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ORIGINAL ARTICLE

Treatment effect of coenzyme Q_{10} and an antioxidant cocktail in fibroblasts of patients with Sanfilippo disease

Leslie Matalonga · Angela Arias · María Josep Coll · Judit Garcia-Villoria · Laura Gort · Antonia Ribes

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Abstract Coenzyme Q_{10} (CoQ_{10}) plays a key role in the exchange of electrons in lysosomal membrane, which contributes to protons' translocation into the lumen and to the acidification of intra-lysosomal medium, which is essential for the proteolytic function of hydrolases responsible -when deficient- of a wide range of inherited lysosomal diseases such as Sanfilippo syndromes. Our aim was to evaluate whether treatment with CoQ_{10} or with an antioxidant cocktail (α-tocopherol, N-acetylcysteine and α-lipoic acid) were able to ameliorate the biochemical phenotype in cultured fibroblasts of Sanfilippo patients. Basal CoQ_{10} was analyzed in fibroblasts and Sanfilippo A patients showed decreased basal levels. However, no dysfunction in the CoQ_{10} biosynthesis pathways was found, revealing for the first time a secondary CoQ_{10} deficiency in Sanfilippo A fibroblasts. Cultured fibroblasts from five patients affected by Sanfilippo A and B diseases were treated with CoQ_{10} and an antioxidant cocktail. Upon CoQ_{10} treatment, none of the Sanfilippo A fibroblasts increased their residual enzymatic activity, but the two Sanfilippo B cell lines showed a statistically significant increase of their residual activity. The antioxidant treatment had no effect on the residual activity in all tested cell lines. Moreover, one Sanfilippo A and two Sanfilippo B fibroblasts showed a statistically significant reduction of glycosaminoglycans accumulation both, after 50 μmol/L CoQ_{10} and antioxidant treatment. Fibroblasts responsive to treatment enhanced their exocytosis levels. Our results are encouraging as some cellular alterations observed in Sanfilippo syndrome can be partially restored by CoQ_{10} or other antioxidant treatment in some patients.

Introduction

Coenzyme Q_{10} (CoQ_{10}) is a lipophilic molecule synthesized in all cells (no dietary uptake is required) and is present in all membranes in eukaryotic organisms. The best known function is its role as electron carrier in the mitochondrial respiratory chain. However, more functions are attributed to CoQ_{10} and it is currently known that it participates in a great number of metabolic processes such as prevention of lipid, protein and DNA oxidation; regulation of mitochondrial uncoupling proteins; formation of mitochondrial permeability transition pores; pyrimidine nucleoside biosynthesis and apoptosis (Turunen et al 2004).

Although CoQ_{10} has a prominent role in the mitochondrial respiratory chain by transporting electrons from complex I and complex II to complex III, both the lysosomal and mitochondrial membranes contain similar proportions of CoQ_{10} (Turunen et al 2004). In fact, several studies have shown that CoQ_{10} also plays a key role in the exchange of electrons in the lysosomal membrane, which contributes to protons' translocation into the lumen and to the acidification of the intra-lysosomal medium (Gille and Nohl 2000). Acidification is essential for the proteolytic function of intra-lysosomal hydrolases since these enzymes require acid pH for optimal activities. As it occurs in the mitochondrial respiratory chain, oxygen is the final electron acceptor in the lysosomal electron transport chain and can potentially trigger the onset of reactive

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L. Matalonga · A. Arias · M. J. Coll · J. Garcia-Villoria · L. Gort · A. Ribes (✉)
Secció d'Errors Congènits del Metabolisme-IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, IDIBAPS, CIBER de Enfermedades Raras (CIBERER), Edifici Helios III, planta baixa, C/Mejía Lequerica s/n, 08028 Barcelona, Spain
e-mail: aribes@clinic.ub.es

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oxygen species (ROS) which are harmful for the cell. The reduced form of CoQ_{10} in the lysosomal bilayer protects the membrane from oxidative stress (Gille and Nohl 2000). Other compounds such as xanthophylls, L-glutathione (Pannuzzo et al 2010), δ- and α-tocopherol (Xu et al 2012), N-acetylcysteine and α-lipoic acid (López-Erauskin et al 2011) have been described as potent antioxidants that are neutralizing ROS, stabilizing lysosomal membranes and exocyting its content.

