PILOT STUDY ABOUT THE
EFFECTS OF CALCITRIOL TREATMENT
IN NEUROLOGICAL FUNCTION AND FRATAXIN LEVELS
PATIENTS WITH FRIEDREICH'S ATAXIA (FA-CALCITRIOL, NCT 480130303)

STUDY PROTOCOL

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1. JUSTIFICATION

Friedriech ataxia (FA) is an autosomal recessive disease whose mutation leads to a deficit of a protein called frataxin, which is responsible for the clinical disease. It is assumed that inducing an increase in the production of frataxin could reverse some of the manifestations of the disease.

Treatments with various drugs that raise frataxin levels have been tried that have either not given the expected result or have induced intolerable side effects. The IRBLleida team (Institut de Recerca Biomèdica de Lleida Fundació Dr. Pifarré), has shown that calcitriol can increase frataxin production by up to 2.5 to 3 times, a higher proportion than any of the drugs tested to date, therefore, the next step of our research would be to check the effects of this drug in patients with FA. On the other hand, calcitriol, the active form of vitamin D, is a drug with a very low rate of adverse effects and decades of use, it is therefore a drug whose tolerability is very well established. The results of this study if positive would lead to the organization of other larger-scale trials and would allow the use of an effective treatment for patients with Friedreich's ataxia.

1.1 Name and description of the investigational medicinal product.

Calcitriol is the active form of vitamin D. Although 25-hydroxycholecalciferol (25-OHD or calcifediol) is produced in the kidneys, calcitriol regulates the plasma concentration of calcium achieving an increase in calcium absorption. It is synthesized locally in the brain and other tissues.

1.2 Summary of findings from non-clinical and clinical studies relevant to the current trial.

Friedreich's ataxia (FA) is a rare inherited recessive disease described by Nikolaus Friedreich in 1863. FA is characterized by progressive gait and limb ataxia with associated weakness of the limb muscles, severe loss of deep sensation, absence of lower extremity reflexes, dysarthria, and decreased proprioception. Most patients with FA also have hypertrophic cardiomyopathy. Thickening of the wall and septum of the left ventricle can be detected with cardiac ultrasound, heart disease is usually asymptomatic in patients who have lost the ability to ambulate. In addition, the electrocardiogram is altered in almost all patients, indicating that subclinical heart disease is universal in patients with FA. In fact, cardiac dysfunction is the leading cause

of death. There is currently no cure or approved treatment for AF. The disease is caused by mutations in the FXN gene (by frataxin). Most patients have repeats of GAA triplets in the first intron of the gene, which leads to lower expression of frataxin. The function of frataxin and the consequences of its deficiency on cellular functions are not fully understood. It has been shown that frataxin is located in the mitochondria where it participates in cellular iron homeostasis, one of the consequences of the lack of frataxin being the deficiency of enzymes with iron-sulfur centers. Alterations in mitochondrial oxidative phosphorylation (OXPHOS) have also been observed in several biological models of frataxin deficiency.

In recent years, cellular models of FA have been used to analyze the ability of various drugs to reverse the consequences of frataxin deficiency. In this sense, we have observed that the active form of vitamin D (calcitriol) is able to significantly improve various phenotypes caused by FXN deficiency in cardiomyocytes and dorsal root ganglia neurons (DRGs). Moreover, calcitriol synthesis may be compromised in AF, as frataxin-deficient cells have decreased levels of CYP27B1 and Fdx1, two proteins necessary for calcitriol synthesis. This synthesis is a mitochondrial process in which the precursor form, 25-OH-vitamin D (or calcidiol), is transformed into 1,25-OH-vitamin D (calcitriol) thanks to the action of CYP27B1. This enzyme has mitochondrial localization and its activity requires the contribution of electrons by ferredoxin 1 (FDX1), a protein containing an iron/sulfur center. Calcitriol synthesis takes place primarily in the kidney, but there is evidence that calcitriol can be synthesized locally in many other tissues, including the brain. In addition, previous studies also indicate that calcitriol may increase mature frataxin levels in various cell types, including frataxin-deficient DRG neurons and lymphoblastoid cell lines derived from patients with FA (Britti et al. see Addendum 1). All these data lead us to consider that calcitriol could be an effective therapeutic agent for the treatment of FA.

