RESEARCH ARTICLE

Unusual Age-Dependent Behavior of Leukocytes Telomere Length in Friedreich's Ataxia

Daniela Scarabino, PhD,[1] Liana Veneziano, PhD,[2,3] Suran Nethisinghe, PhD,[4] Elide Mantuano, MSc,[2] Alessia Fiore, MSc,[5] Giulia Granata, MSc,[5] Nita Solanky, PhD,[4] Ginevra Zanni, MD, PhD,[6] Francesca Cavalcanti, MD,[7] Rosa Maria Corbo, MD,[5] and Paola Giunti, MD, PhD[4*]

[1]*Institute of Molecular Biology and Pathology, National Research Council (CNR), Rome, Italy*
[2]*Institute of Translational Pharmacology, National Research Council (CNR), Rome, Italy*
[3]*Human Functional Genomics Laboratory, IRCCS San Raffaele, Rome, Italy*
[4]*Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom*
[5]*Department of Biology and Biotechnology, La Sapienza University, Rome, Italy*
[6]*Unit of Muscular and Neurodegenerative Disorders, Unit of Developmental Neurology, Bambino Gesù Children's Research Hospital, IRCCS, Rome, Italy*
[7]*Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR), Mangone, Italy*

ABSTRACT: Background: Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by an expanded GAA repeat in the first intron of the *FXN* gene.

Objective: The aim of this study was to analyze leukocyte telomeres length (LTL) in FRDA to verify the possible relationships between LTL and disease progression. We investigated LTL in a cohort of FRDA biallelic patients (n = 61), heterozygous (n = 29), and age-matched healthy subjects (n = 87).

Methods: LTL was measured by real-time polymerase chain reaction quantitative analysis (qPCR).

Results: The results showed that before 35 years of age, leukocyte telomeres were longer in patients than in controls, whereas the reverse applies in patients above 36 years of age. Interestingly, LTL was greater than controls at any age in heterozygous subjects. This picture mirrors what has been previously observed in vitro in FRDA cultured fibroblasts, showing significantly longer telomeres at early passages because of activation of an alternative lengthening of telomeres (ALT)-like mechanism, but showing accelerated telomere shortening as population doubling increases. GAA1 repeat length is positively correlated with the LTL and negatively correlated with the age at blood sampling. The relationship of LTL with clinical parameters (cardiomyopathy, diabetes, dependence on a wheelchair) was also analyzed. Significantly shorter leukocyte telomeres were associated with the presence of cardiomyopathy, but not with diabetes and the dependence on a wheelchair.

Conclusions: Overall, the present study indicates that telomere length analysis in FRDA may be a relevant biomarker for following the stages of the disease. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: Friedreich Ataxia; Leukocyte Telomere Length; Neurodegenerative diseases; Oxidative stress

***Correspondence to:** Dr. P. Giunti, Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, WC1E 6BT, UK; E-mail: p.giunti@ucl.ac.uk.

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Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by an expanded GAA repeat in the first intron of the *FXN* gene (Online Mendelian Inheritance in Man [OMIM] *606829) on the proximal long arm of chromosome 9 coding for frataxin. It is one of the most common forms of autosomal recessive ataxia, occurring in approximately one in 50,000 individuals (OMIM 229300). FRDA is characterized by progressive gait and limb ataxia, poor coordination, dysarthria, dysphagia, eye movement abnormalities, leg weakness, areflexia, sensory loss, scoliosis, foot deformities, cardiomyopathy, and diabetes.[1–5] The neurological phenotype reflects lesions in dorsal root ganglia (DRG), sensory peripheral nerves, corticospinal tracts, and dentate nuclei (DN),[6,7] but the involvement of the heart, skeleton, and endocrine pancreas indicates that FRDA should be viewed as a multisystem condition.[5]