In addition, the deficiency of CoQ_{10} in the mitochondria has an indirect impact on the lysosome through a natural process called mitophagy (mitochondrial autophagy by lysosomes), triggered in the presence of mitochondrial membrane disaggregation. This regulatory process, which is observable under normal conditions, seems to be increased in cells from patients with CoQ_{10} deficiency. When mitophagy occurs there is a significant increase of the activity of all lysosomal enzymes. This effect was reversed when the cell culture medium was complemented with CoQ_{10} (Rodríguez-Hernández et al 2009).

Several evidences related lysosomal diseases with CoQ_{10} deficiency or with an amelioration of the phenotype under antioxidant administration in animal models. In fact, a study of 37 patients affected by Niemann-Pick type C disease showed that CoQ_{10} was low in all of them (Fu et al 2010). Furthermore, in a mouse model study of Krabbe disease, it was observed that mice recovered weight, motility and extended their half-life after the administration of a cocktail of antioxidants (Pannuzzo et al 2010). In addition, Delgadillo et al (2011) observed that CoQ_{10} levels in plasma of Sanfilippo B patients were in the low range of the controls. Moreover, Xu et al (2012) demonstrated that tocopherol reduces cholesterol accumulation and alleviates cellular phenotype of Niemann-Pick type C and Wolman diseases and suggested that the pharmacological effect may be mediated by stimulating an increase in cytosolic Ca^{2+} that enhances lysosomal exocytosis. These authors speculated that this mechanism appears to be independent of either the mutant enzyme or the storage material and might alleviate the phenotype of all lysosomal storage diseases (LSDs) in cells.

Mucopolysaccharidosis type III or Sanfilippo disease is a LSD caused by a deficiency in one of the four enzymes catalyzing the degradation of heparan sulphate: heparan N-sulfatase is deficient in type A (OMIM #252900), α-N-acetylglucosaminidase in type B (OMIM #252920), acetyl-CoA-α-glucosamide-acetyltransferase in type C (OMIM #252930), and N-acetyl-glucosamine-6-sulfatase in type D (OMIM #252940). Clinically these patients are characterized by severe progressive dementia with distinct behavioral disturbances and mild somatic disease. None of the pre-existing treatments, including enzyme replacement therapy (Germain 2005), hematopoietic stem cell transplantation (Krivit 2004), substrate reduction therapy (Jakóbkiewicz-Banecka et al 2007), chaperone mediated therapy (de Ruijter et al 2011) or gene therapy (Ellinwood et al 2010) showed clinical improvement in these patients so far.

The aim of our study was to elucidate whether treatment with CoQ_{10} or a cocktail of antioxidants were able to ameliorate the biochemical phenotype in fibroblasts of Sanfilippo A and B patients before attempting future therapeutic approaches.

Materials and methods

Patients

Primary skin cultured fibroblasts from five patients with either Sanfilippo A (P1, P2, P3) or Sanfilippo B (P4, P5) diseases were grown in Dulbecco's modified Eagles medium (DMEM) with 10 % fetal bovine serum and antibiotics (penicillin and streptomycin), at 37 °C with 5 % CO_2. All reagents were purchased from PAA Laboratories (Velizy-Villacoublay, France). Patients were selected on the basis of fibroblasts availability and measurable residual enzymatic activity. Patients' genotype, enzymatic activity and CoQ_{10} levels are shown in Table 1. The use of human samples was approved by the Ethical Committee of Hospital Clínic, Barcelona.

Treatment and cell viability

Primary cultured human skin fibroblasts between five and nine passages were plated in 6-well plates and were treated during 72 h (according to Xu et al 2012) with different concentrations of CoQ_{10}: 10 μmol/L, 20 μmol/L, 30 μmol/L, 50 μmol/L, and 100 μmol/L or a cocktail of antioxidants at doses previously reported in fibroblasts (López-Erauskin et al 2011): α-tocopherol at 500 nmol/L, N-acetylcysteine at 50 μmol/L and α-lipoic acid at 50 μmol/L, all purchased from Santa Cruz Biotechnology (Santa Cruz, USA).

Cell viability was evaluated for each treatment by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil tetrazolium bromide (MTT) assay (Sigma-aldrich, St.Louis, USA) as described by Sumantran (2011).

