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Long-term treatment with thiamine as possible medical therapy for Friedreich ataxia

Antonio Costantini¹, Tiziana Laureti², Maria Immacolata Pala¹, Marco Colangeli³, Simona Cavalieri⁴, Elisa Pozzi⁴, Alfredo Brusco^{4,5}, Sandro Salvarani⁶, Carlo Serrati⁷, Roberto Fancellu^{6,7,*}

¹*Department of Neurological Rehabilitation, “Villa Immacolata” Clinic, Strada Sammartinese 65A, 01100 Viterbo, Italy*

²*Department of Economics and Management, University of Tuscia, Via del Paradiso 47, 01100 Viterbo, Italy*

³*University Studies Abroad Consortium, University of Tuscia, Via Santa Maria in Gradi 4, 01100 Viterbo, Italy*

⁴*Department of Medical Sciences, University of Turin, 10126 Turin, Italy*

⁵*Medical Genetics Unit, Città della Salute e della Scienza University Hospital, 10126 Turin, Italy*

⁶*Unit of Neurology, ASL3 Villa Scassi Hospital, Corso O. Scassi 1, 16149 Genoa, Italy*

⁷*Unit of Neurology, IRCCS San Martino University Hospital IST, Largo Rosanna Benzi 10, 16132 Genoa, Italy*

***Corresponding author:**

Roberto Fancellu

Unit of Neurology, IRCCS San Martino University Hospital IST, Largo Rosanna Benzi 10, 16132 Genoa, Italy

Tel: +39 010 5555673. Fax: +39 010 5556693

E-mail address: roberto.fancellu@hsanmartino.it

Abstract

Thiamine (vitamin B1) is a cofactor of fundamental enzymes of cell energetic metabolism; its deficiency causes disorders affecting both the peripheral and central nervous system. Previous studies reported low thiamine levels in cerebrospinal fluid and pyruvate-dehydrogenase dysfunction in Friedreich ataxia (FRDA). We investigated the effect of long-term treatment with thiamine in FRDA, evaluating changes in neurological symptoms, echocardiographic parameters, and plasma *FXN* mRNA levels. Thirty-four consecutive FRDA patients have been continuously treated with intramuscular thiamine 100 mg twice a week and have been assessed with the Scale for the Assessment and Rating of Ataxia (SARA) at baseline, after one month, and then every three months during treatment. Thiamine administration ranged from 80 to 930 days and was effective in improving total SARA scores from 26.6 ± 7.7 to 21.5 ± 6.2 ($p < 0.02$). Moreover, deep tendon reflexes reappeared in 57% of patients with areflexia at baseline, and swallowing improved in 63% of dysphagic patients. Clinical improvement was stable in all patients, who did not show worsening even after two years of treatment. In a subgroup of 13 patients who performed echocardiogram before and during treatment, interventricular septum thickness reduced significantly ($p < 0.02$). Frataxin mRNA blood levels were modestly increased in one-half of treated patients. We suppose that a focal thiamine deficiency may contribute to a selective neuronal damage in the areas involved in FRDA. Further studies are mandatory to evaluate thiamine role on *FXN* regulation, to exclude placebo effect, to verify our clinical results, and to confirm restorative and neuroprotective action of thiamine in FRDA.

Key words: Thiamine, Spinocerebellar Ataxia, Triplet Expansion Diseases, Friedreich Ataxia.

Introduction

Friedreich ataxia (FRDA) is a rare autosomal recessive neurodegenerative disorder caused by a mutation in the *FXN* gene, which encodes a protein named frataxin. In the high majority of patients (>95%), the pathogenetic mutation in FRDA is a biallelic GAA trinucleotide repeat expansion in the first intron of *FXN* gene located on chromosome 9q13, that causes a transcriptional gene defect; as a result of the mutation, frataxin is quantitatively reduced but qualitatively normal. Frataxin is a protein localized in the mitochondrial internal membrane and in the mitochondrial matrix. Heterozygous GAA expansion carriers express slightly more than 50% of normal frataxin with no clinical symptoms [1-3].

FRDA is clinically characterized by spinocerebellar ataxia and peripheral neuropathy, variably associated with hypertrophic cardiomyopathy, diabetes mellitus, scoliosis, optic atrophy.