Normal individuals have five to 30 GAA repeat expansions, whereas 96% of FRDA patients have biallelic GAA repeat expansions ranging from 66 to more than 1000 repeats, leading to a drastic decline in the transcription of frataxin mRNA and consequently reduced levels of frataxin protein.[2,8,9] The remaining 4% are compound heterozygotes for a GAA repeat expansion on one allele and a point mutation or deletion on the other.[10] To date, only one family has been reported to have a homozygous point mutation in both alleles.[11] Alleles with an intermediate number of GAA repeats can expand further to cause FRDA, with 34 to 65 GAA triplets the estimated instability threshold.[7] In FRDA biallelic patients, the number of GAA repeats in the smaller allele (GAA1) inversely correlates with the age of onset, with a reduction in age of onset by 2.3 years for every 100 GAA repeats in the smaller allele.[2,12] Onset is usually in the first or second decade, before the end of puberty. However, few cases with late onset of FRDA between 26 and 39 years and very late onset of FRDA at 40 years or older have been reported.[12,13] The size of the smaller GAA1 alleles is also associated with the strongest genotype–phenotype correlation.[14] Recently, we demonstrated that interruption at the 3′ end of the repeat can delay the age at onset by ~10 years. Therefore, the consideration of interruptions at the 3′ end is crucial when delivering a diagnosis to patients because they have an impact on their prognosis.[15]

Individuals who are heterozygous carriers of the triplet GAA expansion have frataxin levels reduced by ~50% of normal levels, but do not manifest clinical symptoms.[16]

Frataxin is a nuclear-encoded mitochondrial iron chaperone involved in iron–sulfur biogenesis and heme biosynthesis. Some studies have also suggested that frataxin may function as an iron storage molecule, an antioxidant, and a tumor suppressor, as well as in lipid peroxidation and calcium deregulation.[17–19]

Frataxin deficiency inhibits mitochondrial respiration and promotes the production of reactive oxygen species (ROS), causing mitochondrial dysfunction, oxidative stress, mitochondrial iron accumulation, and inflammation.[20,21]

Expanded GAA repeats produce fewer *FXN* mRNA transcripts, reducing frataxin protein expression. The amount of residual frataxin in FRDA patients ranges from 30% to 2%, depending on the extent of the silencing, which is linked to the number of GAA-triplet repeats.[22,23] Two mechanisms appear to underlie the reduced expression of the *FXN* gene. It was initially suggested that the reduced expression could be explained by abnormal DNA or DNA/RNA hybrid structures adopted by the GAA repeat expansion, which may interfere with *FXN* gene transcription.[7] Subsequently, examining brain tissue, peripheral blood leukocytes, and lymphoblastoid cells from FRDA individuals with an *FXN* mRNA deficit, it was observed that the region flanking the repeat in these cells was aberrantly methylated and enriched for histone modifications, characteristic of genes that are transcriptionally repressed.[24,25] The data analysis suggested that repeat-induced chromatin changes were responsible for reducing both mRNA transcription initiation and elongation in FRDA cells.[24]

Telomeres are nucleoprotein structures located at the ends of eukaryotic chromosomes and protect the free DNA ends from recombination or degradation events. Human telomeres are characterized by tandemly repeated TTAGGG sequences, typically spanning between 4 and 10 kb in length. Because telomere sequences are not fully replicated during DNA replication, due to the inability of DNA polymerase to replicate the 3′ end of the DNA strand, telomeres shorten with each round of replication. If a critically short telomere length is reached, the cell will undergo apoptosis or enter a state of replicative senescence.[26] A cellular ribonucleoprotein enzyme complex, called telomerase, counteracts telomere shortening,[27,28] but its activity, usually present in the early stages of embryonic development, is almost absent in adult tissues, including skin, kidney, liver, blood vessels, and peripheral leukocytes. Consequently, the telomeres of replicating cells shorten progressively, and this mechanism is thought to underlie aging and age-associated diseases.[29–31] However, some tumor cells, to bypass cellular senescence, can avoid telomere attrition by a telomerase-independent mechanism termed Alternative Lengthening of Telomeres (ALT), which is based on a mechanism of homologous recombination between telomeric repeats, known as telomeric recombination between sister chromatids.[32]

Studies at the population level of leukocyte telomere length (LTL) have provided ample evidence that leukocyte telomere shortening is associated with aging[29–31,33]

and with chronic diseases of advanced age such as cardiovascular disease, type 2 diabetes, or metabolic syndrome, although some inconsistencies have been observed.[34–36] LTL was also investigated in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and polyglutamine diseases (Huntington's disease [HD], spinocerebellar ataxias [SCA]1, SCA2 and SCA3). Recently, we identified differences in telomere length in SCA1, SCA2, and SCA3, highlighting that despite the common type of mutation, each condition has a distinct biological mechanism.[37]