Enzymatic activities

Fibroblasts were harvested and rinsed twice with physiological serum and lysed by three freeze-thaw cycles. Protein concentration was measured using the BioRad DC Protein Assay (Bio-Rad Laboratories, S.A, Alcobendas, Madrid). Equal amounts of protein lysates (10 μL) were seeded in white 96-well plates and the corresponding enzymatic activities, Heparan-N-sulfatase (EC 3.10.1.1) for Sanfilippo A and α-N-acetylglucosaminidase (EC 3.2.1.50) for Sanfilippo B, were determined in triplicate using a fluorimetric assay with

Table 1 Genotype, enzymatic activity and CoQ_{10} levels of patients' fibroblasts

Patient	Disease	Gene	Genotype	Effect on protein	Residual enzymatic activity (nmol/h/mg)	Passage number	CoQ_{10} (nmolCoQ/UCS)
P1	Sanfilippo A	SGSH	c.[1339G>A];[1339G>A]	p.[Glu447Lys];[Glu447Lys]	0.17	7/9	1.3
P2	Sanfilippo A	SGSH	c.[221G>A];[221G>A]	p.[Arg74Cys];[Arg74Cys]	0.15	8/9	0.9
P3	Sanfilippo A	SGSH	c.[1297C>T];[1297C>T]	p.[Arg433Trp];[Arg433Trp]	0.17	8/9	0.7
P4	Sanfilippo B	NAGLU	c.[112C>T];[112C>T]	p.[Arg38Trp];[Arg38Trp]	0.46	6/7	2.91
P5	Sanfilippo B	NAGLU	c.[503G>A];[1696C>T]	p.[Trp168X];[Gln566X]	0.78	7/9	2.74
Reference range	-	-	-	-	27.1-84.7 (Sanfilippo A) 70-486 (Sanfilippo B)	5/10	2.0-2.9

4-methylumbelliferyl artificial substrates (Annunziata and Dimatteo 1978): 4-Methylumbelliferyl-α-N-sulphoglucosaminide purchased from Moscerdam (Oegstgeest, Netherlands) for Sanfilippo A and 4-Methylumbelliferyl-2-acetamido-2-deoxy-α-D-glucopyranoside purchased from Calbiochem (Whitehouse, USA) for Sanfilippo B. Sanfilippo A determination was performed following exactly the recommendations of Moscerdam (Karpova et al 1996). For Sanfilippo B determination, the artificial substrate was prepared with phosphate (0.2 mol/L)/citrate (0.1 mol/L) buffer at pH 4.7 and incubated for 17 h at 37 °C. Both reactions (Heparan-N-sulfatase and α-N-acetylglucosaminidase activities) were stopped with 200 μL of carbonate buffer (0.5 mol/L) at pH 10.7. Fluorescence was measured at 365 nm emission and 465 nm excitation with a microplate reader (POLARstar Omega, BMG LABTECH, Offenburg, Germany). β-hexosaminidase (EC.3.2.1.30) activity was assayed with 4-Methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside (Sigma-Aldrich, St Louis, USA) as artificial substrate. Cells were cultured in triplicate and determinations of the enzymatic activities were also performed in triplicate.

Glycosaminoglycans (GAGs) determination

GAGs quantification was performed using the 1,9-dimethylmethylene blue (DMB) assay adapted from Barbosa et al (2003). Cells were cultured in triplicate in 6-wells plates and harvested after 72 h treatment. DMB absorbance was measured in duplicates at 656 nm with a microplate reader (POLARstar Omega, BMG LABTECH, Offenburg, Germany).

Lysosomal exocytosis assay

Lysosomal exocytosis was assayed by measuring β-hexosaminidase activity in the culture media, as previously described (Xu et al 2012). Briefly, skin fibroblasts derived from patients and a healthy individual were cultured in triplicate in 24-well plates at 30,000 cells/well in 0.4 mL medium for 1 day at 37 °C. After being washed twice with phosphate buffered saline pH 7.4, cells were incubated with 0.3 mL/well treatment medium (0 and 50 μmol CoQ10/L); 30 μL of medium from each well were taken at 0, 5, 8, 24, 32, 48, and 72 h and were aliquoted in triplicate into a 96-well plate. Therefore 10 μL of media was used for each β-hexosaminidase measurement. The enzymatic activities were expressed per mL instead of per protein. Previously, we have measured the proteins at 0, 24, 48, and 72 h (in the pellet) and found no significant differences. We also treated fibroblasts with 80 μmol/L of α-tocopherol according to Xu et al (2012) and exocytosis measurement was performed as describe above.

