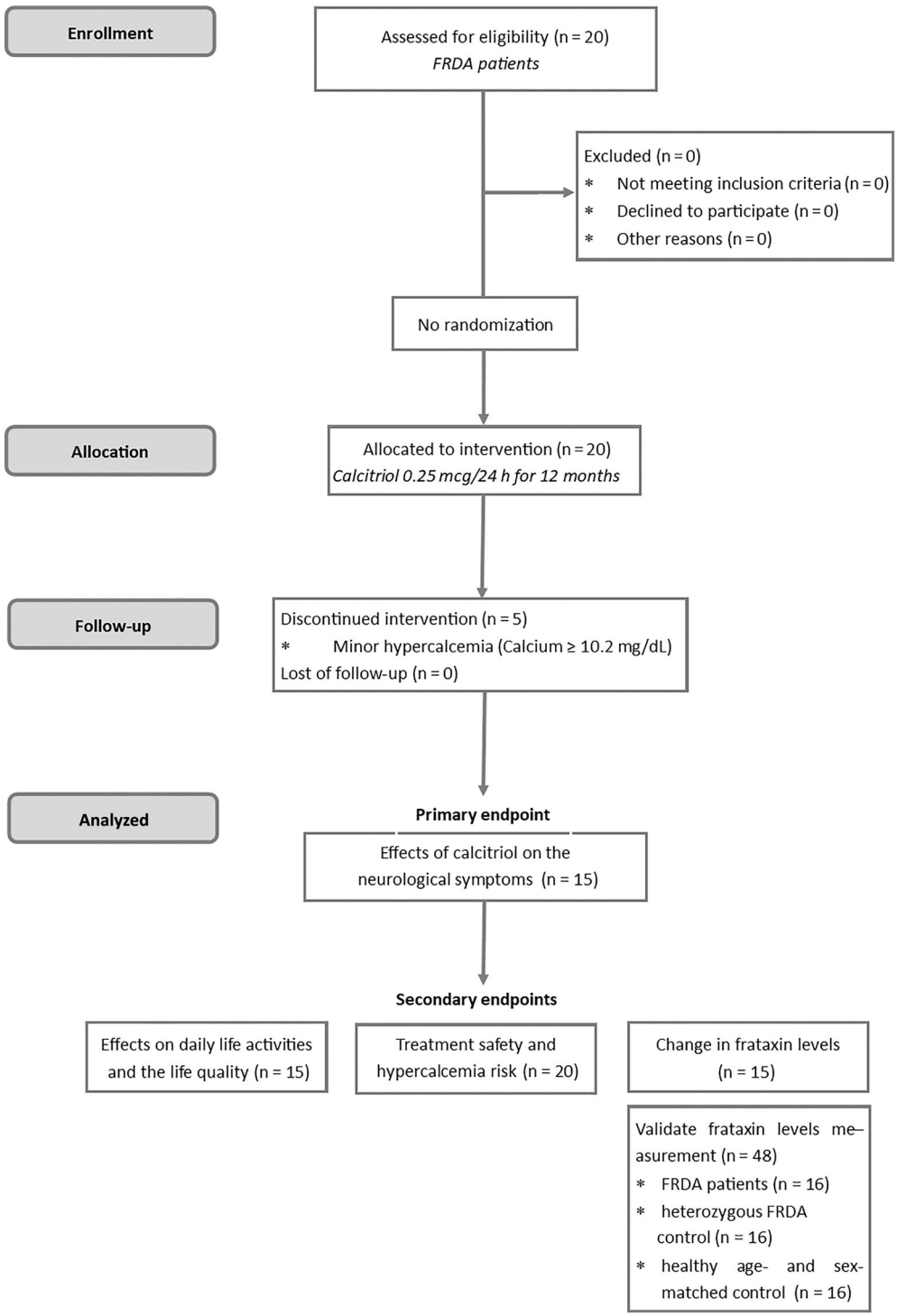

FIG. 1. CONSORT diagram of the clinical trial with calcitriol in FRDA (Friedreich ataxia) patients.

comparison, a Bonferroni correction was performed. Regression line from the fold change in frataxin expression was calculated using *GraphPad Prism* software, and the *P*-value was calculated using an F-test.