Reduced LTL was frequently observed in AD, HD, SCA1, and SCA3,[37–40] but no consistent evidence of shorter telomeres in PD was reported.[41] A common hallmark of AD, HD, SCA1, and SCA3 physiopathology is the triggering of the immune system associated with microglia activation. This, in turn, will result in the production and release of cytokines promoting inflammation and ROS, leading to oxidative stress.[42,43] The involvement of the peripheral immune system may promote leukocyte division and telomere shortening, and the overall rate of leukocyte telomere length reduction would be a measure of the inflammatory component and oxidative damage in disease development.[43–45]

Frataxin deficiency in FRDA leads to oxidative stress, mitochondria dysfunction, and inflammation.[46] Emerging evidence indicates that frataxin knockdown results in direct microglial activation and that neuroinflammation may also be a key feature in the pathophysiology of FRDA.[6,47] In line with these data, reduced telomere length in FRDA peripheral blood leukocytes was observed.[48,49] However, in an *in vitro* study on cultured FRDA human fibroblasts,[49] a significant initial increase in telomere length was observed, followed by an accelerated telomere shortening. This phenomenon was shown to be because of an initial ALT-like activation mechanism, which probably was not sufficient to overcome the action of oxidative stress and defective DNA repair mechanisms of FRDA cells in further cellular replication.

The partial inconsistency between the *in vivo* and *in vitro* FRDA telomere length studies prompted us to analyze LTL in a large cohort with a wide age range, given the known dependence of LTL on age. A further aim of the study was to verify the possible relationships between LTL and disease progression, which in FRDA is associated with cardiomyopathy and diabetes, two diseases known to be associated with reduced LTL.

Materials and Methods

Subjects

A cohort of 61 FRDA biallelic patients, 5 compound heterozygous patients (Table S1), and 29 healthy carriers was collected at the Ataxia Centre, National Hospital for Neurology and Neurosurgery UCL/UCLH, London, United Kingdom (UK) and at the Institute for Biomedical Research and Innovation (IRIB, CNR). Clinical data (age, gender, age at onset, and disease duration) were obtained from participants. Ethical approval was obtained from the London-Queen Square Research Ethics Committee (reference 09/H0716/53).

The control subjects are made up of healthy donors recruited at the Department of Experimental Medicine and Surgery of the University of Tor Vergata, Rome. The control sample consists of age- and sex-matched healthy subjects. The demographic and clinical characteristics of FRDA biallelic patients, carriers, and controls are reported in Table 1.

Written, informed consent was obtained from all participants. The study was conducted according to the guidelines of the Declaration of Helsinki.

TABLE 1 *Demographic and clinical characteristics of FRDA biallelic patients, carriers, and controls*

	Controls n = 87	FRDA carriers n = 29	FRDA patients n = 61
Age at blood sampling (years)	35.66 ± 15.7 (range, 3–81)	38.21 ± 20 (range, 3–80)	31.9 ± 13.7 (range, 4–65)
Sex (males, %)	45.5	44.8	45.8
GAA number (median and range)	NA	GAA1:800, (90–1166)	GAA1: 700, (67–1200) GAA2: 1000, (200–1500)
Age at onset (years)	NA	NA	13.9 ± 9.3
Presence of cardiomyopathy (%)	NA	NA	33.9 (n = 59)
Presence of diabetes (%)	NA	NA	11.9 (n = 59)
Dependence on a wheelchair (%)	NA	NA	51.7 (n = 57)
Disease duration (years)	NA	NA	17.8 ± 12.4
SARA scores	NA	NA	24.8 ± 11.7 (n = 22)

For each FRDA patient and carrier, age- and sex-matching controls were included in the study. Data are shown as mean ± standard deviation.
Abbreviations: FRDA, Friedreich ataxia; NA, not applicable; SARA, Scale for the Assessment and Rating of Ataxia.