CoQ_{10} and citrate synthase (CS) measurements

CoQ_{10} concentration was measured by HPLC-MS/MS as described by Arias et al (2012). CS activity was determined in fibroblasts by a previously described spectrophotometric method (Srere 1969) using 0.1 mmol/L DTNB, 0.2 % Triton X100 and 30-50 μg of protein in a final volume of 500 μL. Results were expressed in nmol CoQ_{10}/unit of CS (UCS).

Biosynthesis of CoQ_{10} in fibroblasts

Biosynthesis of CoQ_{10} was determined as described by Buján et al (2013). Briefly, skin derived fibroblasts were treated with two stable isotope labeled precursors: $^{13}C_6$-parahydroxybutyrate ($^{13}C_6$-PHB) and $^{2}H_3$-mevalonate ($^{2}H_3$-MV) for 72 h. Pelleted-cells were resuspended with 300 μL of buffer solution (0.25 mmol/L sucrose, 2 mmol/L EDTA, 10 mmol/L Tris and 100 UI/mL heparin at pH 7.4) and sonicated twice for 5 s. Homogenates were used to determine CoQ_{10} biosynthesis from labeled substrates, total protein content and CS activity. Results were expressed in nmol CoQ_{10}/UCS.

Statistical analysis

Statistic significance of data was assessed using T-student statistical test. All data are presented as mean ± standard deviation (SD), with the level of significance set at $P<0.05$.

Results

In this study we have analyzed the potential treatment efficacy of CoQ_{10} and of an antioxidant cocktail (α-tocopherol, N-acetylcysteine and α-lipoic acid) in fibroblasts from three patients affected by Sanfilippo A syndrome and two patients affected by Sanfilippo B syndrome. Treatment response was evaluated by measuring the increase of the enzymatic activities and the decrease of the abnormal GAGs accumulation. Exocytosis was also evaluated.

CoQ_{10} in fibroblasts

As basal CoQ_{10} levels in fibroblasts of the three Sanfilippo A patients were low (Table 1), we analyzed CoQ_{10} biosynthesis in fibroblasts of two of them (P1 and P3) in order to know if the deficient level might be due to a dysfunction of its biosynthesis. Results showed normal biosynthesis with both precursor substrates, which excluded any primary defect of CoQ_{10} pathway (Supplementary Table 1).

Treatment and cell viability

Fibroblasts were treated with different concentrations of CoQ_{10}. At 200 μmol/L cell viability was around 70 %. Therefore, this concentration was toxic to the cells and was excluded to be used in further experiments. Plotted concentration of CoQ_{10} in the culture media versus intracellular CoQ_{10} concentration showed a linear regression up to 50 μmol/L (Fig. 1), on the other hand cell viability was almost 100 % in all tested concentrations except for 100 μmol/L of added $CoQ_{10,}$ which was around 90 %. Therefore, we decided to use 30 and 50 μmol/L in further experiments. After treatment, we also evaluated the intracellular content of CoQ_{10} in all the patients, and results were within the values of controls (data not shown).

Enzymatic activity

To evaluate the effect of treatment on the enzymatic activity, basal and treated fibroblasts were measured. Results are shown in Fig. 2a and Supplementary Table 2. None of the fibroblasts from patients affected by Sanfilippo A disease (P1, P2 and P3) increased their enzymatic activity, neither with CoQ_{10} nor with the antioxidant cocktail, while the two patients (P4 and P5), suffering from Sanfilippo B disease, showed a significant increase both, after treatment with 30 μmol/L CoQ_{10} ($p<0.01$ and $p<0.05$ respectively) as well as after treatment with 50 μmol/L CoQ_{10} ($p<0.05$ and $p<0.01$ respectively), but the antioxidant cocktail had no effect on the enzymatic activity. Fluorescence ranges for both enzymatic activities are shown in Supplementary Table 3. Cell viability was not altered in any of the cell lines after treatment at the concentrations used in this study (data not shown).