Neurological symptoms are progressive gait and limb ataxia, dysarthria, deep tendon areflexia, loss of position sense, and progressive motor weakness [1]. Neuropathological studies found alterations in dorsal root ganglia, sensory peripheral nerves, corticospinal tracts, cerebellar dentate nuclei, heart, and endocrine pancreas. At present, several therapeutic studies are in progress, based on the different pathogenetic mechanisms of this disease, although there is no effective or disease-modifying therapy for FRDA [1, 2, 4, 5].

We focused our attention on thiamine (vitamin B1) which plays a relevant role in the functions of the central and peripheral nervous systems. Its deficiency causes beri-beri neuropathy and Wernicke-Korsakoff encephalopathy [6-8]. Thiamine is a cofactor of enzymes involved in fundamental pathways of energetic cell metabolism (transketolase, alpha-keto-acid decarboxylase, piruvate dehydrogenase, alpha-keto-glutarate dehydrogenase). Thiamine-dependent processes are critical in glucose metabolism, and recent studies described the role of thiamine in oxidative stress, protein processing, peroxisomal function, and gene expression [9-11]. In fact, new studies suggest that thiamine has also non-coenzymatic roles, potentially

relevant in neuroprotection [10].

Thiamine deficiency is a complication of severe malnutrition associated with chronic alcoholism, HIV/AIDS, and gastrointestinal disease, frequently resulting in Wernicke-Korsakoff encephalopathy, a subacute neurologic disorder characterized by ophthalmoplegia, gait ataxia, confusion, and memory loss [6, 12, 13]. The pathophysiology of thiamine deficiency is multifactorial and involves a number of events, resulting in focal neuronal cell death. It involves several molecular alterations, such as reduced activity of alpha-ketoglutarate dehydrogenase, impaired oxidative metabolism, increased oxidative stress, and selective neuronal loss in specific brain regions. These are also reported among the pathological mechanisms involved in different neurodegenerative diseases; thiamine deficiency could then be a useful model in neurodegeneration [9].

Several factors may link thiamine to FRDA. It is noteworthy that both FRDA and thiamine deficiency (Wernicke encephalopathy and beri-beri) have the same main targeted systems: central nervous system, peripheral nervous system, and heart. Cerebellum is one of the most involved brain areas in thiamine deficiency [7].

Previous studies have demonstrated significantly low levels of thiamine and thiamine monophosphate in the cerebrospinal fluid of patients with FRDA [14-16]. In addition, several authors observed a dysfunction of pyruvate dehydrogenase complex in FRDA [17-20]. More recently, a study reported the clinical efficacy of high doses of thiamine in few FRDA patients, who showed an improvement in gait and limb coordination, speech, and swallowing, as well as reduction of fatigue, after three months of treatment [21].

The aim of our study was to investigate whether a long-term treatment with thiamine in patients with FRDA could improve the neurological symptoms and upregulate *FXN* expression in an attempt to restore frataxin concentration towards those in healthy carriers.

Materials and Methods

Patients

Starting from July 2012, we prospectively evaluated 34 outpatients affected by FRDA attending the Department of Neurological Rehabilitation of Villa Immacolata Clinic (Viterbo, Italy), the Unit of Neurology of ASL3 Villa Scassi Hospital (Genoa, Italy), and the Unit of Neurology of IRCCS San Martino University Hospital IST (Genoa, Italy).

The diagnosis of FRDA had been made by standard routine genetic tests, performed in primary Italian laboratories of Medical Genetics. All the participants signed an informed consent to the study. The Ethical Committee of our hospital approved the study.

Evaluations and Treatment

All the patients have been evaluated at baseline and 1, 3, 6, 9, 12, and 24 months after the beginning of treatment with thiamine, with an extended neurologic examination including the Scale for the Assessment and Rating of Ataxia (SARA) [22]. According to the disease severity, disease stages of ataxia were measured by using a 5-point scale ranging from 0 to 4 [23]. At each visit during the study, a subgroup of 20 patients was also assessed with the Fatigue Severity Scale (FSS) [24] and with Archimedes' spiral [25], administered as simple functional test. A subgroup of 13 patients underwent echocardiogram before and during the treatment (at 472 ± 282 days after baseline).

At baseline, all the patients underwent a blood sample to dose plasma level of thiamine, which was measured by using high-performance liquid chromatography [26].

After baseline evaluation, the patients have been continuously treated with intramuscular 100 mg of thiamine twice a week, without any change to personal pharmacological therapy or rehabilitation program.

