Letter to Editor

Title

Peripapillary retinal nerve fibre layer thickness in Friedreich's ataxia: a biomarker for trials?

Running Title

RNFL thickness in FRDA as a biomarker for trials

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Keywords

Friedreich's Ataxia, Optical Coherence Tomography, Biomarker, Scale for Assessment and Rating of Ataxia

Abbreviations

AAO - Age at Onset

FARS - Friedreich’s ataxia rating scale

FDA - Food and Drug Administration

FRDA - Friedreich’s ataxia

GAA1 - GAA repeat sequence length of the shorter allele

ICARS - International cooperative ataxia rating scale

NICE - National Institute of Clinical Excellence

PROM - Patient reported outcome measure

RNFL - Retinal nerve fibre layer

SARA - Scale for assessment and rating of ataxia

TD-OCT - Time domain optical coherence tomography

VA - Visual acuity

Word Count - 1500

Introduction

Friedreich's ataxia (FRDA) is the commonest inherited ataxia (Parkinson *et al.* 2013). The causative mutation is a recessively inherited GAA expansion in intron 1 of the frataxin (*FXN*) gene (Campuzano *et al.* 1997), leading to reduced transcription at the *FXN* locus and thus a decreased amount of the mitochondrial protein frataxin. Insufficient frataxin causes mitochondrial dysfunction and lipid peroxidation causing cell death in dorsal root ganglia, cardiomyocytes and pancreatic islet cells (Abeti *et al.* 2015, 2016). This leads to the clinical phenotype of ataxia with loss of deep tendon reflexes, dysarthria and hypertrophic cardiomyopathy (Parkinson *et al.* 2013).

Many clinical trials have been carried out in FRDA patients though none have shown efficacy in modifying disease progression. Therefore there is a pressing need to identify more sensitive biomarkers which are able to capture disease progression in an objective, quantifiable manner.

Oculomotor abnormalities are common in patients with FRDA, reflecting disruption of brainstem–cerebellar circuits. Findings include saccadic dysmetria and interrupted smooth pursuit, but most commonly square-wave jerks (Furman *et al.* 2008). Optic neuropathy is seen in around 60-70% of patients with FRDA (Fortuna *et al*. 2009) with disc pallor being clinically apparent in 30% (Harding, 1981). Several studies have characterized the retinal changes associated with FRDA using optical coherence tomography (OCT) and described correlations with measures of disease severity, including the International Cooperative Ataxia Rating Scale (ICARS), Friedreich's Ataxia Rating Scale (FARS) and disease duration (Fortuna *et al.* 2009, Noval *et al.* 2011, Seyer *et al.* 2013, Dag *et al.* 2014) (table 1). Our group have investigated a large cohort of subjects with a range of genetically

proven ataxias using OCT and have shown FRDA to be associated with the greatest degree of RNFL thinning (Parkinson *et al.* 2018). We decided to look at this cohort and for the first time demonstrate that retinal nerve fibre layer (RNFL) thickness correlates with the other major ataxia scoring system, the Scale for Assessment and Rating of Ataxia (SARA) (Schmitz-Hubsch *et al.* 2006) and also a quality of life outcome measure the Activities of Daily Living Questionnaire (ADLQ) from the modified FARS assessment. Indeed, the last is a patient reported outcome measure (PROM) that is now increasingly desirable for approval of new drugs by the relevant regulatory authorities.

Materials and Methods

Study population

Fifty two FRDA patients were recruited from the Ataxia Centre of the National Hospital for Neurology and Neurosurgery (NHNN) in London, UK. All patients gave informed consent, the study was given approval by the London Brent Research Ethics Committee (reference 12/LO/1291) and complied with the Declaration of Helsinki.

Ophthalmological assessment

Pupils were first dilated with 1% tropicamide eye drops. RNFL thickness was measured in a circle around the optic disc using the 'Fast RNFL Thickness' acquisition protocol on a time-domain OCT (T-D OCT) device (Stratus, Carl Zeiss Meditec). OCT data were analysed using the proprietary software (Stratus version 4.07), which provides estimates of RNFL thickness in each quadrant around the disc and an average measurement of RNFL thickness around the entire circumference of the disc. Scans were repeated in triplicate, and the results for both eyes averaged. In a small number of cases, imaging of both eyes was not possible for technical reasons. Best corrected visual acuity (VA) using the Snellen chart was recorded from thirty seven out of fifty two subjects and converted into an ordinal scale (supplementary table 1). For analysis, the visual acuity in the better eye was used.

Clinical data

Each participant with FRDA underwent a full neurological examination, including SARA score using a standard protocol. Age at onset (AAO) was taken as the age of first symptoms compatible with a diagnosis of FRDA and disease duration ascertained by subtracting this number from age at examination. Participants filled the ADLQ from the modified FARS examination and also the EQ-5D-3L (Greiner *et al.* 2003).