There is currently no effective treatment to correct frataxin deficiency in PA, so a drug that has the ability to increase frataxin production to levels at least similar to those of heterozygotes (healthy carriers) could correct or reduce neurological and cardiological deficits in this disease. (Britti et al., Calcitriol increases frataxin levels and restores altered markers in cell models of Friedreich Ataxia. Under revision in "Cell Death & Disease").

Several investigations have at some point obtained an increase in the production of frataxin with other drugs (eg: EPO, nicotinamide, interferon gamma, resveratrol or rosuvastatin). These products achieved smaller increases in frataxin production in the laboratory than those achieved with calcitriol. Although most of them have been followed by the corresponding trials, none of them have provided results that show a significant effect on the clinical situation of patients. Others are ongoing as a study with etravirine (a drug that increases frataxin production by 50%), or are repeated with modifications due to adverse effects or ineffectiveness (nicotinamide, resveratrol).

1.3 Summary of known and potential risks and benefits.

Calcitriol is a drug on which there is vast experience, it has been used for decades in all diseases related to inability of renal synthesis of calcitriol. It has few contraindications: hypersensitivity to the active substance or to any of the excipients included. It should not be administered to patients with hypercalcemia or evidence of vitamin D toxicity. The potential adverse effects are well known (see Addendum 6). Since the most common adverse reaction is hypercalcaemia, successive monitoring of calcium levels will be performed throughout the study and patients will be monitored for symptoms related to hypercalcaemia.

1.4 Description and justification of route of administration, dose, dosage schedule and treatment period.

The ideal dose of calcitriol to raise frataxin levels in patients with Friedreich's ataxia is unknown. Levels of the precursor (25-OH-VitD, known as calcidiol) are below normal in patients with Friedreich's ataxia (Eigentler at al, 2014), but it is not possible to determine calcitriol levels in this research. Therefore, the most prudent option is, in this first study, to use a low dose of calcitriol and check if this is sufficient to raise frataxin levels, in addition to carefully and repeatedly measuring calcemia levels to control possible adverse effects of treatment. This will therefore be the first "in vivo" study in which the possible effect of calcitriol on the production of frataxin in patients with a severe deficit in its production will be analyzed and will allow to know on the one hand the tolerability and safety of the drug in these patients and if a low dose is sufficient to induce changes in neurological function. A single daily dose of 25 micrograms of calcitriol will therefore be used throughout the study treatment period (12 months).

1.5 Description of the population to be studied.

The effects of calcitriol on the neurological function of patients with FA will be studied, among this population will be selected patients who still retain ambulatory capacity, that is, patients who do not yet have a significant degree of disability.

1.6 References

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2. OBJECTIVES AND PURPOSE OF THE TRIAL.

2.1 Main objective.

To assess the effects of treatment with calcitriol on the neurological function of patients with FA. The increase in frataxin levels could modify the neurological function of patients with FA, and also the cardiac function. For this reason, a comparative study will be carried out between the neurological data obtained at the beginning, at 6 and at 12 months of treatment with calcitriol.

2.2. Secondary objectives.

a) This study also aims to control the effects that said drug may have on the calcium level of the monitored individuals. Calcitriol supplementation is considered safe since the recommended doses are well known. However, it is necessary to control the effects that such supplementation may have on the calcium level of Friedreich's ataxia patients.

b) Analyze the effect of calcitriol administration on frataxin levels and other biochemical parameters in platelets obtained from the patients under study. Frataxin is undetectable in serum or plasma, while its detection in blood is not recommended for the objectives of the project, since it is present in erythrocytes which have a high half-life (around 115 days). This could make it difficult to observe changes in frataxin content due to the treatments carried out. Therefore, its platelet content will be analyzed, since these have a half-life of 10 days. Platelets can be easily obtained from

circulating blood and contain active mitochondria that sustain most of their ATP production. Various mitochondrial proteins will also be quantified throughout the study and the effect of calcitriol on their production.

c) Effect of treatment with calcitriol on activities of daily living with the Barthel Index and quality of life using the SF36 questionnaire, at the beginning and at the end of treatment.