Laboratory Methods

Genomic DNA was extracted from patient peripheral blood leukocytes using a FlexiGene DNA kit (QIAGEN Hilden, Germany), according to the manufacturer's instructions. FRDA molecular diagnosis was performed according to Ciotti et al[50] and Filla et al.[13]

LTL was measured by monoplex real-time polymerase chain reaction quantitative analysis (monoplex qPCR) on a 7300 real-time PCR instrument (Applied Biosystems, Waltham, MA, USA). This method allows the determination of the number of copies of telomeric repeats (T) compared to a single-copy gene (S) used as a quantitative control (T/S ratio).[51] The telomere and single-copy gene β-globin (HGB) were analyzed on the same plate to reduce inter-assay variability. DNA (35 ng) was amplified in a total volume of 20 µL containing 10 µL of SYBR Select Master Mix (Applied Biosystems); primers for telomeres and the single-copy gene were added to final concentrations of 0.1 µM (Tel Fw), 0.9 µM (Tel Rev), and 0.3 µM (HGB Fw), 0.7 µM (HGB Rev), respectively. The primer sequences were: Tel Fw 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; Tel Rev 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′; HGB Fw 5′-GCTTCTGACACAACTGTGTTCACTAGCAAC-3′; HGB Rev 5′-CACCACCAACTTCATCCACGTTCACCTTGC-3′. The enzyme was activated at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. In addition, two standard curves (one for HGB and one for telomere reactions) were prepared for each plate using a reference DNA sample (Control Genomic Human DNA, Applied Biosystems) diluted in series (dilution factor of 2) to produce five concentrations of DNA ranging from 50 to 6.25 ng in 20 µL. Measurements were performed in triplicates and are reported as a T/S ratio relative to the calibrator sample to allow comparison across runs. Replicate assays of the same sample were carried out to calculate the inter-assay variation. The average standard deviation over three different assays was 4.2%. Therefore, assuming a normal distribution, samples differing in average telomere length by as little as 8.3% (1.96 × standard deviation) should be distinguishable by this method at the 95% confidence interval (CI).[51] No amplification of the negative controls with both primer sets (HGB and telomeres) was observed.

Statistical Analysis

Statistical analyses were performed using Statistix software (version 8.0; Analytical Software, Tallahassee, FL, USA). Parametric (analysis of variance) and non-parametric (Kruskal–Wallis/Wilcoxon rank-sum test) tests were used to compare the distribution of quantitative variables between patients, carriers, and controls and the distribution of the mean T/S ratio across age and repeat number classes. The level of significance was set at $P < 0.05$. The relationship between the T/S ratio and age at blood sampling was evaluated by regression analysis. When necessary, LTL was adjusted for age by multiple regression. A comparison of the regression lines slopes was carried out by a *t* test.

Results

Subjects

The FRDA cohort comprised 61 patients with biallelic GAA repeat expansions, 5 compound heterozygous patients (Table S1), 29 heterozygous subjects, and 87 controls. The demographic, genetic, and clinical features of the FRDA cohorts and controls are summarized in Table 1. No significant differences were observed among the ages of blood sampling among the three groups ($P = 0.11$). The number of GAA repeats of the shorter allele (GAA1) ranges from 67 to 1200 and that of the larger allele (GAA2) from 200 to 1500. The mean age at onset was 14 years, and the mean disease duration was 18 years (Table 1). At the time of blood collection, 51.7% of patients were dependent on a wheelchair, 34% were affected by cardiomyopathy, and 12% by diabetes (Table 1). For a subgroup of patients (n = 22), Scale for the Assessment and Rating of Ataxia (SARA) scores were available (mean, 24.8).

LTL Analysis

LTL is expressed as a T/S ratio, where T is the number of copies of telomeric repeats, and S is a single-copy gene used as a quantitative control. LTL was measured in FRDA biallelic patients, carriers, and controls. No significant difference ($P = 0.08$) was observed between patients and controls, with a slight trend of longer LTL in FRDA patients (median, 1.03; Q1: 0.96; Q3:1.13) compared to controls (median, 1.02; Q1: 0.97; Q3:1.08) (Fig. 1). In the heterozygous subjects, the median LTL value was 1.09 (Q1: 1.01; Q3: 1.17) and was significantly different from controls ($P = 0.012$), but not from FRDA biallelic patients ($P = 0.11$) (Fig. 1). There were five compound heterozygous patients in our cohort whose LTL showed a similar trend to the biallelic patients (Table S1).

No difference in median LTL was observed between males and females in the three groups (controls: $P = 0.35$, FRDA patients: $P = 0.37$, FRDA carriers: $P = 0.84$).