Glycosaminoglycans

We evaluated the capability of CoQ_{10} and the antioxidant cocktail to decrease GAGs' accumulation in fibroblasts (Supplementary Table 2, Fig. 1b). Results showed that GAGs' levels in two of the patients (P1 and P2) remained

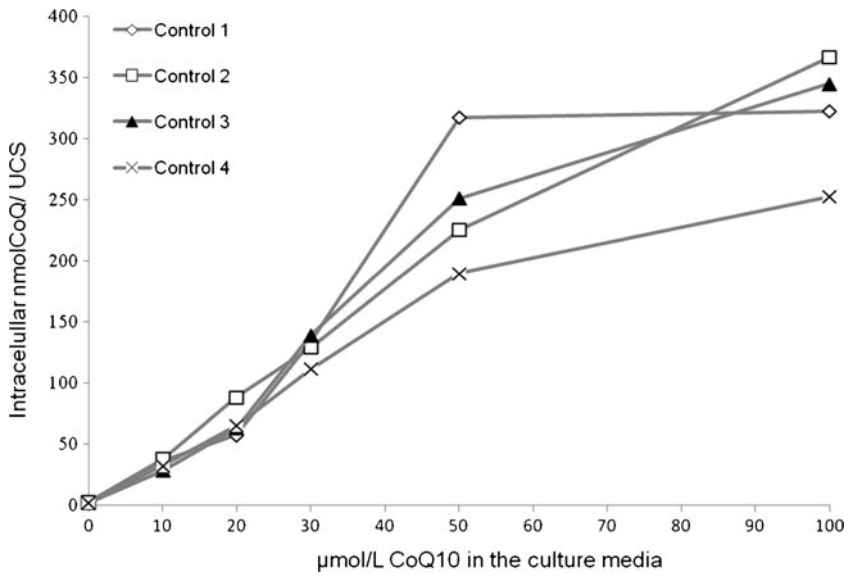

Fig. 1 Intracellular incorporation of CoQ_{10} after treatment at different concentrations in control fibroblasts. Plot of μmol CoQ_{10}/L in the culture media against intracellular nmol CoQ_{10}/unit citrate synthase (UCS)