3. TRIAL DESIGN

Given the purpose of the trial, the neurological function of the patients will be evaluated prior to the start of treatment, at 6 months and at 12 months. The duration is determined by the characteristics of the disease, which is neurodegenerative and therefore changes in neurological function are not expected until after a long period of time. Neurological function will be assessed using standardized tests aimed at assessing cerebellar motor function, gait, dysarthria and manual dexterity. This includes the SARA scale for assessing and grading cerebellar function, the eight-meter walk test (time taken by the patient to travel 8 meters in both directions and calculating round trip and average), a dexterity test, the 9-Hole Peg test (the time spent placing nine pegs in 9 holes, with the dominant and non-dominant hand, is assessed, and the PATA speed test, for assessing dysarthria, in which the number of times is counted that the patient emits these syllables in two periods of 10 seconds, calculating the number in each series and the average of both.

All these tests will be videotaped and the files obtained will be archived on an external hard drive for conservation, evaluation, reproduction and subsequent comparison. In this first visit, measures of activities of daily living will be taken using the Barthel Index and also the SF36 questionnaire (Spanish version of SF-36v2™).

Control visits will be made at 15 days, 4, 8 and 12 months, to check calcium levels (among other analytical determinations), symptoms of adverse effects, and ECG. Communication with patients will be facilitated with various methods (telephone, email) to assist them in any eventuality.

In these same visits and with the same extraction, samples will be obtained to determine frataxin levels. Frataxin will be quantified by western blot of platelet lysate.

In the same lysate, various mitochondrial proteins will be quantified by SRM-based mass spectrometry.

3.1 Specific description of the main and secondary variables.

As primary variables, the parameters obtained from the aforementioned neurological function tests will be specifically evaluated: SARA scale values, time to travel 8 meters, time to complete the 9-hole test, number of syllables of the dysarthria test.

Secondary variables will be evaluated: calcium levels and kidney function, frataxin levels, Barthel index values and SF36 quality of life questionnaire.

3.2 Description of the type of trial.

The present study is a pilot study aimed at verifying the effects of treatment with calcitriol on neurological function, calcium levels, frataxin levels, and quality of life in patients with FA.

This is an open study, all patients will receive treatment, and the results obtained before and after treatment will be compared, using parameterized tests and video filming.

3.3 Description of trial treatment.

The drug used, calcitriol, is on the market under the registered trademark Rocaltrol©. The lowest dose will be used: 25 micrograms, orally once a day for one year (Rocaltrol 0.25 micrograms soft capsules, presented in a PVC blister containing 20 capsules).

3.4 Expected duration of participation and all trial periods.

The study will continue over one year.

It will begin with a recruitment period, a screening period that includes the evaluation of inclusion and exclusion criteria, the assessment of neurological function, a determination of calcium, vitamin D, kidney function, albumin, proteins, sodium, potassium, phosphate. and pregnancy test in pre-menopausal women, and extraction for basal determination of frataxin, evaluation of the quality of life questionnaire. An ECG will also be performed.

The treatment period will begin from here.

Fifteen days after the start of this, the next visit will be carried out with the first extraction for determination of frataxin, calcium, kidney function, sodium, potassium, phosphate, ECG and assessment of possible symptoms related to possible adverse effects of the treatment.

This procedure will be repeated at 4, 8 and 12 months with determination of frataxin, calcium, albumin, kidney function, ECG and assessment of possible symptoms related to possible adverse effects of the treatment (Adendum 6).

After 6 months of treatment, the first assessment of neurological function will be carried out.

At the last visit of the 12 months, a new assessment of neurological function, Barthel index and final quality of life questionnaire will also be carried out (Adendum 5.1, Addendum 5.2, Addendum 5.3 and Addendum 5.4).

Post-study follow-up, the data obtained will be analyzed and its statistical evaluation will be carried out. Patients who wish to continue treatment will be allowed to continue, at least until the first results of the comparison of the initial neurological function with the second and final ones are obtained. If the results obtained are positive, treatment will be maintained in these patients with regular analytical controls. If the result does not demonstrate a statistically significant result, this will be discontinued in all patients. Detection of hypercalcemia will lead to immediate withdrawal from the study.