Relationship between LTL and Age at Blood Sampling

Because there is a recognized relationship between LTL and age, linear regression analysis of LTL and age at blood sampling was carried out. The analysis showed in the control group an expected significant

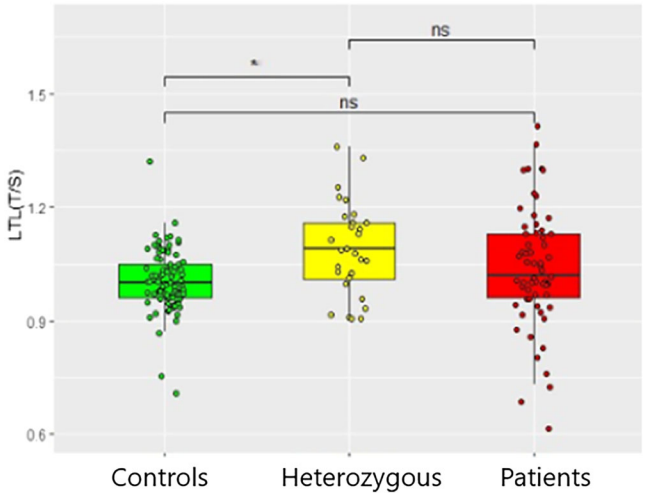

FIG. 1. Distribution of Leukocyte Telomeres Length (LTL) in controls, Friedreich ataxia (FRDA) heterozygous, and biallelic patients. Box plots showing the distribution of LTL expressed as a relative telomere length T/S ratio. Comparison of LTL between control subjects and FRDA patients showed no significant difference ($P = 0.08$). The LTL value of heterozygous subjects was statistically different from controls ($P = 0.012$,) but not from patients ($P = 0.11$). [Color figure can be viewed at wileyonlinelibrary.com]

negative relationship ($y = -0.004x + 1.13$, $P < 0.0001$, 95% CI, $-0.0050 \leq \beta \leq -0.003$). A similar negative relationship of LTL with age at blood draw was observed in the FRDA patient group ($y = -0.009x + 1.33$, $P < 0.0001$, 95% CI, $-0.011 \leq \beta \leq -0.007$) and in FRDA heterozygous group ($-0.005x + 1.27$, $P < 0.0001$, 95% CI, $-0.006 \leq \beta \leq -0.004$) (Fig. 2).

The regression lines of controls and heterozygotes showed similar slopes ($P = 0.12$), but significantly different intercept values ($P < 0.0001$), indicating that the LTL values in heterozygous subjects were higher than controls at any age. On the contrary, the slopes of regression lines of controls and patients differed significantly ($P < 0.0001$) and intersected at the age of ~35 years. These results indicate that at the age of <35 years, LTL values are greater in patients than in controls, but after 35 years, LTL values are lower in patients than in controls. Regression lines of patients and heterozygotes differed significantly for both intercept ($P < 0.0001$) and slopes ($P < 0.001$) and intersected at the age of about 20 years. The LTL of young patients was higher than that of heterozygote carriers. As shown in more detail in Table 2, LTL values in FRDA patients age 35 years or younger were significantly higher than in controls, whereas they were significantly lower than controls in patients age 50 and over. In heterozygous carriers, the trend for longer LTLs than both controls and patients (older than 20 years) is confirmed.

Relationship between LTL and GAA1 Repeat Number

The relationship between LTL and the GAA1 allele, the allele with the greatest influence on the age at onset and the strongest genotype–phenotype correlation, was then analyzed. A significant trend ($P < 0.0001$, $R = 0.48$) for the LTL to increase with increasing number of GAA1 repeats was observed