The correlation between frataxin levels and the following variables, short GAA repeat allele and time of disease progression, was performed using Spearman's test.

The analyses were performed using statistical software *R*, version 4.2.1, with the "rstatix" and "compareGroups" packages. All *P*-values were two-sided, and a *P*-value <0.05 was considered statistically significant.

Results

The initial pool of participants was 20 patients (10 female and 10 male) aged from 16 to 55 years (mean: 29.8 years) (Fig. 1). From those 20 patients, 18 were homozygous for GAA trinucleotide expansions, and 2 patients harbored a heterozygous compound mutation (one with a GAA repeat and a point mutation and the other with a GAA repeat and a deletion in exon 1). Four patients had a late-onset FRDA. The patients' characteristics are summarized in Table 1.

The 16 heterozygous carriers comprised 8 women and 8 men aged 22 to 67 years (mean: 48.6 years), and the 16 unaffected participants comprised 8 women and 8 men aged 19 to 58 years (mean: 33 years).

Five patients could not complete the 1-year treatment because of minor hypercalcemia and therefore were excluded from the study. The final sample of participants comprised 15 patients, who received a daily dose of 0.25 mcg/24 h of Rocaltrol (calcitriol) for 53 weeks.

All participants met the inclusion criteria and signed the consent form to participate in the study.

Clinical Measures

The primary objective of this research project was to evaluate the neurological changes in FRDA patients treated with low doses of calcitriol. Neurological symptoms were assessed using the SARA, the 9-HPT, the 8-MWT, and the PATA velocity test (PATA test).

There were no significant changes in the SARA scores (Table 1). The total SARA values increased an average of 0.73 points in 1 year (M0: 14.8, M12: 15.53, $P = 0.133$), indicating neurological deterioration.

Similarly, there were no observed significant changes in the 9-HPT (M0: 53.94 seconds, M12: 56.90 seconds, $P = 0.120$) and the PATA test (M0: 21.25 seconds, M12: 22.25 seconds, $P = 0.477$). The patients experienced significant worsening in the 8-MWT (M0: 22.71 seconds, M12: 36.51 seconds, $P = 0.0067$) (Table 2), which falls in line with the observed variation in the "gait item" of the SARA (M0: 4, M12: 5, $P = 0.0337$). One patient was not able to complete the 8-MWT.

The score in the Barthel scale remained steady (M0 and M12: 85%, $P = 0.144$), and the patients did not experience any significant variation in the quality-

TABLE 1 *Demographics of the 20 FRDA patients*

Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
Sex	M	F	F	F	F	M	M	M	F	M	M	F	M	M	M	F	M	F	F	F
Genetic status																				
Short GAA	40	466	>66		416	Exp	>200	>160	83	Exp	>66	Exp	Exp	>66	>700	>66	300	>66	>66	
Long GAA	500	466	>66	>66	834	Exp	>200	>160	83	Exp	>66	Exp	Exp	>66	>700	>66	300	>66	>66	650
Heterozygous compound				Yes																Yes
Age (y)																				
Disease onset	39	8	13	10	20	11	17	15	27	17	11	13	27	10	6	23	19	35	12	5
Dysarthria	49	21	16	18	28	28	No	ND	55	26	18	18	31	11	6	30	24	50	No	16
Use of a crutch	48	ND	18	ND	29	22	No	No	50	No	No	18	32	18	13	27	ND	48	No	No
Use of a walker	55	19	No	23	36	25	No	No	55	No	No	No	33	No	15	33	26	50	No	No
Disease duration (y)	16	17	5	14	19	16	1	6	32	15	8	5	7	8	12	22	15	19	6	11
Age at examination	55	29	18	24	39	27	18	21	59	32	19	18	34	18	18	45	34	54	18	16
SARA score	12	19	15.5	17.5	23.5	23	6	10	12	12	11.5	15.5	18	14	33	19.5	27.5	23	8.5	9

Abbreviations: FRDA, Friedreich ataxia; M, male; F, female; Exp, expansion; ND, no data; SARA, Scale for the Assessment and Rating of Ataxia.