If possible, patients who show improvement will be followed up in our Ataxia Unit.

3.5 Prescription and dispensing of treatment. Accounting for study medication

The investigational medication is marketed under the name ROCALTROL 0.25 MCG 20 capsules.

Conservation: at room temperature (20–25ºC).

If a patient agrees to participate in the study, the research team will contact the Pharmacy Service to inform of this inclusion.

The Neurology service will prescribe the medication (Calcitriol 0.25 mcg capsules (ROCALTROL®) through a prescription for specially prescribed medications, a model for internal use in the hospital.

Information to be completed by the research team:

- Patient identifying data: patient name and surname, medical record number and assigned patient number
- Prescribing doctor: Name, registered number, signature and date
- Code and/or abbreviated name of the study protocol
- Visit number within the study calendar

The Pharmacy service will dispense to the patient based on said prescription and the period that must be covered until the next study visit (4 months, equivalent to 120-123 days).

The study medication will be properly relabeled in the Pharmacy service as a clinical trial sample. A label will be used for the medication carton with the following information:

Active principle
Pharmaceutical Specialty
Route of administration
Batch and expiration
Name and Number of the patient in the study

and another for the dispensing bag, according to the following model:

STUDY: FA-CALCITRIOL

Promoter: Joint IAS/ICS Neurology Service

Address: Dr. Castany, s/n 17190 SALT Tel. 972 182500

Researcher: Dr. Berta Alemany Perna

Patient number ....

Patient Name.....

_____ packaging ROCALTROL 0.025 mcg 20 Capsules

Store at room temperature.
Protect from sunlight.
KEEP OUT OF THE REACH OF CHILDREN

Sample for clinical research

From the IAS Pharmacy Service, control of the dispensing and return of all dispensed containers will be carried out, counting the medication returned by the patient after

each visit, without prejudice to the fact that said count is also carried out by the research team. , so that there can be a double check on adherence to treatment, by the research team and by the Pharmacy service.
The returned medication will be kept in the Pharmacy service itself, for final counting by the research team. The accounting of medication as well as patient dispensations and returns will be carried out through the Fundanet application.
Expiration control of the study medication will be carried out.

SCHEDULE

TASKS	MES														
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
RECLUTAMIENTO	X	X													
TREATMENT			X	X	X	X	X	X	X	X	X	X	X	X	X
FRATAXIN LEVELS			X	X Día 15			X				X				X
CALCEMIA MONITORING			X	X			X				X				X
ECG			X	X			X				X				X
NEUROLOGICAL AVALUATION			X						X						X

4. SELECTION AND WITHDRAWAL OF SUBJECTS

Patient recruitment will be proposed to patients with FA controlled in the Ataxia Unit of the joint Neurology service of the Dr. Josep Trueta University Hospital and the Santa Caterina Hospital who meet the inclusion criteria and lack exclusion criteria and wish to participate in the study. To reach the desired “n” (20 patients), an invitation to participate will be made through a press release on the websites and publications of

FEDAES (Federation of Ataxias of Spain) and ACAH (Catalan Association of Hereditary Ataxias) (Adendum 2). This invitation will include: the title of the study, description of the objectives, duration of the research, contact email address from which more detailed information will be sent to those interested in participating. The information will be expanded by letter/mail (Adendum 3) to those who request to participate and they will be scheduled for the screening visit.

4.1 Inclusion criteria.

In the screening visit, the inclusion criteria will be assessed:

Patients with FA confirmed by molecular genetic studies, who are between 16 and 65 years old and maintain the ability to walk, even with external support of any type.

4.2 Exclusion criteria.

During the screening visit, a first blood draw will be carried out to assess frataxin levels and a determination of calcium, albumin, glomerular function, vitamin D, sodium, potassium and phosphorus. Patients with hypercalcemia, or impaired glomerular function or elevated creatinine levels will be excluded. A pregnancy test will be carried out in pre-menopausal women.