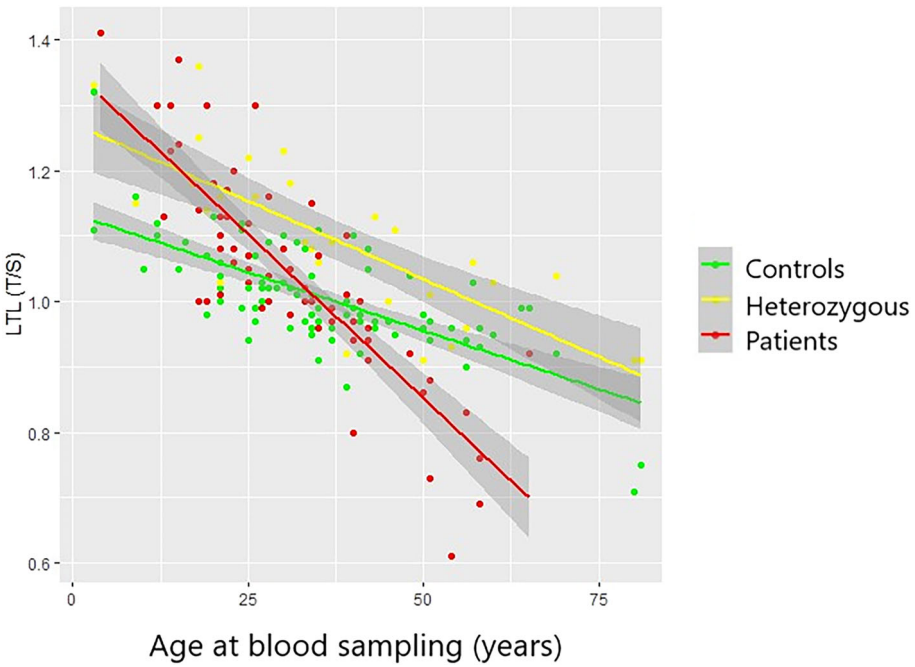

FIG. 2. Relationship between leukocyte telomeres length (LTL) and age at blood sampling. LTL expressed as a relative telomere length T/S ratio as a function of age at blood sampling in Friedreich ataxia (FRDA) biallelic patients (n = 61; red dots), controls (n = 87; green dots), and FRDA heterozygous carriers (n = 29; yellow dots). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 *LTL (T/S) distribution by age at blood sampling classes (median, Q1, and Q3 [n]) in controls, FRDA heterozygous, and biallelic patients*

Age class (years)	LTL controls (n)	LTL FRDA carriers (n)	LTL FRDA patients (n)	*P* value (KW)
≤20	1.10, 0.98–1.13 (12)	1.22, 1.15–1.34 (6)	1.24, 1.13–1.30 (12)	0.009
21–35	1.02, 0.99–1.05 (38)	1.16, 1.07–1.20 (9)	1.07, 1.0–1.13 (28)	0.0001
36–50	0.98, 0.96–1.05 (21)	1.05, 0.92–1.12 (6)	0.95, 0.92–0.99 (14)	0.14
≥51	0.95, 0.92–0.97 (16)	0.99, 0.92–1.04 (8)	0.76, 0.69–0.88 (7)	0.002

There is a statistically significant difference at all ages between patients, controls, and carriers except in the 36–50 age group.
Abbreviations: LTL, leukocyte telomeres length; FRDA, Friedreich ataxia; KW, Kruskal–Wallis test.

TABLE 3 *Distribution of LTL (T/S) and age at blood sampling (median, Q1, and Q3) by GAA1 repeat number classes*

GAA1 repeat number (n)	LTL FRDA patients (T/S)	Age at blood sampling (years)
67–467 (13)	0.94, 0.90–1.01	40, 33.5–54.5
468–680 (11)	1.0, 0.97–1.06	28, 23–40
681–834 (17)	1.08, 1.0–1.14	25, 20.5–36.5
835–1200 (12)	1.13, 1.05–1.30	22, 14.3–33.3
P value	0.0012	0.0008

The LTL values increase with increasing number of GAA1 repeats ($P = 0.0012$, Kruskall– Wallis), whereas at the same time age at blood sampling decreases ($P = 0.0008$).
Abbreviations: LTL, leukocyte telomeres length; FRDA, Friedreich ataxia.

(Spearman rank correlation test). However, the relationship between the number of GAA1 and LTL became statistically less significant ($P = 0.01$, $R = 0.33$) when LTL was adjusted for age at blood sampling. The complex relationship between LTL, age at collection, and number of GAA1 repeats is easily understood by looking at Table 3. The four GAA1 classes were established according to the values of the quartile distribution of the GAA1 alleles in the sample under study. As you can see in Table 3, the relationship between the GAA1 allele repeat number and LTL was, at least in part, dependent on the negative relationship between age at collection and the GAA1 number. A significant association was observed between LTL adjusted for age and GAA2 repeat number ($P = 0.003$, $R = 0.41$) as well, whereas no relationship was observed between GAA repeat number and LTL in heterozygotes ($P = 0.52$, $R = 0.14$).