TABLE 2 *SARA score evolution for 15 patients who completed 1-year treatment on calcitriol*

SARA score (n = 15)	M0	M12	*P*-value
SARA total score	14.8 (±6.8)	15.5 (±6.2)	0.133
SARA gait	4 [2, 4.5]	5 [2, 6]	0.034
SARA stance	2 [2, 3]	2 [2, 3]	0.317
SARA sitting	1 [0.5, 1]	1 [1, 1.5]	0.025
SARA speech disturbance	1 [1, 2]	1 [1, 2]	1
SARA finger chase	1.5 [1, 1.75]	1 [1, 1.5]	0.011
SARA nose-finger test	1 [0.25, 1.25]	1 [0.5, 1]	1
SARA fast alternating hand movements	1.5 (±0.8)	1.267 (±1.0.)	0.169
SARA heel-shin	2.5 [2, 3]	2.5 [2, 3]	0.305

In the M0 column are expressed the total score and individual item score before the beginning of the treatment and in the M12 the total score and individual item score after 1-year treatment. Scores are expressed as the mean and standard deviation when the variable follows a normal distribution or as the median and the interquartile range when the variable does not follow a normal distribution.

of-life questionnaire (M0: 55.99%, M12: 59.336%, $P = 0.89$) (Table 3). The only exception was in the overall health perception in the SF-36 questionnaire, in which patients were asked to rate their health on a five-point scale at the time the questionnaire was delivered, compared to 1 year prior. At the end of the study, patients perceived their health condition had significantly improved by one point, from "somewhat worst now than one year ago at the beginning of the study" to "about the same" at the end of the clinical trial (M0: 25%, M12: 50%, $P = 0.019$) (Table 4).

Side Effects

Side effects were assessed through patient interviews, electrocardiograms, and blood control analyses. Results showed that neither moderate nor severe side effects occurred during the treatment.

Some patients complained about mild side effects, mainly during the first 15 days of treatment. Four patients had minor side effects that subsided in a few days (1 had a headache, 1 had nausea, 1 had constipation, and 1 had pollakiuria). At the fourth-month appointment only 1 patient reported a mild headache, which lasted for an hour after taking the drug, but this side effect lasted only 1 month. There were no more calcitriol side effects during the rest of the trial.

As mentioned earlier, 5 patients were excluded from the trial due to mild hypercalcemia. None of these patients reported side effects. The hypercalcemia levels were always between 10.2 and 10.3 mg/dL (normal calcium levels 8.6–10.1 mg/dL), which returned to normal levels a week after the treatment was stopped.

Frataxin Quantification

To validate the frataxin quantification method, frataxin was first measured in platelets obtained from patients and from groups of age-/sex-matched carriers and healthy donors. As expected, frataxin level was significantly lower ($P < 0.001$) in patients (5.7 pg/µg, SD ± 2.5, n = 16) than in carriers (10.1 pg/µg, SD ± 3.1, n = 16) or healthy donors (18.1 pg/µg, SD ± 6.1, n = 16) (Fig. 2A), validating the accuracy of the assay.

TABLE 3 *9-HPT total scores and scores for DM and NDH, 8-MWT total scores and scores for trial 1 (8 meters going-way) and trial 2 (8 meters returning-way), and PATA test score changes for 15 patients who completed 1-year treatment with calcitriol*

9-HPT score (n = 15)	M0	M12	*P*-value
9-HPT total score (s)	53.94 [51.475, 64.255]	56.90 [51, 73.185]	0.120
9-HPT DM (s)	50.98 [42.52, 57.945]	54.45 [46.455, 62.66]	0.761
9-HPT NDM (s)	60.27 [53.085, 70.67]	61.52 [54.045, 86.355]	0.073
8-MWT score (n = 14)	**M0**	**M12**	***P*-value**
8-MWT total score (s)	22.71 [15.685, 36.335]	36.51 [18.925, 57.785]	0.007
8-MWT trial 1 (s)	12.58 [7.7125, 17.8575]	15.41 [9.375, 25.7475]	0.008
8-MWT trial 2 (s)	14.44 [9.2725, 21.3225]	21.69 [9.05, 32.0375]	0.091
PATA test score (n = 15)	**M0**	**M12**	***P*-value**
PATA test total score (number of PATA syllables)	21.25 [18.75, 23.25]	22.25 [19.875, 25]	0.477