More specifically, patients affected by some other disease that interferes with the patient's assessment, whether medical or neurological, will be excluded. Patients with severe visual or auditory deficits will also be excluded, as well as those with cognitive impairment (dementia or cerebellar cognitive affective syndrome), or mentally or legally incapacitated subjects. Those affected by serious psychiatric illnesses, or those with a history of substance abuse within the 6 months prior to screening, will also not be admitted. Avoid including patients who have suffered severe allergies to medications, those who present heart disease of a certain entity (detailed in Addendum 4.1 and Addendum 4.2) and those who have taken investigational drugs in the 30 days prior to inclusion. Patients under treatment with digoxin, thiazide diuretics, cholestyramine, corticosteroids, laxatives with magnesium, barbiturates and antiepileptics, calcium or vitamin D will also not be accepted for the study.

Pregnant or breastfeeding women will not be admitted.

Patients who meet the inclusion criteria and those in whom no exclusion criteria are detected will be admitted, then the patient will sign the informed consent.

Instructions will be given for the dosage of treatment with the drug calcitriol (Rocaltrol© 0.25mcgs).

The patient will be provided with the schedule of visits and appointments for the entire study.

4.3. Participation of heterozygotes and healthy subjects

In order to verify the effectiveness of treatment with calcitriol, it is essential to have the levels of frataxin in two situations, firstly, that of healthy controls who do not carry the AF mutation, and secondly, the levels presented by heterozygous individuals. . It is known that the latter have intermediate figures in frataxin levels: higher than those of affected individuals, but lower than those of the population that does not carry the mutation. Heterozygotes are free of FA symptoms and do not present any alterations; in them, although reduced, the amount of protein is sufficient to keep them asymptomatic throughout their lives. It would therefore be a hypothetical ideal level to approach in order to reach a situation close to clinical normality.

Therefore, we will proceed to draw blood to determine levels of frataxin and mitochondrial proteins in these people. Determinations will therefore be carried out either in parents of those affected or in known heterozygous siblings.

The same determinations will be carried out on healthy controls, who, in theory, do not carry the mutation (the conditions of the study do not allow us to verify the presence of the mutation in this group). The prevalence of the FA mutation in the general population is 1/100).

In these determinations, the levels of frataxin and mitochondrial proteins will be assessed. The data obtained will allow a comparative study between the values of healthy individuals, heterozygotes, and those affected by FA, and in these their basal levels and those obtained during treatment with calcitriol.

Therefore, the following will be chosen for each patient included in the study:

- A healthy control heterozygous for FA, choosing the volunteer parent who attends the screening visit or, if possible, a heterozygous sibling. It will be verified that the heterozygous healthy control does not have any neurological disease, nor any disease related to the metabolism of calcium or Vitamin D and that it is not being treated with Calcium or Vitamin D.

- A non-familial healthy control and therefore supposedly not heterozygous for FA (n= 20). You must not have any neurological disease, any disease related to calcium or vitamin D metabolism and you must not be under treatment with calcium or vitamin D.

Once the healthy controls have been chosen, blood will be drawn, after signing the specific informed consent for this type of collaboration.

4.4. Subject withdrawal criteria.

Will be removed from the study:

to. Any patient who presents hypercalcemia during the controls carried out throughout the trial.

b. Subjects who present with ECG abnormalities indicative of possible hypercalcemia or arrhythmias during treatment with calcitriol.

c. Those who repeatedly skip taking treatment.

d. Those affected who present a new disease or require supplementary treatment that may make the interpretation of the results difficult.

Patients with hypercalcemia and those with ECG abnormalities will continue to be monitored until resolution of symptoms or recorded abnormalities (see Addendum 6).

Telephone or email contact will also be maintained with the patients in section d), although they will not re-enter the study.

Controls with those in section c) will not be followed.

A notification of a serious adverse effect will be made if this occurs (see Addendum 7).

The date of withdrawal from the study and the reason for this, as well as the evolution followed by the patient, will be noted in the CRD.

Subjects who drop out of the study will not be replaced by others as long as the “n” of the study remains between 15 and 20.