Gene Expression

Total mRNA was extracted from whole blood collected with PAX gene tubes according to the manufacturer's protocol (PreAnalytiX GmbH, Switzerland); one milligram of mRNA was retro-transcribed using M-MLV Reverse Transcriptase (Life Technologies Europe, Monza, Italy). The level of frataxin mRNA was measured with quantitative Real Time RT-PCR using a TaqMan®Universal PCR Master Mix and TaqMan Gene Expression Assay for Frataxin (Hs00175940_m1). Ct values were normalized to the human TBP (TATA-Binding Protein) Endogenous control (VIC® probe, Life Technologies Europe). Gene expression was calculated in each sample relative to the mean of controls, using the delta-delta Ct method. Each sample was examined in triplicate.

Statistical analysis

Normal distribution of data was assessed using the Shapiro-Wilk test. Because of the execution of multiple assessment during time for each patient, baseline and follow-up scores at clinical scales for normally distributed data have been compared first by using a one-way analysis of variance (ANOVA) for repeated measures, followed by the Box's conservative test, and then using t-test for paired data. Wilcoxon matched-pairs signed-ranks test has been used for the analysis of not normally distributed data. Comparisons between data of different subgroups of patients, examined by gender, disease stage, or disease onset (early ≤ 14 years, intermediate 15–24 years, and late ≥ 25 years) have been performed with t-test for unpaired data. *FXN* gene expression was analyzed using the t-test for unpaired data (two-tailed) and the Wilcoxon ranksum test. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA 92037, USA) and STATA13 software (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP, College Station, TX). Differences with $p < 0.05$ have been considered statistically significant.

Results

Clinical assessment

We performed an open-label trial treating with thiamine 34 patients with FRDA; 13 were males and 21 were females. Mean age was 36.3 ± 11.1 years, while mean age of disease onset was 17.1 ± 9.9 years. The duration of treatment with thiamine, and therefore the duration of patients' follow-up, ranged from 80 days to 930 days (mean \pm sd, 332 ± 257 days). Demographic data and scores in clinical scales at baseline are shown in Table 1.

In all the patients, the basal levels of plasma thiamine were within the normal range. Routine biochemical and hematological investigations were normal including thyroid hormones, TSH, folic acid, and B12 vitamin.

We found that the treatment with thiamine led to significant improvement of motor symptoms: mean total SARA score improved from 26.6 ± 7.7 at baseline to 21.5 ± 6.2 at the last control visit ($p < 0.02$). Detailed scores for each item of SARA at baseline evaluation at and follow-up visits are displayed in Table 2.

In normally distributed groups of SARA scores (visits at 3, 6, 12, and 24 months), we performed the analysis of data with ANOVA for repeated measures (on the subjects with complete evaluation at 3 and 6 months) founding significant differences in mean total SARA scores ($F(3, 29) = 4.87$, $p < 0.005$). The comparisons with t-test for paired data between baseline and each time-point of follow-up (at 3, 6, 12, 24 months) during thiamine treatment were also statistically significant (Table 2). The change in total SARA scores at baseline and month 9 was assessed using the Wilcoxon matched-pairs signed-ranks test, as these data were not normally distributed. This analysis did not reach the statistical significance.

In order to analyze the changes in total SARA scores during time for our whole dataset, which is characterized by missing data points, we used a mixed model for repeated measures.

Significant improvement by time of visit in total SARA scores was confirmed ($p < 0.0001$).

Analysis of SARA scores in patients grouped according to gender (males vs. females) or to disease onset (early vs. intermediate vs. late onset) did not show significant differences.

The analysis of patients according to disease stage (stage 1—walking independently, and 2—permanent use of walking aids, vs. stage 3—permanent use of wheelchair) found improvements in both subgroups, with the subgroup more impaired (stage 3) ameliorating more than the subgroup of patients in stages 1 and 2 ($p=0.0001$ vs. $p=0.026$ in the total SARA score at the last follow-up versus baseline, respectively).

We also evaluated whether our sample of patients confirmed the mean progression annual rate (increase of 0.86 points in SARA total score) described by Reetz *et al.* [27] in FRDA patients.

We used a t-test being the differences in SARA scores after 12 months of treatment normally distributed. The results have shown that the null hypothesis is rejected against the alternative hypothesis that in our study the progression rate is lower than 0.86 ($t=-3.9634$; $p=0.0008$).