In the M0 column is expressed the score just before starting the treatment and in the M12 column the score after 1-year treatment. Scores are expressed as the median and the interquartile range because the variables do not follow a normal distribution.
Abbreviations: 9-HPT, 9-Hole Peg Test; DM, dominant hand; NDH, nondominant hand; 8-MWT, 8 Meters Walking Test.

TABLE 4 *Barthel scale total score and SF-36 health-survey total score and individual item score variations for 15 patients who completed 1-year treatment with calcitriol*

Barthel score (n = 15)	**M0**	**M12**	***P*-value**
Barthel total score (%)	85 [80, 95]	85 [80, 90]	0.144
SF-36 score (n = 15)	**M0**	**M12**	***P*-value**
SF-36 total score (%)	55.99 (±14.70)	59.36 (±12.98)	0.890
SF-36 physical functioning (%)	35.5 (±23.39)	42 (±22.50)	0.395
SF-36 role functioning (%)	56.562 (±31.18)	55 (±26.85)	0.359
SF-36 bodily pain (%)	70.9 (±19.12)	66 (±22.59)	0.299
SF-36 general health (%)	46.2 (±20.40)	42.8 (±13.08)	0.067
SF-36 vitality (%)	51.875 (±19.77)	52.084 (±20.95)	0.609
SF-36 social functioning (%)	75 [59.375, 90.625]	62.5 [43.75, 100]	0.147
SF-36 emotional role (%)	83.33 [68.75, 100]	91.66 [79.15, 100]	0.859
SF-36 mental health (%)	69.75 (±20.61)	69.333 (±14.74)	0.807
SF-36-reported health transition (%)	25 [25, 50]	50 [25, 50]	0.020

In the M0 column are expressed the scores before the beginning of the treatment and in the M12 column the scores after 1-year treatment. Scores are expressed as the mean and the standard deviation when the variable follows a normal distribution or as the median and the interquartile range when the variable does not follow a normal distribution.

The correlation between frataxin levels with the age of symptom onset and the genetic status was also assessed (Fig. 2B,C). The frataxin levels at M0 correlate directly with the age of symptom onset (M0 Rs = 0.69, $P = 0.0007$) in the 20 patients initially enrolled, meaning that frataxin is higher in patients with a later disease onset. On the contrary, the frataxin levels at M0 correlate inversely with the short allele expansion,