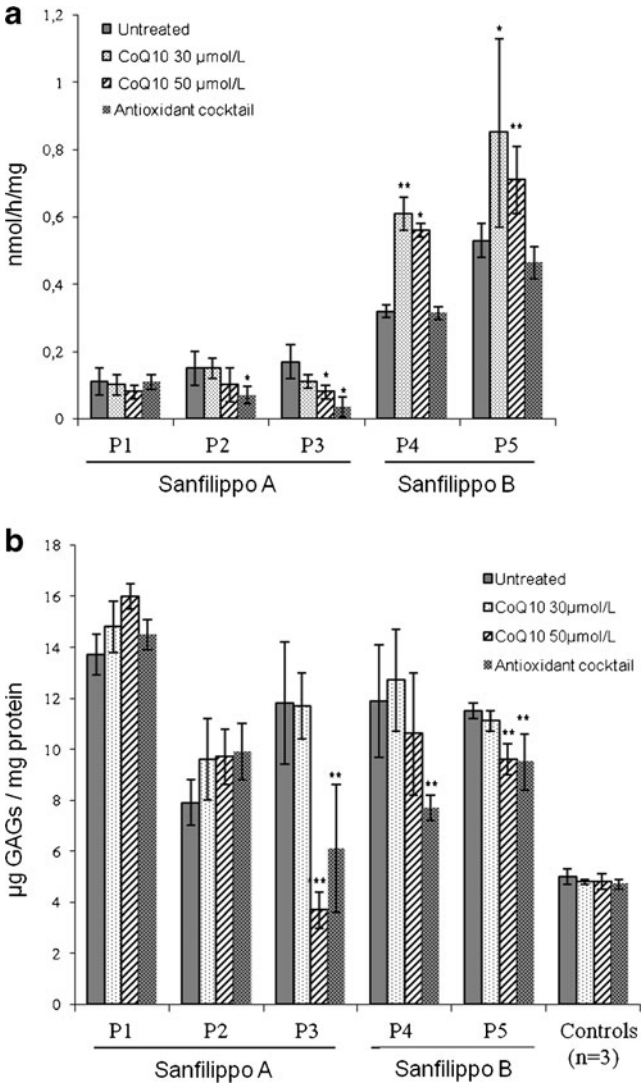

Fig. 2 Enzymatic activity and glycosaminoglycan concentration in Sanfilippo A and B patients' fibroblasts before and after treatment with CoQ_{10} and an antioxidant cocktail. **a** Heparan-N-sulfatase (Sanfilippo A syndrome) and α-N-acetylglucosaminidase (Sanfilippo B syndrome) activities in patients' fibroblasts treated with 30 and 50 µmol/L CoQ_{10} compared with the antioxidant cocktail (α-tocopherol at 500 nmol/L, N-acetylcysteine at 50 µmol/L and α-lipoic acid at 50 µmol/L). **b** Glycosaminoglycans concentration in patients' fibroblasts treated with 30 and 50 µmol/L CoQ_{10} and the antioxidant cocktail (α-tocopherol at 500 nmol/L, N-acetylcystein at 50 µmol/L, α-lipoic acid at 50 µmol/L). All data are expressed as the mean and standard deviation from three independent experiments in triplicate. Differences were analyzed using the student's *T* test. (*$p<0.05$; **$p<0.01$, ***$p<0.001$)

unaltered after both, CoQ_{10} and the antioxidant cocktail treatment. Three patients (P3, P4 and P5) showed a variable decrease with both treatments. After treatment with 30 µmol/L CoQ_{10}, P3 did not show any significant decrease of GAGs, but at 50 µmol/L the decrease was impressive ($p<0.001$) reaching control levels. This patient also exhibited a significant decrease ($p<0.01$) with the antioxidant cocktail. P4 only showed a significant decrease with the antioxidant cocktail ($p<0.01$), while the behavior of P5 followed the rule of P3, that is, a significant decrease after 50 µmol/L CoQ_{10} treatment and also a significant decrease ($p<0.01$) with the antioxidant cocktail.

Lysosomal exocytosis

In addition to the antioxidant capability, already described for both compounds, we wondered if the decrease of GAGs was due to an increase of lysosomal exocytosis. Therefore, we monitored lysosomal exocytosis by measuring β-hexosaminidase activity in fibroblasts' media, as it had recently been described for tocopherol (Xu et al 2012). After 72 h incubation, results showed a tendency to increase exocytosis in all cell lines (including controls), but it was only statistically significant in the three responsive patients, with both CoQ10 and α-tocopherol treatment (Fig. 3). We have not corrected for the biomass because we did not compare between patients we compared each cell line with itself, with and without treatment, at each time point and at each concentration (Fig. 3). In addition proteins (in the pellet) for each cell line were measured at 0, 24, 48, and 72 h without any significant change. Moreover, we have monitored the cell viability after each treatment and it was unaltered. Therefore, we assume that growth and death parameters are stable.

Discussion

The main clinical symptoms of Sanfilippo syndromes are mental retardation with behavioral problems and somatic disease (Neufeld and Muenzer 2001) and are characterized by impaired lysosomal enzymatic activities and abnormal GAGs storage inducing a pathogenic cascade that impacts on multiple cellular systems and organelles. Previously reported evidences on lysosomal diseases showed increased ROS, dysfunctional mitochondria, aberrant inflammatory and apoptotic signaling and perturbed calcium homeostasis, among other biochemical alterations (Platt et al 2012), and modulators of exocytosis have been proposed as general treatment for lysosomal diseases (Xu et al 2012). Central nervous system is the main target for the treatment of these diseases but, to date, no effective therapy exists and most of the compounds used are unable to cross the blood–brain barrier (Valstar et al 2008). CoQ_{10} and other antioxidants play a vital role in maintaining the structure and function of the lysosome enabling membrane fluidity, protecting this organelle from ROS, acidifying intralysosomal medium and restoring calcium homeostasis (Fu et al 2010; Pannuzzo et al 2010; Delgadillo et al 2011; Xu et al 2012; Cornelius et al 2013). Much evidence showed that CoQ_{10} (Artuch et al 2004), α-tocopherol (Vatassery et al 1998; Gabsi et al 2001), lipoic acid (Malińska and Winiarska 2005), and N-acetylcysteine (Erickson et al 2012), the last three included in the antioxidant cocktail used