5. SUBJECTS TREATMENT

5.1 Trial treatment.

The only treatment in the study is calcitriol (Rocaltrol©) at 0.25 micrograms/day orally.

5.2 Allowed medications.

Patients under treatment with idebenone or coenzyme Q10 for years.

De novo inclusion of idebenone will only be permitted in patients who develop signs of cardiomyopathy during the study.

5.3 Not allowed medications.

They will not be able to enter the study, but patients who require treatment with digoxin, amiodarone or thiazide diuretics, corticosteroids, cholestyramine, magnesium laxatives, barbiturates and antiepileptics, calcium or vitamin D will not be included or withdrawn.

Nor those who continue treatment with other tested drugs other than idebenone, which include: diferiprone, EPO, resveratrol, nicotinamide, etravirine, rosuvastatin, interferon of any type, EPI743 (vatiquinone), CTI-1601, RT001 (stabilized linoleic acid), pioglitazone, leriglitazone (MIN-102), epicatechin, ovameloxolone, VP 20629 (propionic acid), varenicline, ginko biloba, alpha-tocopheryl-quinone.

If a patient has followed a treatment in a clinical trial with any of these drugs, they will be allowed to enter the study only after a washout period of 6 months.

Other drugs will be admitted as long as these drugs do not interfere with the assessment of the results or the neurological function of the patients or modify the calcium levels directly or indirectly.

5.4 Hypercalcemia treatment

The risk of hypercalcemia with Calcitriol 0.25mcg/24h is very low. It increases in patients in situations of prolonged immobilization, treatment with digoxin and thiazide diuretics among others, these patients being already excluded from the study.

The treatment of hypercalcemia will be based on the severity of the hypercalcemia (see Addendum 8):

- Mild hypercalcemia (Calcium 10.2-12mg/dl): withdrawal of treatment, abundant water intake and analytical control after 7 days.
- Asymptomatic moderate hypercalcemia (Calcium 12-14mg/dl): similar to the treatment of mild hypercalcemia, possibility of treatment with Prednisone 20-40mg/24h
- Moderate symptomatic hypercalcemia (Calcium 12-14mg/dl): withdrawal of treatment. Hospital admission for hydration with physiological saline, treatment with Pamidronate (a bisphosphonate), analytical control at 12-24 hours.

- Severe symptomatic hypercalcemia (Calcium >14mg/dl): withdrawal of treatment. Hospital admission for hydration with physiological saline, treatment with Zolendronate (a bisphosphonate), evaluate treatment with subcutaneous Calcitonin and Prednisone, analytical control at 12-24 hours.

5. 5 Procedures to monitor subject compliance. Patient's diary.

At each visit the patient will be given a "Patient's Diary" in which they will be asked to write down the date, check a box if they take the treatment, and write a note if any new symptoms appear or any abnormality occurs related to taking the treatment. The patient will provide this list at each visit (see Addendum 9).

6. ASSESSMENT OF EFFICACY

6.1 Specification of efficacy variables.

The efficacy variables for improving neurological function would be the initial, intermediate and final comparison of:

- Partial and total values of the SARA scale (Scale for the assessment and grading of cerebellar function in ataxias).

-SCAFI index (composite Z-score) result of the transformation of the results of:

.. Average time to travel 8 meters round trip.

.. Time spent completing the 9-hole test with dominant and non-dominant hands.

.. Number of syllables PA-TA repeated in two series of 10 seconds.

- Total partial scores of the Barthel Index.
- Total score and each dimension of the SF36 quality scale.
- Increase in frataxin levels compared to baseline measurement figures.
- Significant change in the values of the mitochondrial proteins studied.

6.2 Methods and schedule for evaluation, registration and analysis of efficacy parameters.

- Evaluations of efficacy parameters will be carried out at the beginning, after 6 months and after one year of treatment for neurological function. The quality of life questionnaire and the Barthel index will be administered at the screening visit and after one year of treatment.
- The registration of these data will be carried out in the corresponding CRD.

- The parameters will be assessed and compared at the end of the study.

