Quantitative Real Time PCR in Friedreich’s Ataxia: implications for diagnosis and clinical trial design

Giorgia Puorro¹, Antonella Antenora¹, Angela Marsili¹, Alessandra Denaro¹, Raffaele Piro², Pierpaolo Sorrentino¹, Chiara Pane¹, Alessandra Tessa², Sergio Cocozza³, Filippo Santorelli², Giuseppe De Michele¹, Alessandro Fill¹, Francesco Sacc¹

INTRODUCTION
Ataxia is the loss of the ability to coordinate voluntary muscular movement. Friedreich’s ataxia (FRDA), an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia among Caucasians. The molecular defect in FRDA is the trinucleotide GAA expansion in the first intron of the FXN gene. Most patients are homozygous for this mutation. Two to 5% of patients harbor a point mutation on one allele and a GAA expansion on the other allele. The FXN gene encodes a 210 amino acid mitochondrial protein named frataxin, that is involved in iron–sulphur cluster and heme biogenesis, iron binding/storage and iron chaperone activity. Aim of the study was to screen a population of FRDA patients, carriers, and controls for FXN mRNA levels, and to determine the utility of q-PCR as a biomarker and diagnostic tool.

RESULTS
We enrolled 27 patients with classic FRDA phenotype (cFA), 6 late onset FRDA (LOFA), 5 compound heterozygotes for expansion and point mutations (pFA; 1154F, IVS4+3delA, R165P), 33 healthy expansion carriers, and 30 healthy controls. In cFA, FXN mRNA was profoundly reduced to 19.4% of controls (range 0.35-0.85, p<0.0001). In LOFA, FXN mRNA was reduced to 50.4% (range 0.35-0.85, p<0.0001, area=1.00). Comparison of pFA with controls resulted in a profoundly reduced to 19.4% of controls (range 0.06-0.48, p<0.0001). In healthy expansion carriers, FXN mRNA dosage was assessed constructing ROC curves. Correlation analysis was performed calculating Pearson’s coefficient. P values of less than 0.05 were considered statistically significant.

Table 1: demographics and genotyping for all enrolled subjects: classic FRDA (cFA), late onset FRDA (LOFA), carriers, and controls. All data are presented as mean±SD.

Figure 1: FXN mRNA levels in PBMCs. Box and whiskers plots (min to max) of FXN mRNA relative expression levels in cFA (n=27), LOFA (n=6), carriers (n=33), controls (n=30), and pFA (n=5). Statistical significance for all groups compared to controls is p<0.0001.

DISCUSSION
We report the first explorative study on FXN mRNA levels in PBMCs from a cohort of FRDA patients, carriers and healthy controls. FRDA patients showed reduced levels of FXN mRNA to one-fifth of control levels. Messenger RNA levels proved to be diagnostic when comparing cFA to controls with 100% sensitivity and specificity. In contrast, q-PCR is not able to differentiate pFA and controls. Clinical trials should be designed to include FXN mRNA levels as an endpoint.

ACKNOWLEDGEMENTS
This study was supported in part by “Associazione il Cuore in un Dono” Cardito, Napoli, “Associazione Italiana per la lotta alle Sindromi Atassiche” (AISA) sez. Campania, Napoli and grants from Fondazione “Cassa di Risparmio di Livorno” and the Italian Ministry of Health. We thank all the patients and their families for their willingness to take part to this research project.