Moreover, we also considered as null hypothesis the lower limit of the mean progression annual rate (increase of 0.75 points in SARA total score, as the lower limit of confidence interval) as reported by Reetz *et al.* [27]. Also in this case the null hypothesis is rejected, thus we have evidence that the progression rate is lower than 0.75 ($t=-3.81$; $p=0.001$). Therefore, the clinical progression of our group of patients was significantly better than the natural progression of the disease.

Our patients showed also some other clinical changes: 16 out of 28 patients with absence of deep tendon reflexes at baseline, revealed presence of deep tendon reflexes after three months of treatment; swallowing difficulties improved in 14 out of 22 patients with dysphagic symptoms at baseline.

None patient experienced adverse events or discontinued the treatment. We carefully monitored diabetic patients treated with insulin, who showed a slight increase of glycemia levels and subsequent mild increase in insulin dosage; however, glycosylated hemoglobin was

unchanged.

The time needed to complete Archimedes' spiral improved with a tendency to statistical significance, from 73.9 ± 48.2 seconds at baseline to 44.3 ± 26.3 at 6-month follow-up ($p=0.081$) and to 41.5 ± 22.1 seconds at 24-month follow-up ($p=0.098$).

FSS scores did not change significantly.

Echocardiographic study

A subgroup of 13 patients underwent echocardiogram at baseline and during therapy. The follow-up echocardiogram was performed 449.5 ± 276.2 days (range 73-890 days) after the beginning of thiamine treatment. The thickness of the interventricular septum decreased from 9.54 ± 1.76 mm to 8.85 ± 2.00 mm ($p=0.016$), while the thickness of the left ventricle posterior wall was unchanged (from 8.57 ± 1.80 to 8.61 ± 2.08 mm), as well as the ejection fraction (from 63.18 ± 7.86 to 61.55 ± 7.22 mm).

Analysis of FXN gene expression

In six of the 34 patients enrolled, we could measure *FXN* blood expression at baseline and after 12 months of treatment. One patient (FXN8) showed ~40% increment of *FXN* expression ($p=0.0002$), in three patients a 20% increase was seen (FXN2, FXN4 and FXN7; $p<0.05$), while in the remaining two (FXN1 and FXN3), no mRNA change was apparent. A combined analysis of all the patients did not show a statistically significant increase ($p=0.078$, Wilcoxon matched-pairs signed-ranks test) (Figure 1).

Discussion

Currently there is no effective therapy for the treatment of most cerebellar ataxias, and mainly for FRDA [2, 28]; several promising treatments did not show significant results in further studies, such as for idebenone [29, 30], erythropoietin [31, 32], and deferiprone [33], or need to be confirmed, such as for histone deacetylase inhibitors [34, 35], nicotinamide [36], riluzole [37], varenicline [38], and resveratrol [39]. Current treatment remains symptomatic, while clinical and experimental studies are in progress, aiming to find effective molecules [5, 28]. In some of these studies, the effects on frataxin mRNA and protein levels were also evaluated with conflicting results. Then, a potential drug stopping or slowing the disease progression may be clinically relevant.

In our open-label pilot study, we have investigated the clinical effect and the possible influence on *FXN* expression of treatment with thiamine in FRDA: long-term administration of high doses of parenteral thiamine was effective in improving motor symptomatology in our series of 34 patients with FRDA. Our patients treated with thiamine showed a significant decrease of SARA score of -1.82 ± 0.68 points after treatment for 12 months ($p=0.018$). The natural disease progression in FRDA was estimated with an annual increase at SARA score of 1.36 ± 2.3 points according to Marelli *et al.* [40] or in a range between 0.56 and 1.17 point according to Reetz *et al.* [27]. We found that the annual progression rate in our patients is significantly lower ($p=0.0008$) than the medium estimated annual worsening of SARA [27]. Therefore, the improvement in the SARA scores of our patients treated with thiamine is clinically relevant, especially considering the rate of clinical impairment in natural disease progression [27, 40-42]. This clinical improvement was stable over time in all the patients: even after two years of treatment, motor performances did not impaired but remained significantly better than those at baseline ($p=0.019$).

The thiamine effects on SARA scores were significant in all the subgroups of patients,

without differences of age, gender, disease duration, disease severity, or degree of functional disability according to disease stage.