Genetic analysis

GAA1 repeats were detected following the methods described by Montermini et al. (Montermini *et al.* 1997).

Statistical analysis

Statistical analysis was undertaken using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, 2012) and Microsoft Excel (2010). Data were assessed for normality using Kolmogorov-Smirnov test. Associations between RNFL thickness, clinical data (SARA, ADLQ, EQ-5D-3L, GAA1) and demographic data (age at onset, disease duration) were undertaken using Spearman's rank test for non-parametric data and Pearson's correlation coefficient for normally distributed data. For all analyses, only $p<0.05$ was accepted as statistically significant.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Cohort Characteristics

Demographic and ophthalmic characteristics of our cohort are shown in table 2. Values shown are the mean of the measurements from both eyes. The average peripapillary RNFL thickness across the cohort was 76.3 μm with a range from 39.1 μm to 104.2 μm.

RNFL thickness correlates with multiple predictors and measures of disease severity.

Relationships between the RNFL thickness and the AAO, GAA1 and disease duration were analysed using the Spearman's rank test. The relationship between these three known indicators of disease severity were correlated with RNFL thickness and all found to be significant (r=0.338, p=0.0141, r=-0.422, p=0.003 and r=-0.339, p=0.0143 respectively) demonstrating that the earlier the AAO, the larger the GAA1 and the longer the disease duration, the thinner the RFNL. Analysis using Pearson's correlation coefficient showed that SARA score was also correlated with RNFL thickness (r=-0.457, p=0.0007) demonstrating that as cerebellar function declines, the RNFL becomes thinner. This was also reflected in the ADLQ score from the FARS examination, with higher scores reflecting greater difficulty in completing normal daily activities; here too there was a significant correlation between ADLQ score and RNFL thickness (r=-0.367, p=0.0075). A multiple regression analysis of factors affecting ADLQ score using SARA score and best eye's VA as independent variables gives an adjusted R^2 of 0.768 ($p<0.001$) with both variables making a significant contribution. This demonstrates that degeneration of the optic nerve as well as cerebellar deterioration could be taken as measurements of the day-to-day functioning in the FRDA population.

Discussion

To our knowledge, this is the first time a correlation between the peripapillary RNFL and SARA score in FRDA patients has been demonstrated. This has implications for the use of peripapillary RNFL thickness as a possible biomarker of disease progression in clinical trials. OCT is quick, non-

invasive and widely available making it an attractive method to assess disease progression for large clinical trials. New software allows us to reliably measure the exact area assessed in previous visits, enabling accurate comparison between sequential visits with obvious benefits for trial design. Our results demonstrating the strong correlation between disease duration and RNFL thickness also add weight to the hypothesis set out by Alldredge et al. (Alldredge *et al.* 2003) and back up the findings of Seyer et al. and Noval et al. This correlation was not seen by Fortuna et al. though their analysis only just fell short of achieving significance (p=0.068) and did not have as many subjects nor as great a range of ages as our study. We also confirm the finding that the AAO correlates with peripapillary RNFL thickness, corroborating the data of Fortuna et al. and Seyer et al. In summary, we have demonstrated that the level of neurological disability as measured by the SARA and ADLQ score correlate with RNFL thickness, further supporting the use of OCT as a potential biomarker of disease progression in future clinical trials. The finding that RNFL thickness correlates with the ADLQ score of subjects with FRDA is important because current Food and Drug Administration (FDA) guidelines state that a good outcome measure should capture how a patient "feels, functions or survives". The National Institute for Health and Care Excellence (NICE) also favours the use of PROMs, though in our analysis their current favoured general PROM measure, the EQ-5D-3L did not show any correlation with RNFL thickness (data not shown). This is likely due to the fact that the EQ-5D-3L is not particularly sensitive to disease-specific or patient-important outcomes.

To summarise, the results from this study show that OCT measurements of RNFL thickness have the potential to become a robust and objective biomarker for future treatment trials in FRDA for the following reasons; a) we have demonstrated a correlation with a measure of clinical severity, the SARA score b) in line with the FDA's edicts, RNFL thickness correlates with a patient reported outcome measure and c) OCT is a direct measure of affected neural tissue. Future work will focus on exploring the possibility that RNLF thickness in FRDA will change with the progression of the disease, therefore a longitudinal study is required. If a prospective study proves positive, OCT of the

RNFL will be the first quantifiable biomarker that is readily available and cost effective, thus suitable for multi-centric trials in FRDA.

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Competing interests

The authors report no conflicts of interest.

Supplementary material

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