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Review

Using human pluripotent stem cells to study Friedreich ataxia cardiomyopathy

Duncan E. Crombie [a,b], Martin F. Pera [c], Martin B. Delatycki [d,e,f], Alice Pébay [a,b,*]

[a] Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Australia
[b] Ophthalmology, Department of Surgery, The University of Melbourne, Australia
[c] Department of Anatomy and Neurosciences, The University of Melbourne, Florey Neuroscience & Mental Health Institute, Walter and Eliza Hall Institute of Medical Research, Australia
[d] Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Department of Paediatrics, The University of Melbourne, Australia
[e] School of Psychology and Psychiatry, Monash University, Australia
[f] Clinical Genetics, Austin Health, Australia

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ABSTRACT

Friedreich ataxia (FRDA) is the most common of the inherited ataxias. It is an autosomal recessive disease characterised by degeneration of peripheral sensory neurons, regions of the central nervous system and cardiomyopathy. FRDA is usually due to homozygosity for trinucleotide GAA repeat expansions found within first intron of the *FRATAXIN* (*FXN*) gene, which results in reduced levels of the mitochondrial protein FXN. Reduced FXN protein results in mitochondrial dysfunction and iron accumulation leading to increased oxidative stress and cell death in the nervous system and heart. Yet the precise functions of FXN and the underlying mechanisms leading to disease pathology remain elusive. This is particularly true of the cardiac aspect of FRDA, which remains largely uncharacterized at the cellular level. Here, we summarise current knowledge on experimental models in which to study FRDA cardiomyopathy, with a particular focus on the use of human pluripotent stem cells as a disease model.

1. Introduction

Friedreich ataxia (FRDA) was first described over 150 years ago in a series of papers by physician Nikolaus Friedreich. It is a hereditary degenerative condition that includes neurological and non-neurological symptoms (reviewed in [1]). The predominant neuronal manifestations occur through degeneration of dorsal root ganglia, cerebellar neurons and long tracts of the spinal cord leading to development of ataxia, dysarthia and areflexia of the lower limbs. The main cause of death in FRDA is cardiomyopathy. Approximately 96% of individuals with FRDA are homozygous for an unstable expanded GAA repeat mutation within the first intron of *FXN* [2]. The remaining 4% are compound heterozygous for a GAA expansion in one allele and point mutation/deletion in the other. FXN is a nuclear-encoded mitochondrial protein [3]. The intron 1 GAA expansion interferes with transcription and results in reduced amounts of structurally normal FXN [4]. This interference is thought to be caused by abnormal DNA structures at the site of the GAA repeats as well as aberrant methylation and altered chromatin formation [5]. Furthermore, studies in patient cohorts demonstrated that the length of the GAA repeats, and in particular the shorter of the two alleles, correlate with disease severity whilst being inversely correlated with the age of onset and FXN protein levels [3,6].

2. Cardiac manifestations of FRDA

Cardiomyopathy in individuals with FRDA is usually hypertrophic, with dilated cardiomyopathy being an inconsistent and generally late manifestation. Arrhythmias are also common [7]. In cross-sectional studies, about two-thirds of individuals with FRDA have cardiac hypertrophy on echocardiogram [6,8,9]. The most common findings on echocardiography are increased relative wall thickness with left ventricular wall thickness and left ventricular mass index also commonly seen [9]. Diastolic dysfunction is also commonly seen [9,10]. Over time, in a small number of patients, this evolves to a dilated cardiomyopathy. Systolic function has been reported as generally being normal until late in disease progression [11]. Almost all have T-wave inversion on electrocardiogram [12]. Studies of myocardial energy assessed by ^{31}P nuclear magnetic resonance spectroscopy found a correlation between the level of energy deficit and the extent of the hypertrophy [13]. Arrhythmias and cardiac failure are the major causes of death in people with FRDA [6,7]. In the largest study of mortality in FRDA, cardiac failure alone was the cause of death in 29%, isolated arrhythmia in 5% and a combination of cardiac failure and arrhythmia in a further 5% [7]. The progressive decline of the left ventricular ejection fraction is also associated with a worse prognosis [14]. Some studies have found that the

* Corresponding author at: CERA, University of Melbourne, 32 Gisborne Street, East Melbourne, VIC 3002, Australia.
E-mail address: apebay@unimelb.edu.au (A. Pébay).

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presence of cardiomyopathy does not correlate with the neurological measures or GAA repeat expansion sizes [11,15], whilst others have identified a correlation with severity of the cardiomyopathy and the length of the shorter GAA repeat [14,16]. Recently, a 22-year longitudinal follow up study of patients with FRDA confirmed that patient survival is correlated to cardiac complications, themselves linked to the size of the GAA repeat, where patients with a longer GAA repeat of the smaller allele showed a worse cardiac outcome [14]. This study also identified two prognostic groups: the more common (79%) had relatively stable ejection fraction over time while the less common (21%) had declining ejection fraction and this was associated with worse survival.

Iron accumulation and oxidative stress have generally been considered as the mechanism leading to cardiac dysfunction (Fig. 1). Histological studies of FRDA hearts reveal detectable iron accumulation but also evidence of myocarditis even in hearts from individuals who have had a short disease duration (4 years) [17]. There is also evidence of reduced ATP production and reduced mitochondrial OXPHOS pathway function in the FRDA heart based upon enzyme assays performed on endomyocardial biopsies [18].