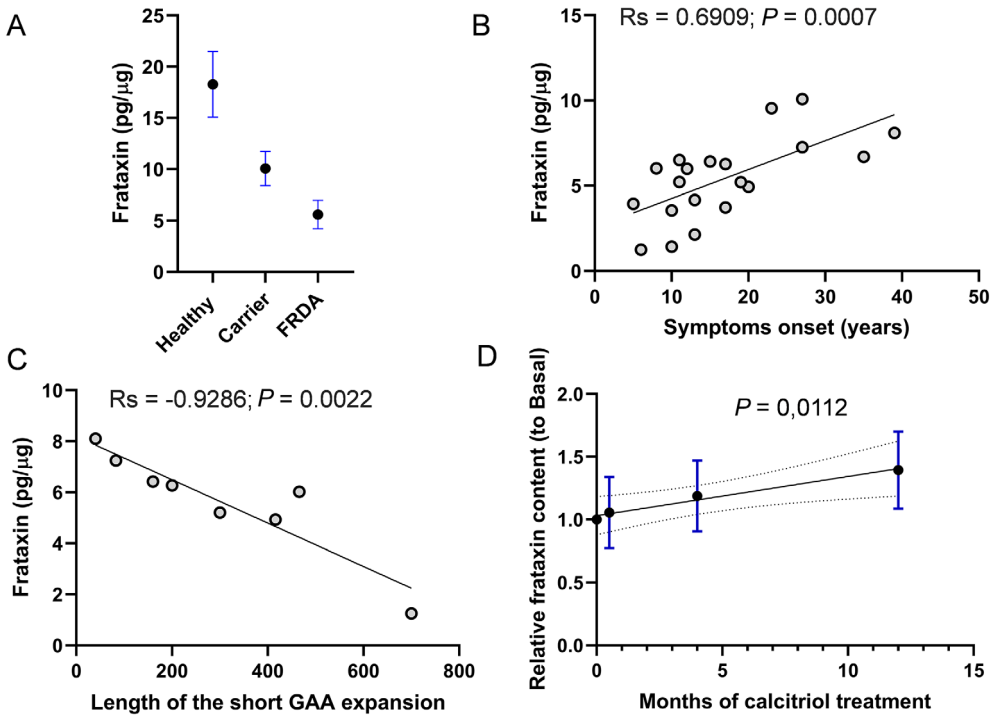

FIG. 2. Calcitriol treatment increases frataxin protein content in FRDA (Friedreich ataxia) patients' platelets. (**A**) Frataxin levels at baseline of 16 healthy, 16 carrier, and 16 FRDA individuals. Mean ± 95% confidence intervals are shown. (**B**) Correlation between frataxin levels at M0 and age of symptom onset. (**C**) Correlation between frataxin levels at M0 and short allele expansion. Linear regression and Spearman's correlation Rs and *P*-values are shown. Data in (**B**) correspond to the 20 patients who initiated the study. Data in (**C**) correspond to those patients with known length of the GAA expansion (n = 8). (**D**) Fold change in frataxin expression relative to baseline observed over 12 months of calcitriol treatment in 15 FRDA patients completing the trial. Slope from the regression line is significantly nonzero ($P = 0.0112$). Dashed lines indicate the 95% confidence bands of the regression line. [Color figure can be viewed at wileyonlinelibrary.com]

indicating that frataxin levels are lower in patients who harbor a longer GAA repeat (M0 $Rs = -0.92$, $P = 0.0022$).

These results are in line with the current knowledge of the disease, indicating that individuals with longer GAA repeats have an earlier disease onset due to lower frataxin levels.[8,37,42]

Frataxin was then measured in platelets obtained from patients before starting calcitriol treatment (M0) and at 15 days, 4 months, and 12 months of treatment. Results showed that frataxin levels increased gradually in the 15 patients on calcitriol treatment ($P = 0.0112$; Fig. 2D). There were significant differences ($P = 0.007$) in frataxin levels in the 15 patients who took calcitriol for a year, from 5.5 pg/μg (SD ± 2.6) at the beginning of the treatment to 7.0 pg/μg at the end of the study (SD ± 3.2).

Discussion

Effects of Calcitriol in Neurological Symptoms, Daily-Life Activities, and Patients' Quality of Life

Calcitriol treatment did not improve patients' neurological function, showing an increase of 0.73 points in the SARA score after 1 year of treatment. Considering that the average of the patients who participated in the clinical trial had a moderate FRDA disease stage (total SARA score = 14), it cannot be ruled out that starting the treatment at early stages of the disease could yield significant benefits in clinical progression. Besides, although at the end of the study frataxin showed a 25% increase, this was progressive over the year analyzed, and presumably a longer period would be needed to observe further frataxin increase as well as clinical improvements.

The changes observed in this cohort of patients cannot be compared with an FRDA natural history, as there have been no studies measuring changes in the SARA in the time span of 1 year. However, two studies have been carried out by the European Friedreich's Ataxia Consortium for Translational Studies, which has assessed the rate of disease progression in FRDA patients after 2 and 4 years.[30,31] It observed an average SARA progression rate of 0.77 per year for a 2-year study and 0.82 per year for a 4-year study. Because the present study assessed patients only over a year, it was not possible to calculate an annual progression rate, and thus comparison with the aforementioned studies should be made with care. That being said, the SARA scores in the present study showed that the patients' progression (0.73 per year) was similar to the progression rate calculated for the 2-year (0.77 per year) and 4-year (0.82 per year) studies. It is possible that the progression observed in the FRDA patients treated with calcitriol is similar to what would have been observed if the patients had not received calcitriol treatment, suggesting that the treatment did not cause an exacerbation of neurological deterioration in our FRDA patients.