7. SAFETY ASSESSMENT

7.1 Specifying security variables.

- The main safety variable is the determination of corrected calcium levels, measured simultaneously with albumin and proteins at all visits.
- Another variable is the anomalies of the ECG that will be carried out and checked at each visit.
- The deterioration of neurological function at the 6-month visit, or at the time it occurs and can be attributed to the treatment, with no other cause found to justify it (fever, intercurrent illness, etc.).

7.2 Methods and schedule for evaluation, recording and analysis of security parameters.

En el control visitado en 15 days, 4, 8 y 12 months, the following must be evaluated:

. Calcium, albumin, kidney function, sodium, potasium and phosphate levels.

. ECG.

The data obtained will be registered in the corresponding CRD.

The normality or not of the corrected calcemia parameters will be assessed. Hypercalcemia, depending on its severity, can cause increased contractility, arrhythmias, and atrioventricular block. For this reason, the typical ECG abnormalities in hypercalcemia will be especially evaluated: shortening of the QT, especially shortened QTc (defined as the period from the beginning of the QRS to the end of the T wave) and other alterations: increased duration of the wave T (severe hypercalcemia), increased amplitude of the QRS complex, elevation of the ST segment, arrhythmias.

All these parameters will be assessed in each ECG compared with the baseline recording.

7.3 Reporting of adverse events and intercurrent illnesses and registration of adverse reactions.

Adverse events and intercurrent illnesses will be recorded in the corresponding section of the CRD.

Serious adverse events will be especially noted on a separate sheet (Adendum 7).

Each serious adverse event will be reported to the corresponding authorities.

7.4 Type and duration of follow-up after adverse events (AEs).

- Patients with adverse effects will be followed until resolution. If the AE is mild and does not require attention by the researchers, follow-up will be carried out remotely. Otherwise, the patient will be examined at our center in an out-of-program visit.
- If it is a serious adverse effect, the patient will be followed until it is resolved, the necessary means will be put in place to solve it. Subsequently, the serious adverse effect form will be filled out and said SAE will be communicated through the Spanish Pharmacovigilance System for medicines for Human Use: www.notifyam.es.

8. DATABASE AND REGISTRATION

8.1 Creating and implementing a database

An external group will be hired to create and implement a database.

8.2 **Database features**.

It contains all the fields contemplated in the CRD in the specific format for each one according to the type of data obtained.

Allow online data entry.

Access by multiple users as long as a username and password are available.

Access will be possible both from the computers of the participating hospitals and from an external computer.

You will have appropriate systems in place to maintain data integrity.

Backups can be made repeatedly to avoid data loss.

The data from the fields can be obtained to be analyzed by the statistical program IBM SPSS26 Statistics and R software.

Hosting of said database will be requested for a period of more than one year.

8.3 Data registration, authorizations and database control

. Access with username and password.

. Only the following will have access to the database: the principal investigator, authorized study collaborators, the person carrying out the statistical studies, the study coordinator in charge of monitoring the study.

. The data will be entered by: researchers and study coordinator.

. The data will be controlled and corrected if necessary by the study coordinator.

- The company hired to prepare it will take care of maintenance, security and that backup copies are made regularly.

9. STATISTICS

9.1 Sample size for a pilot study.

Number of subjects planned: 20

Given the type of study design, a formal calculation of the sample size is not required. However, general rules for determining the appropriate sample size for a pilot study have been described in the scientific literature. For two-arm studies, it is recommended to recruit between 24-40 patients in total. For single-group studies, the calculation can be adapted by applying an adjustment factor of 0.5. Therefore, in our study it is estimated that it will be necessary to recruit a minimum of between 15-25 patients, assuming a loss rate of 20%.

9.2 Statistics methods

A descriptive analysis will be carried out using the usual statistics: mean and standard deviation, or median and interquartile range for quantitative variables with non-normal distribution. The normality of the data will be checked using the Shapiro-Wils test and/or Q-Q plots. The qualitative variables will be described according to the number and percentage of subjects in each category.

The effect of treatment with calcitriol on neurological function will be evaluated using a mixed linear model with the levels of neurological function tests as a dependent variable and the measurement time (screening, 6-month control and final control) as an independent variable included as an effect. random. To take into account possible confounding effects, covariates will be introduced as fixed effects. The same analysis will be repeated for frataxin levels and other biochemical parameters in platelets.