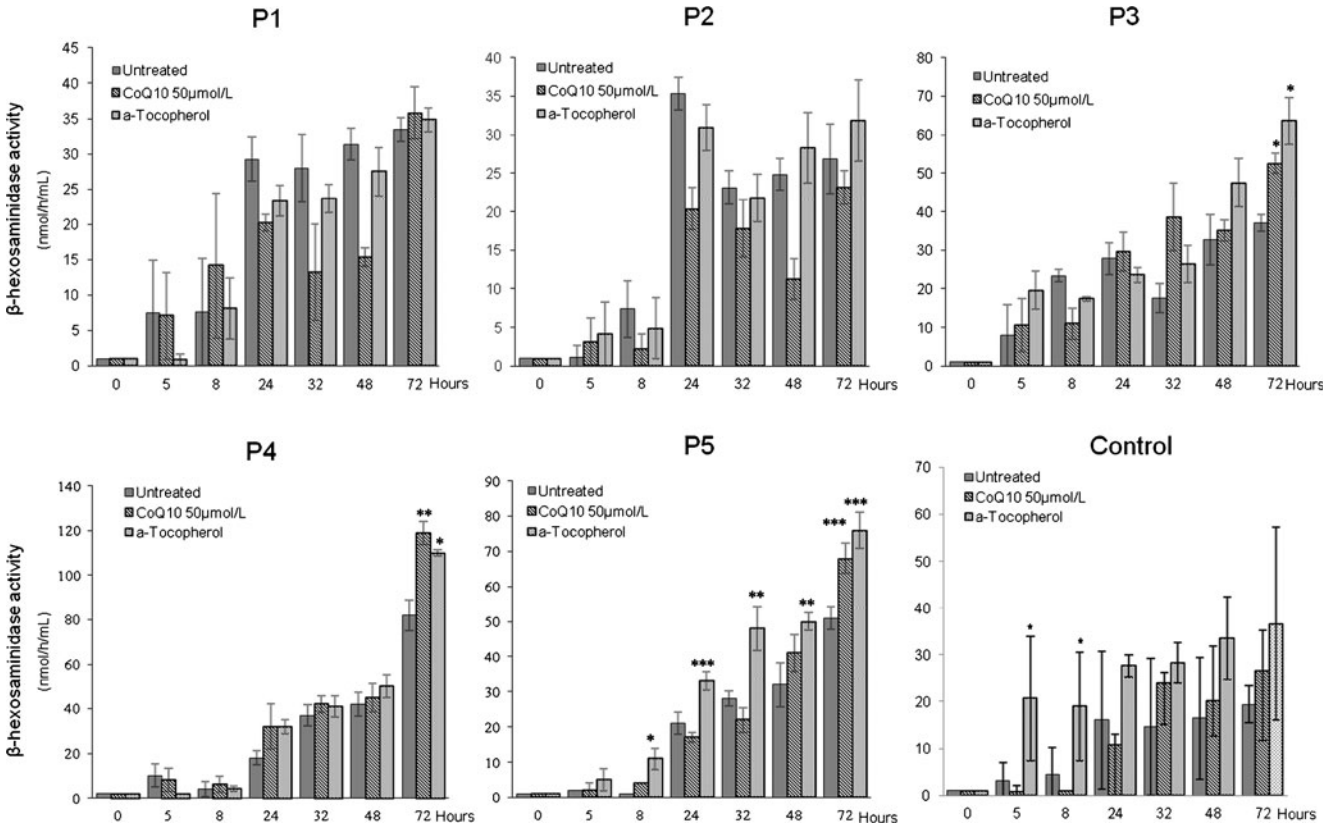

Fig. 3 Lysosomal exocytosis in CoQ_{10} and α-tocopherol treated fibroblasts. Results were obtained by measuring β-hexosaminidase activity in the fibroblasts culture media at different incubation times. All data are shown as mean and standard deviation obtained from three independent experiments in triplicate. T-student statistical test was performed to determine the significance of the results (*$p<0.05$; **$p<0.01$, ***$p<0.001$)

in this study, were able to cross the blood–brain barrier. Therefore, in this work we have studied if CoQ_{10} or an antioxidant cocktail treatment were able to ameliorate the biochemical phenotype in fibroblasts of both Sanfilippo A and B patients.