There were changes neither in the daily-life activities nor in the general perception of the quality of life. The only exception was the patient's perceived health condition, which improved after the 1-year treatment. A reason for this change could be that patients were attending a more frequent follow-up, which could have helped them improve their health perception.

Safety and Hypercalcemia Risk with Low Doses of Calcitriol

Results confirm that calcitriol at low doses is a safe drug for FRDA patients. Only minor side effects were observed (eg, headache, nausea, constipation, and pollakiuria), which subsided in a few days or weeks. The only moderate side effect observed during the calcitriol treatment was low-level asymptomatic hypercalcemia in 25% of the patients, and levels returned to normality a week after patients stopped taking the drug. Thus, the minimal and manageable side effects, along with the reversible nature of hypercalcemia, highlight the drug's safety and tolerability.

Frataxin Levels Change during Calcitriol Treatment

One of the main findings of this work is that the levels of frataxin increase significantly during calcitriol treatment, which leads to the question of why calcitriol could lead to frataxin increase in FRDA patients. Although we cannot rule out other possibilities, one of them could be by increasing *FXN* gene expression. In support of this assumption, it is worth mentioning that in silico data on the EPDnew database (https://epd.expasy.org/epd/) reveal that within the promoter region of the frataxin gene there are five predicted vitamin D receptor (VDR)–binding motifs with a high probability score (*P*-value = 0.001). One should bear in mind that VDR can exert its function only when calcitriol is bound to it,[16] thus highlighting the role of calcitriol in triggering gene expression. However, the treatment with 0.25 mcg/24 h of calcitriol did not result in significant neurological improvement. This falls in line with results from prior studies with other treatments in FRDA patients, which have also shown an increase in frataxin levels[28,32] but have not found any change in neurological function.[33,34]

A few studies have assessed frataxin levels in FRDA patients. These studies have measured frataxin in fibroblasts, lymphocytes, muscle biopsies,[35] buccal cells,

peripheral blood,[36,37] and platelets.[29,38] These studies have shown that frataxin levels in peripheral tissues range from 30% to 40% of control levels in FRDA patients and from 60% to 80% of control levels in FRDA carriers.[39-42] In this work, we have observed similar differences between the three groups. Moreover, we have observed a correlation between frataxin levels and age of onset or length of the shorter GAA expansion, as previously reported.[42] Therefore, we can conclude that the results obtained are accurate.

Conclusions

Calcitriol, with a minimum dose of 0.25 mcg/24 h, increases frataxin levels in FRDA patients and causes minimum side effects, although the lack of significant neurological improvement highlights the intricate nature of FRDA progression.

Based on previous results with cell models of FRDA, it is possible to speculate that calcitriol may have a potential beneficial effect on mitochondrial function and on oxidative stress parameters in FRDA patients. A clinical trial with higher doses of calcitriol (eg, 0.50 mcg daily, which is still a normal dose), a larger patient cohort, and/or a more prolonged treatment can be considered for future interventions. This would allow us to confirm if the increase in frataxin level is sustained and if it leads to a decline in disease progression after 1 year of treatment.