Relationship between LTL and Disease Duration

The effect of disease duration on telomere length was examined in FRDA patients. A negative linear relationship was observed between LTL and duration of the disease ($P < 0.0001$), which was highly dependent on age because the relationship was no longer significant ($P = 0.79$) by applying a multiple regression model, including age at collection as an independent variable.

Relationship between LTL and Clinical Parameters

LTL values adjusted for age at blood sampling were compared between subjects affected or not by cardiomyopathy, diabetes, and dependence on a wheelchair (Fig. 3). Lower LTL values were associated with the presence of cardiomyopathy ($P = 0.033$). The association with the presence of diabetes was not significant ($P = 0.057$), the former possibly because of the small number of affected patients (n = 7) and was also absent with wheelchair

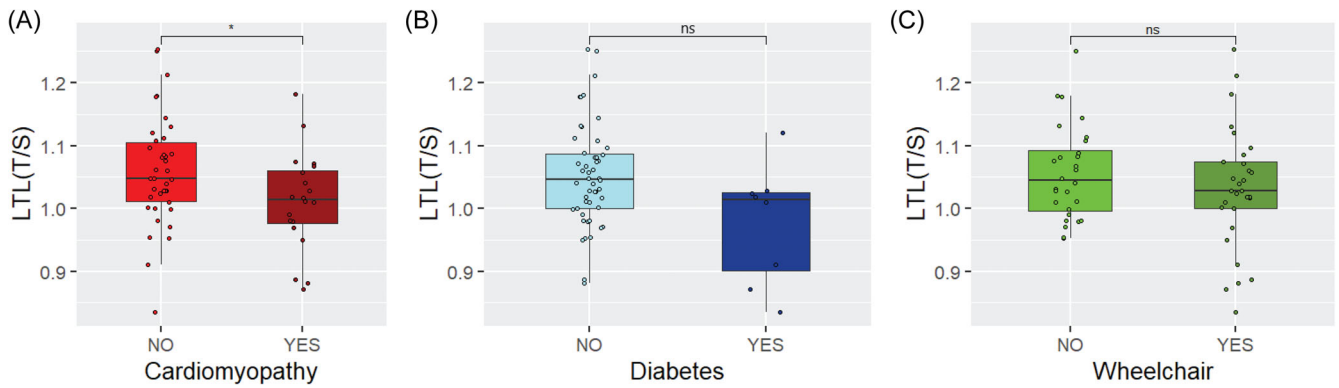

FIG. 3. Relationship between leukocyte telomeres length (LTL) and clinical parameters. Box plots showing the distribution of LTL expressed as a relative telomere length T/S ratio, compared between subjects affected or not by (**A**) cardiomyopathy, (**B**) diabetes, and (**C**) dependence on a wheelchair. [Color figure can be viewed at wileyonlinelibrary.com]

dependence ($P = 0.38$). For a subgroup of patients (n = 22), SARA scores were available corresponding to age at sampling. No statistically significant relationship was observed between SARA scores and LTL (Spearman rank correlation test, $R = 0.19$, $P = 0.40$).

Discussion

There is wide evidence that alterations in telomere length are critical factors in conditions associated with increased systemic oxidative stress and chronic inflammation,[52] such as neurodegenerative disorders. Leukocyte telomere shortening was found to be a common hallmark of AD, HD, and some SCAs,[37–40,45,53,54] but the same was not observed for PD or amyotrophic lateral sclerosis, and conflicting data have been reported.[41,55–57]

In the present study, the LTL data in FRDA showed unusual characteristics. Examining the cohort as a whole, the median LTL values of FRDA biallelic patients were similar to those of controls, but a more detailed analysis highlighted a complex relationship between LTL and age. Leukocyte telomeres were longer in patients with an age at collection of 35 years or less compared to controls, whereas after 36 years, the LTL values of FRDA patients were reduced compared to controls, as if a more rapid shortening occurred in patients after this age. This result is partially inconsistent with another study,[48] in which reduced LTL values were reported in FRDA patients compared to controls, a discrepancy that may depend on the size and age composition of the two study cohorts. The number of FRDA patients in that study was 37 with an age at sampling of 30.6 ± 10.9 S.E. (Standard Error) and GAA1 repeats of 661.65 ± 35.02 S.E. Our cohort has a higher number of participants and a more variable phenotype including age at onset, age at sampling, but also number of GAA1 repeats better representing the complexity of the disease. This allowed us to identify a significant positive correlation between LTL and GAA1 repeat sizes that was not observed in the previous study. A significant association was also observed between LTL and the GAA2 allele, probably because of the fact that the age at onset is inversely correlated with the GAA2 allele as well, although to a reduced degree compared with the GAA1 allele.[2]

The absence of association between the GAA number and LTL in heterozygotes can be explained by hypothesizing that a 50% reduction in frataxin is sufficient to activate ALT, but not to induce disease. Therefore, telomere shortening in heterozygotes is only because of age, with a trend similar to that observed in controls, as shown in Figure 2.