Basal CoQ_{10} was analyzed in fibroblasts of both Sanfilippo A and B patients. Sanfilippo A fibroblasts showed decreased basal levels. However, no dysfunction in the CoQ_{10} biosynthesis pathways was found, revealing for the first time a secondary CoQ_{10} deficiency in Sanfilippo A fibroblasts.

After CoQ_{10} or the antioxidant supplementation, fibroblasts from P1 and P2 showed neither an increase in Heparan-N-sulfatase activity nor a decrease in GAGs concentration (Fig. 2, Supplementary Table 2). Surprisingly, although P3 did not show a significant increase in the residual enzymatic activity (Fig. 1a), GAGs accumulation decreased drastically (Fig. 2b). Concerning P4 and P5, both affected of Sanfilippo B disease, it is interesting to remark a slight increase in the residual enzymatic activity (Fig. 2a) in parallel with a significant decrease of GAGs (Fig. 2b). The possibility that the differences in the enzymatic activities are influenced by the basal intracellular levels of CoQ_{10} has been excluded as the intracellular concentrations, after this supraphysiological supplementation, do not differ among patients (data not shown). Nevertheless, it has been reported (Turunen et al 2004; Crane 2001) that after CoQ_{10} supplementation, changes in physicochemical properties of the lysosomes occur. It might be that the different response to treatment between Sanfilippo A and Sanfilippo B is due to different physicochemical characteristics of both enzymes, that may allow a slight recovery of the α-N-acetylglucosaminidase activity, while Heparan-N-sulfatase would present different physicochemical characteristics insensitive to any modulation by supraphysiological levels of CoQ_{10}. However, GAGs decrease could not be explained by this slight increase of the enzymatic activity. In addition, P3 showed the most significant decrease of GAGs while the enzymatic activity, remained unaltered. We wondered if, as it has been described for tocopherol (Xu et al 2012), the reduction of accumulated substrate may be due to an increase in exocytosis. We measured it through the activity of β-hexosaminidase in the culture media. Results showed that patients (P3, P4, and P5) which decreased their intracellular content of GAGs (Fig. 2b, Supplementary Table 2) significantly increased β-hexosaminidase activity in the culture media (Fig. 3), indicating an enhanced exocytosis. These results are also supported by the role of CoQ_{10} in calcium

homeostasis regulation, which is necessary for the exocytosis (Crane 2001). Concerning treatment with the antioxidant cocktail, we observed similar results to those found for CoQ_{10}, as is shown in Fig. 3. To avoid any influence of the changes that may occur during 72 h of incubation we monitored treated and untreated fibroblasts at each time point and at each concentration within the same cell line. Therefore, the most likely explanation for an increased exocytosis is the treatment, but it does not explain differences between patients. Xu et al (2012) suggested that treatments enhancing the exocytosis should be independent of either the mutant enzyme or the storage material, but this logical principle could not be verified in our patients.

In theory, the genotype is indirectly related with the enzymatic activity but not with the exocytosis mechanism. Both patients suffering from Sanfilippo B disease (P4 and P5) showed a slight increase of their enzymatic activity (Fig. 1a). The mutation carried by P4 in *NAGLU* gene (p.Arg38Trp in homozygosity) is a non-conservative amino acid substitution which disrupts the secondary structure without affecting the active site of the protein (Beesley et al 2005). Mutations of P5 p.[Trp168*];[Gln566*] lead to premature termination codons (PTC) potentially recognized and degraded by the nonsense mediated mRNA decay (NMD) surveillance mechanism. However, it is known that RNA transcripts with a PTC located in the last exon of the gene, as it occurs for p.Gln566* mutant, may elude the NMD surveillance mechanism (Singh and Lykke-Andersen 2003). In both cases treatment may help a small percentage of the mutant protein to reach the lysosome, leading to a less severe pathogenic cascade of events. Nevertheless, the different exocytosis response remains to be explained.

Altogether, our results point that some biochemical alterations in fibroblasts caused by Sanfilippo disease can be partially restored by CoQ_{10} or other potent antioxidant supplementations with different efficiencies depending on the characteristics of each patient. Results are encouraging, but further studies, including those in mouse models are necessary before clinical trials in patients can be considered.

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Conflict of interest None.

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