Our trial had an open design, but we observed some objective clinical and cardiological changes in treated patients. In fact, thiamine administration was associated with a significant reduction of thickness of interventricular septum in a subgroup of 13 patients who complete the echocardiographic study. Moreover, a portion of patients experienced the re-occurrence of deep tendon reflexes (47% of total patients, 57% of areflexic patients) and the improvement of dysphagia (41% of total patients, 63% of dysphagic patients).

Furthermore, in the last years several open-label trials with different molecules has been published, with different results, both in FRDA [39, 43-47] and in spinocerebellar ataxias [48-51]. All these studies had shorter duration and smaller sample size of patients compared to our study.

Based on the role of the *FXN* gene in the pathology, we evaluated the effect of thiamine on *in vivo* blood levels of *FXN* mRNA, in a subgroup of six patients. We showed a not uniform response, with an upregulation from 20% to 40% in four patients and no effect in the remaining two. Although we think that *FXN* increment was too modest to support the clinical improvement observed, we were intrigued by the possible correlation between gene expression and SARA score improvement. We may speculate that thiamine may have a direct influence on *FXN* expression warranting further *in vitro* study to verify this hypothesis.

However, the more likely explanation is that thiamine acts independently from *FXN* in improving the phenotype, possibly on other genes related to the disease status [52].

The long-lasting treatment with thiamine, as demonstrated by our study, is also safe; we did not observe any serious adverse event, in accordance with the literature, who did not report any thiamine-related adverse effects even at high doses and for long periods of administration [53].

The absence of blood thiamine deficiency at baseline and the efficacy of continuous treatment with high doses of thiamine in our patients might indicate that FRDA symptomatology is the manifestation of focal neuronal thiamine deficiency, possibly due either to dysfunctions of the active intracellular transport of thiamine or to any structural enzymatic abnormality. The defects in thiamine-dependent processes could be overcome by diffusion transport at elevated thiamine concentrations. We then hypothesize that symptoms of FRDA could partly derive from a chronic intracellular thiamine deficiency.

It has been experimentally described that thiamine deficiency reduces the activity of thiamine-dependent enzymes (e.g. alpha-ketoglutarate dehydrogenase) with regional selectivity, being different cerebral areas affected with different severity [7, 54]. However, new data suggest that thiamine has also non-coenzymatic roles [10]. Since there is no correlation between the brain levels of thiamine diphosphate or the activities of thiamine-dependent enzymes and the positive effects of thiamine administration, the potential non-coenzymatic action of thiamine should not be neglected in patients with neurodegenerative diseases [10]. A dysfunction of thiamine-dependent metabolic pathways, either via coenzymatic or non-coenzymatic processes, could cause a selective neural damage in the centers affected by this disease and might be a fundamental molecular event provoking neurodegeneration [9, 10, 13]. Thus, high-dose thiamine administration may elevate both thiamine coenzymatic and the non-coenzymatic forms, which may be responsible for its therapeutic effects [10]. The clinical efficacy of continuous treatment with high doses of thiamine in our patients with FRDA could indicate that FRDA symptomatology is the manifestation of neuronal TD. The primary cause of FRDA could be highly expressed in cerebellar and sensitive ganglia cells and cardiomyocytes, causing severe thiamine deficiency and motor symptoms, and less expressed in all other cells, causing mild thiamine deficiency and related disorders [21].

The precise role played by thiamine in FRDA pathogenesis was not extensively investigated

so far, but some possible links have been described. In the past years, several studies reported low levels of thiamine and thiamine monophosphate in the cerebrospinal fluid of patients with FRDA [14-16]. In pre-genetic era, searching for a possible metabolic cause of FRDA, some authors observed a dysfunction of pyruvate dehydrogenase complex in this disease [17-20]; these data were not confirmed by other studies [55, 56]. Some authors found selective loss of the alpha-ketoglutarate dehydrogenase complex in the cerebellum of patients with FRDA [57, 58]. In a more recent proteomic study, the authors evaluated the expression of proteins in cardiac tissue of frataxin knockout mice; the knockout cardiac cells exhibited increased and early expression of pyruvate dehydrogenase complex, oxoglutarate dehydrogenase and branched chain ketoacid dehydrogenase, all enzymes whose thiamine is a cofactor [59]. Finally, other authors, evaluating the structural assembly of Fe-S clusters and the protein-protein interactions, found a number of interactors with IscS protein, the enzyme initiating sulfur transfer; among these partners there are CyaY, analogue of frataxin, and Thil, involved in thiamine biosynthesis and tRNA modification [60].