Interestingly, the results reported here appear to be in line with an *in vitro* study on FRDA cultured fibroblasts,[49] where telomere length was found to be significantly longer than controls after the initial seven passages, but it decreased more rapidly than control fibroblasts after 15 and 26 passages. The same authors also showed that FRDA fibroblasts did not maintain long telomeres because of telomerase activity, but rather because of the activation of a mechanism similar to ALT. The activation of ALT, possibly induced by oxidative damage present in FRDA cells, however, would not be sufficient to counteract cellular senescence because the telomeres shorten more quickly than the controls in the following steps.[49]

The picture we observed *in vivo*, in leukocytes of FRDA patients, seems to follow closely what was observed *in vitro* in FRDA cultured fibroblasts. This observation may suggest that in leukocytes of younger patients, a presumably ALT-like mechanism is active, which would keep telomeres longer. The observation of longer telomeres in heterozygotes at all ages seems to indicate that a 50% reduction in frataxin[58] is sufficient to activate the ALT mechanism. This mechanism would exhaust its efficiency as the disease progresses in patients over 35 years of age. It could be hypothesized that in addition to oxidative damage, a neuroinflammatory component induced by frataxin deficiency and consequent activation of microglia[46] may contribute to the fast LTL shortening in patients over 35 years. Leukocyte infiltration in the cardiomyopathies of FRDA patients[59] confirms the role of inflammation in the pathology that accompanies FRDA. In line with this picture, the presence of cardiomyopathy showed a significant shortening effect on LTL, in agreement with previous studies where LTL reduction was frequently found to be a common hallmark of cardiovascular disease.[34] Taking into account that no significant relationship was observed between LTL and disease duration, the overall data seem to indicate that telomere shortening, rather than disease duration, may depend on disease progression and the development of non-neurological features such as cardiomyopathy and probably reflects the inflammatory processes that accompany this disease.[52] It is conceivable that the presence of cardiac complications in FRDA patients at this clinical-stage could make a supposed ALT-like mechanism insufficient to counteract leukocyte telomeres shortening. No significant effect of LTL was associated with the dependence on a wheelchair or SARA score. This was unsurprising because this result is similar to what we have observed in HD and SCA,[40] (Scarabino 2023, personal communication). We believe that the LTL is a biomarker capturing oxidative stress and inflammatory processes, whereas SARA and wheelchair use capture the disability aspects of the disease. The presence of diabetes has been frequently associated with short LTL,[34] a trend that was observed in the present cohort as well, despite the number of FRDA patients with diabetes in the sample being quite small (n=7).

A longitudinal study in a larger cohort of patients at different stages of the disease is needed to confirm our data. The present study indicates that telomere length

analysis in FRDA may provide a relevant tool to follow, at the molecular level, the stages of the disease. Further studies in this field could make LTL measurement a useful biomarker of FRDA progression, supporting clinical trial design and further insights into disease mechanisms.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Ataxia Centre, National Hospital for Neurology and Neurosurgery UCL/UCLH, London, UK (ethical approval references: 09/H0716/53).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data that support the findings of this study areavailable from the corresponding author on request. Data are not publicly available due to privacy restrictions.

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Supporting Data

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

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(1) Research project: A. Conception, R.M.C., D.S., L.V., and P.G. B. Organization, D.S., L.V., E., G.Z., F.C., N.S., and P.G: C. Execution, A.F. and D.S. (2) Statistical Analysis: A. Design, R.M.C. B. Execution A.F., G.G. and R:M: C:, C. Review and Critique R.M.C., S.N., L.V., D.S., E.M. G.Z. and P.G., (3) Manuscript: A. Writing of the First Draft: R.M.C., B. Review and Critique: D.S., R.M.C., L.V., E.M., S. N., G.Z. and P.G.

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