3. Functions of FXN

The precise functions of FXN are not clearly understood. Nuclear-encoded FXN protein is synthesised as an immature form that is transported to the mitochondria where it is cleaved by mitochondrial processing peptidase to become a mature protein [19]. Immunoprecipitation and yeast 2-hybrid systems have provided evidence that FXN interacts with the ISCU/NFS1/ISD11 iron–sulphur cluster assembly, which forms a complex that synthesises iron–sulphur clusters [20,21] (Fig. 1). Iron–sulphur cluster containing proteins perform a range of functions that range from electron transfer, enzymatic catalysis, protein structure stability and function as biological sensors of iron and oxidative stress [22] (Fig. 1). Key mitochondrial and extramitochondrial iron–sulphur-containing proteins include complexes I–III of the oxidative phosphorylation (OXPHOS) pathway and aconitase, part of the tricarboxylic cycle. There is substantial evidence in both higher and lower order organisms that reduced FXN expression reduces iron–sulphur cluster synthesis, increases mitochondrial iron-levels and reduces OXPHOS in both higher and lower order organisms [18,23–32]. In addition to its involvement in iron–sulphur cluster synthesis, FXN has been reported to interact with mitochondrial aconitase, where it is thought to provide iron to repair iron–sulphur clusters damaged by oxidative stress, within the enzyme [33]. Therefore at a cellular level, any iron accumulation will affect mitochondrial membrane potential, ion channel function and could increase generation of reactive oxygen species *via* Fenton chemistry, if present as free iron [34]. Furthermore, reduction of iron-clusters and subsequent alteration in the activity of iron–sulphur-containing enzymes, including those in the OXPHOS pathway and aconitase, potentially contribute further to oxidative stress and reduce available energy in the form of ATP for normal cellular functions [35,36]. Additionally, iron–sulphur clusters are sensitive to reactive oxygen species and further exacerbate their deficiencies [22]. Taken together this suggests that reduced FXN increases mitochondrial iron levels, increases oxidative stress, decreases iron–sulphur cluster synthesis and decreases ATP production (Fig. 1).

4. Models of FRDA

Studies of oxidative stress in *Saccharomyces cerevisiae null* mutants, lacking the yeast *fxn* homologue, demonstrate mitochondrial iron

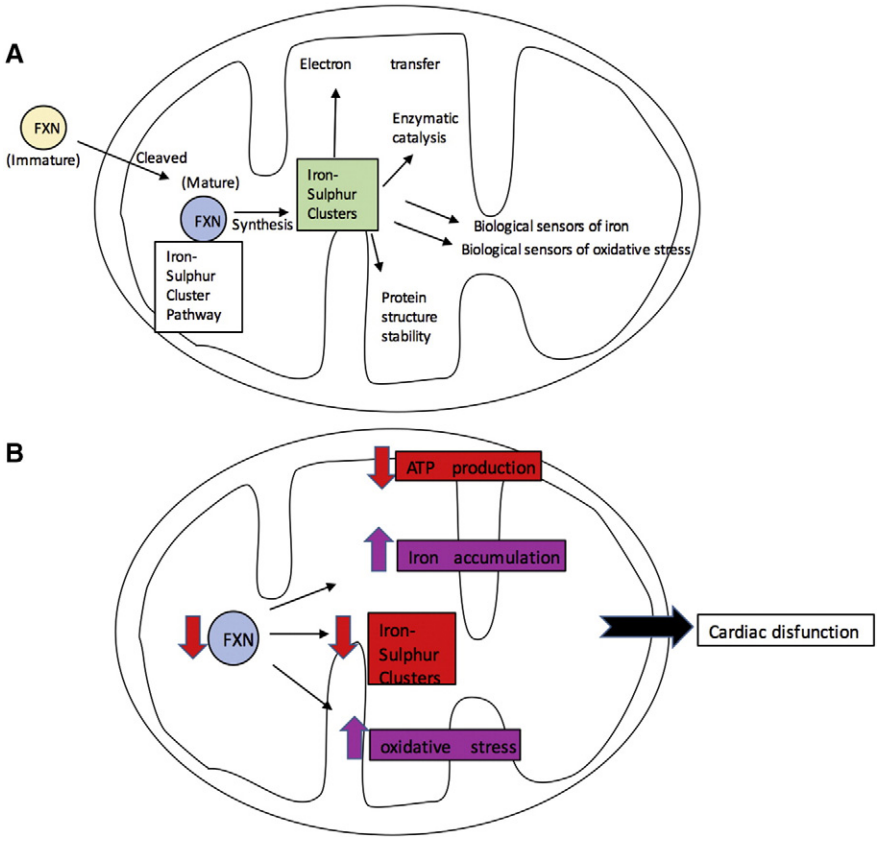

Fig. 1. Schematic illustration of the role of FXN in the mitochondria. (A) The nuclear-encoded immature FXN protein is transported to the mitochondria and cleaved into a mature protein. There, FXN interacts with the iron–sulphur cluster assembly pathway, leading to the synthesis of iron–sulphur clusters. Iron–sulphur cluster containing proteins perform a range of functions that range from electron transfer, enzymatic catalysis, protein structure stability and function as biological sensors of iron and oxidative stress. (B) With reduced FXN levels, there is a reduction of iron-clusters and subsequent alteration in the activity of iron–sulphur-containing enzymes, increase in mitochondrial iron levels, increase in oxidative stress, decrease in iron–sulphur cluster synthesis and decreases in ATP production. These mitochondrial events most likely lead to cardiac dysfunction.