Limitations

The study has a few limitations that should be acknowledged. First, the sample size was small. Second, the clinical trial was an open trial without a placebo. The decision to have an open trial without a placebo was precisely due to the fact that obtaining a larger sample of FRDA patients was challenging. Similarly, the exclusion of 5 patients during the clinical trial because of minimal hypercalcemia further reduced the sample size. Third, the patient sample was heterogeneous. Two patients had late-onset FRDA, and 2 patients had compound heterozygous mutations (one with a GAA repeat and a punctual mutation, and another with a GAA repeat and one deletion). The GAA length was not available from genetic studies reported by the patients, and therefore it was not possible to compare the frataxin levels with the genetic status of every FRDA patient. Further research with a larger sample and a longer follow-up period could overcome these limitations.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Campuzano V, Montermini L, Molto MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 1996;1979(271):1423–1427.
2. Pandolfo M. Molecular basis of Friedreich ataxia. Mov Disord 2001;16:815–821.
3. Cossée M, Dürr A, Schmitt M, Dahl N, et al. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. Ann Neurol 1999;45:200–206.
4. Galea CA, Huq A, Lockhart PJ. Compound heterozygous FXN mutations and clinical outcome in Friedreich ataxia. Ann Neurol 2016;79(3):485–495. https://doi.org/10.1002/ana.24595
5. Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 1981;104(3):589–620. https://doi.org/10.1093/brain/104.3.589
6. Parkinson MH, Boesch S, Nachbauer W, Mariotti C, Giunti P. Clinical features of Friedreich's ataxia: classical and atypical phenotypes. J Neurochem 2013;126(Suppl):103–117.
7. Durr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 1996;335:1169–1175.
8. Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, Cocozza S. The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet 1996;59(3):554–560.
9. Adinolfi S, Iannuzzi C, Prischi F, et al. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat Struct Mol Biol 2009;16:390–396.
10. Yoon T, Cowan JA. Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. J Biol Chem 2004;279:25943–25946.
11. Lodi R, Rajagopalan B, Blamire AM, et al. Cardiac energetics are abnormal in Friedreich ataxia patients in the absence of cardiac dysfunction and hypertrophy: an in vivo 31P magnetic resonance spectroscopy study. Cardiovasc Res 2001;52:111–119.
12. Berridge MJ. Vitamin D cell signalling in health and disease. Biochem Biophys Res Commun 2015;460:53–71.
13. Sevrioukova IF, Li H, Zhang H, Peterson JA, Poulos TL, Park H-W. Structure of a cytochrome P450-redox partner electron-transfer complex. Proc Natl Acad Sci U S A 1999;96:1863–1868.
14. Pedersen JI, Ghazarian JG, Orme-Johnson NR, DeLuca HF. Isolation of chick renal mitochondrial ferredoxin active in the 25-hydroxyvitamin D3-1alpha-hydroxylase system. J Biol Chem 1976;251:3933–3941.
15. Strushkevich N, MacKenzie F, Cherkesova T, Grabovec I, Usanov S, Park H-W. Structural basis for pregnenolone biosynthesis by the mitochondrial monooxygenase system. Proc Natl Acad Sci 2011;108:10139–10143.
16. Berridge MJ. Vitamin D, reactive oxygen species and calcium signalling in ageing and disease. Philos Trans R Soc Lond B Biol Sci 2016;371:20150434.
17. Nakai K, Fujii H, Kono K, et al. Vitamin D Activates the Nrf2-Keap1 Antioxidant Pathway and Ameliorates Nephropathy in Diabetic Rats. Am J Hypertens 2014;27:586–595.