Moreover, a dysfunction of intracellular thiamine transport has been described in genetic diseases characterized by mutations in thiamine-transporters genes in which clinical improvements can be documented after thiamine administration, such as biotin-thiamine-responsive basal ganglia disease [61] or Wernicke-like encephalopathy [62]. Genetic disorders of thiamine metabolism and transport that lead to neurological diseases can be treated with large doses of thiamine [63-65]. On the other hand, in patients with thiamine deficiency and low cerebrospinal fluid levels of 5-hydroxyindoleacetic acid, the thiamine treatment markedly increased 5-hydroxyindoleacetic acid [66].

The exact mechanism of thiamine responsiveness in FRDA patients is still unknown. We could argue that even if frataxin is a useful biomarker for drug response in FRDA treatment [36], other peripheral biomarkers related to disease status (i.e. defects in mitochondria, cell

cycle, and lipid metabolism) could be evaluated for assessing thiamine response and efficacy [52].

Our study has some limitations, the most relevant being the absence of a placebo-controlled group. Although clinical improvement of our patients is continuous and stable for a long period of follow-up (even more than two years), the lack of a placebo group suggests taking these results carefully. On the other hand, considering the smaller entity of the placebo effect described in previous studies [31-38], the longer duration of follow-up of present study, and the significant changes in objective parameters such as deep tendon reflexes and echocardiographic measures, we cannot exclude that the improvement of SARA score is not to be accounted for placebo effect only.

We then suppose that parenteral thiamine supplementation in FRDA may play a role in restoring survivor neurons and in limiting the disease progression, and that the dysfunction of thiamine-dependent processes could be a primary pathogenic pathway leading to the apoptotic death of cerebellar and sensitive neurons in FRDA.

In fact, thiamine-dependent processes are impaired in cerebral tissues of patients with several neurodegenerative diseases; the activity reduction of thiamine-dependent enzymes can be readily linked to symptomatology and pathology of these disorders. Thus, most neurodegenerative diseases share similarities and could be responsive to high doses of thiamine [67, 68].

In conclusion, on the basis of the present data we cannot rule out that thiamine may have a beneficial action in FRDA. Further studies are necessary to exclude placebo effect, to verify the possible synergy with other therapies, to investigate its mechanism of action at cell and tissue level, and to evaluate potential symptomatic and neuroprotective effects of thiamine in FRDA.

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Authors' Roles:

Costantini A, Laureti T, Pala MI, Colangeli M, Cavalieri S, Pozzi E, Brusco A, Salvarani S, Serrati C, Fancellu R: Conception and design, acquisition of data, analysis and interpretation of data, drafting, critical revision, supervision.

Figure 1. Effect of thiamine treatment on *FXN* mRNA expression. We measured *FXN* mRNA levels at T0 (baseline) and T1 (after 12 months of treatment). The overall change in *FXN* mRNA levels after treatment was not statistically significant (n=3 in triplicate, Wilcoxon ranksum test, p=0.078).

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Table 1. SARA scale scores for each item at baseline and during follow-up

	Baseline (N=34)	Month 3 (3.38±0.48) (N=29)	Month 6 (6.30±0.45) (N=20)	Month 9 (9.44±0.36) (N=9)	Month 12 (12.54±0.64) (N=14)	Month 24 (24.54±2.90) (N=8)
Total SARA score	26.59±7.75	23.38±8.06^a	22.55±8.16^a	22.83±7.15	22.71±6.44^b	21.50±6.23^b
1) Gait	6.91±1.73	6.76±1.94	6.45±1.85	6.33±2.29	6.64±1.91	6.63±1.69
2) Stance	5.12±1.57	4.86±1.87	4.75±2.02	4.78±1.92	4.93±1.69	4.88±1.55
3) Sitting	2.65±1.35	2.00±1.46^a	1.65±1.57^c	2.00±1.12	1.71±1.27^c	1.38±1.30
4) Speech disturbance	2.50±1.02	1.79±1.08^a	1.90±0.91^a	2.11±0.33	1.86±0.53	1.38±0.74^b
5) Finger chase	2.00±1.05	1.60±1.06^c	1.55±0.81^c	1.61±0.74	1.64±0.74	1.38±0.69
6) Nose-finger test	1.56±1.52	1.14±1.17^c	1.10±1.17	0.39±0.42	0.43±0.62	0.56±0.73
7) Fast alternating hand movements	2.60±0.86	2.36±0.96^c	2.35±0.90	2.72±0.44	2.43±0.76	2.25±1.00
8) Heel-shin slide	3.25±0.95	2.86±1.28^c	2.80±1.26	2.89±1.34	3.07±1.19	3.06±1.24