Comparison of baseline frataxin levels between healthy controls and patients with ataxia will be carried out using the Student's t test for independent data or the Mann-Whitney U test, if the data do not meet the assumption of normality. The relationship between the increase in frataxin production and clinical improvement will be

evaluated using a logistic regression model where the dependent variable will be defined dichotomously by jointly evaluating frataxin levels against increases of more than one point on the scale. SARA, two or more points of the SCAFI composite index (which would add the results of the 8 m walk test, time taken from the 9-HPT, the number of syllables from the PATA test), the values of the Barthel Index in 10% and the SF36 questionnaire by 20% on the values obtained in the first evaluation.

The results will be expressed in odds ratio (OR).

The effect of treatment with calcitriol on the quality of life of the patients at the beginning and end of treatment will be evaluated through the t-student test for paired data or the Wilcoxon test, if normality is not met in the distribution of the data.

To assess toxicities, a table of frequencies of occurrences will be created.

Statistical analysis will be performed with o IBM SPSS Statistics version 26 and R software.

10. ETHICAL CONSIDERATIONS

Given that FA is a disabling and progressive disease that shortens the patient's life expectancy and lacks treatment, it is justified to follow the line established by basic research related to FA that identifies possible therapeutic solutions to improve the situation of these patients. especially if the drug used has a good margin of tolerability, as is the case in this case.

The patient's freedom will be respected as established in the Declaration of Helsinki and is reflected in the researchers' commitment to respect the patient's decisions in the informed consent (Adendum 10.1 and 10.2). Healthy controls will also sign the corresponding informed consent (Adendum 10.3 and 10.4).

The health of the individual will be respected through the repeated controls to which they will be subjected to guarantee this through the necessary tests, measurement of calcium, ECG, contact facilities.

11. FUNDING

11.1 Provided budget.

This project will be submitted to the competition for a FEDAES Scholarship (Federation of Ataxias of Spain) which, if accepted, would provide €6,000 for the study.

The Ataxia Unit would contribute, if necessary, up to €1,500 to carry out this study.

11.2 Trial expenses.

. **Personal:**

The salary of the IRBLLeida staff is borne by said institution.

The salary of the nurse, the study monitor, and the statistics are provided by the Neurodegeneration and Neuroinflammation Research Group of the IdIBGi.

The statistical analysis, with an estimated time of 40 hours, is calculated at €1,416.

. Consumable material for 140 extractions.

. Analytical shipping expenses to IRBLleida.

. Analytical (calcemia and others) total: 100 determinations.

. Determination of frataxin in platelets (run by IRBLleida): 140 determinations

. Database creation and implementation price: €1950.

. Medical-health civil liability insurance (will be requested if the study is approved by the CEIC and the scholarship is obtained): €1,500 (budget)

*The necessary material is available to carry out the neurological tests (9-HPT).

- The requirements of current regulations in Spain will be taken into account.

PROJECT FUNDING			
CONCEPT	**CONTRIBUTIONS**	**EXPENSES**	**SUBTOTALS**
FEDAESA CONTRIBUTIONS	6.000€		+6.000
ATAXIA UNIT	1.600€		+1.600
PERSONAL		1.416€	
FUNGIBLE MATERIAL		429€	
SENDINGS		250€	

BLOOD ANALYSIS		1.741€	
DATABASE		1.950€	
INSURANCE		1.500€	
SUBTOTALS		7.536€	7.600€
		TOTAL	

12. PUBLICATION POLICY

Researchers commit to:

. Publish the results of this trial, regardless of their positivity or negativity, in a medical journal related to the topic studied.

. Present the results to national and international conferences interested in Friedreich's ataxia.

. Study authorship will include the study investigators (PI and collaborating investigators).

. It will be mentioned in all cases that the financing of the study has been granted through a scholarship from FEDAES (Federation of Ataxias of Spain), and ACAH (Associació Catalana d'Atàxies Hereditaries) will be thanked for its collaboration.