18. Forster RE, Jurutka PW, Hsieh J-C, et al. Vitamin D receptor controls expression of the anti-aging klotho gene in mouse and human renal cells. Biochem Biophys Res Commun 2011;414:557–562.
19. Kajta M, Makarewicz D, Ziemińska E, et al. Neuroprotection by co-treatment and post-treating with calcitriol following the ischemic and excitotoxic insult in vivo and in vitro. Neurochem Int 2009;55: 265–274.
20. Karam C, Scelsa SN. Can vitamin D delay the progression of ALS? Med Hypotheses 2011;76:643–645.
21. Rodriguez M, Munoz-Castaneda JR, Almaden Y. Therapeutic use of calcitriol. Curr Vasc Pharmacol 2014;12:294–299. https://doi.org/10.2174/15701611113119990021
22. Britti E, Delaspre F, Sanz A, et al. Calcitriol increases frataxin levels and restores mitochondrial function in cell models of Friedreich ataxia. Biochem J 2021;478(1):1–20. https://doi.org/10.1042/BCJ20200331
23. Chacko BK, Kramer PA, Ravi S, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Lab Invest 2013;93:690–700.
24. Machin D, Campbell MJ, Tan SB, Tan SH. Sample Sizes for Clinical, Laboratory and Epidemiology Studies, Fourth Edition. Hoboken, New Jersey: John Wiley and Sons; 2018.
25. Whitehead AL, Julious S, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Meth Med Res 2016;25(3):1057–1073.
26. Birkett MA, Day SJ. Internal pilot studies for estimating sample size. Stat Med 1994;13:2455–2463.
27. Browne RH. On the use of a pilot study for sample size determination. Stat Med 1995;14:1933–1940.
28. Igoillo-Esteve M, Oliveira AF, Cosentino C, et al. Exenatide induces frataxin expression and improves mitochondrial function in Friedreich ataxia. JCI Insight 2020;5(2):e134221.25.
29. Guo L, Wang Q, Weng L, et al. Liquid chromatography-high resolution mass spectrometry analysis of platelet frataxin as a protein biomarker for the rare disease Friedreich's ataxia. Anal Chem 2018; 90(3):2216–2223.
30. Reetz K, Dogan I, Hilgers R-D, et al. Progression characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS): a 2 year cohort study. Lancet Neurol 2016;20:1346–1354.
31. Reetz K, Dogan I, Hilgers R-D, et al. Progression characteristics of the European Friedreich's ataxia Consortium for Translational Studies (EFACTS): a 4-year cohort study. Lancet Neurol 2021;20:362–372.
32. Boesch S, Sturm B, Hering S, Goldenberg H, Poewe W, Scheiber-Mojdehkar B. Friedreich's ataxia: clinical pilot trial with recombinant human erythropoietin. Ann Neurol 2007; 62(5):521–524.
33. Libri V, Yandim C, Athanasopoulos S, et al. Epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia: an exploratory, open-label, dose-escalation study. Lancet 2014;384(9942):504–513.
34. Mariotti C, Fancellu R, Caldarazzu S, et al. Erythropoietin in Friedreich ataxia: no effect on frataxin in a randomized controlled trial. Mov Disord 2012;27(3):446–449.
35. Campuzano V, Montermini L, Lutz Y, et al. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum Mol Genet 1997;6(11):1771–1780.
36. Nachbauer W, Wanschitz J, Steinkellner H, et al. Correlation of frataxin content in blood and skeletal muscle endorses frataxin as a biomarker in Friedreich atàxia. Mov Disord 2011;26(10):1935–1938.
37. Lazaropoulos M, Dong Y, Clark E, et al. Frataxin levels in peripheral tissue in Friedreich ataxia. Ann Clin Transl Neurol 2015;2(8): 831–842.
38. Selak MA, Lyver E, Micklow E, et al. Blood cells from Friedreich ataxia patients harbor frataxin deficiency without a loss of mitochondrial function. Mitochondrion 2011;11(2):342–350.
39. Willis JH, Isaya G, Gahk O, Capaldi RA, Marusich MF. Lateral-flow immunoassay for the frataxin protein in Friedreich's ataxia patients and carriers. Mol Genet Metab 2008;94(4):491–497.
40. Deutsch EC, Santani AB, Perlman SL, Farmer JM, Stolle CA, Marusich MF, Lynch DR. A rapid, noninvasive immunoassay for frataxin: utility in assessment of Friedreich ataxia. Mol Genet Metab 2010;101(2–3):238–245.
41. Saccà F, Marsili A, Puorro G, et al. Clinical use of frataxin measurement in a patient with a novel deletion in the FXN gene. J Neurol 2013;260:1116–1121.
42. Rojsajjakul T, Wu L, Grady CB, Hwang W-T, Mesaros C, Lynch DR, Blair IA. Liquid chromatography−mass spectrometry analysis of frataxin proteoforms in whole blood as biomarkers of the genetic disease Friedreich's ataxia. Anal Chem 2023;95(8):4251–4260.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.