Data are expressed as mean ± standard deviation. Significant differences are marked in bold.

^a p<0.001; ^b p<0.02; ^c p<0.05; (p-test for paired data)

SARA: Scale for Assessment and Rating of Ataxia

Table 2. SARA scale scores for each item at baseline and during follow-up

	Baseline vs month 3 (N=29)		Baseline vs month 6 (N=20)		Baseline vs month 9 (N=9)		Baseline vs month 12 (N=14)		Baseline vs month 24 (N=8)	
	Baseline	Month 3 (3.38±0.48)	Baseline	Month 6 (6.30±0.45)	Baseline	Month 9 (9.44±0.36)	Baseline	Month 12 (12.54±0.64)	Baseline	Month 24 (24.54±2.90)
Total SARA score	26.14±8.01	23.38±8.06^a	24.90±8.10	22.55±8.16^a	23.72±6.93	22.83±7.15	24.54±6.00	22.71±6.44^b	24.94±6.58	21.50±6.23^b
1) Gait	6.83±1.85	6.76±1.94	6.60±1.76	6.45±1.85	6.44±2.30	6.33±2.29	6.71±1.90	6.64±1.91	6.75±1.67	6.63±1.69
2) Stance	5.00±1.67	4.86±1.87	4.85±1.69	4.75±2.02	4.67±1.87	4.78±1.92	4.93±1.59	4.93±1.69	5.00±1.41	4.88±1.55
3) Sitting	2.66±1.37	2.00±1.46^a	2.35±1.42	1.65±1.57^c	2.56±1.24	2.00±1.12	2.43±1.22	1.71±1.27^c	2.50±1.31	1.38±1.30
4) Speech disturbance	2.52±1.09	1.79±1.08^a	2.35±0.93	1.90±0.91^a	2.22±0.67	2.11±0.33	2.29±0.61	1.86±0.53	2.13±0.64	1.38±0.74^b
5) Finger chase	1.93±1.04	1.60±1.06^c	1.83±1.05	1.55±0.81^c	1.61±0.74	1.61±0.74	1.68±0.64	1.64±0.74	1.81±1.00	1.38±0.69
6) Nose-finger test	1.45±1.48	1.14±1.17^c	1.20±1.44	1.10±1.17	0.50±0.56	0.39±0.42	0.75±0.91	0.43±0.62	0.81±1.07	0.56±0.73
7) Fast alternating hand movements	2.60±0.87	2.36±0.96^c	2.65±0.80	2.35±0.90	2.72±0.57	2.72±0.44	2.61±0.56	2.43±0.76	2.75±0.46	2.25±1.00
8) Heel-shin slide	3.16±0.98	2.86±1.28^c	3.08±0.94	2.80±1.26	3.00±0.83	2.89±1.34	3.14±0.74	3.07±1.19	3.19±0.75	3.06±1.24

Data are expressed as mean ± standard deviation. Significant differences are marked in bold: ^a p<0.001; ^b p<0.02; ^c p<0.05; vs. respective baseline (t-test for paired data)
SARA: Scale for Assessment and Rating of Ataxia

Figure 1

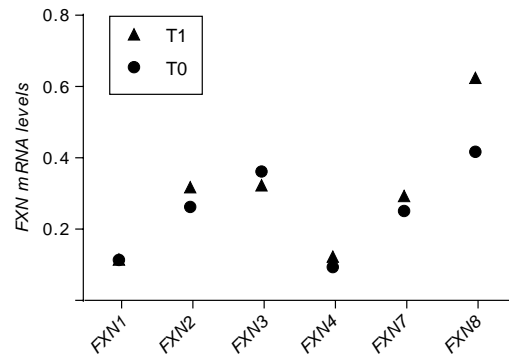


Figure 1S

