Clinical Investigation

Serum versus Imaging Biomarkers in Friedreich Ataxia

to Indicate Left Ventricular Remodeling and Outcomes

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Patients with Friedreich ataxia typically die of cardiomyopathy, marked by myocardial fibrosis and abnormal left ventricular (LV) geometry. We measured procollagen I carboxyterminal propeptide (PICP), a serum biomarker of collagen production, and characterized genotypes, phenotypes, and outcomes in these patients.

Twenty-nine patients with Friedreich ataxia (mean age, 34.2 ± 2.2 yr) and 29 healthy subjects (mean age, 32.5 ± 1.1 yr) underwent serum PICP measurements. Patients underwent cardiac magnetic resonance imaging and outcome evaluations at baseline and 12 months.

Baseline PICP values were significantly higher in the patients than in the control group (1,048 ± 77 vs 614 ± 23 ng/mL; P <0.001); severity of genetic abnormality did not indicate severity of PICP elevation. Higher PICP levels corresponded to greater LV concentric remodeling only at baseline (r=0.37, P <0.05). Higher baseline PICP strongly indicated subsequent increases in LV end-diastolic volume (r=0.52, P=0.02). The PICP levels did not distinguish between 14 patients with evident myocardial fibrosis identified through positive late gadolinium enhancement and 15 who had no enhancement (1,067 ± 125 vs 1,030 ± 98 ng/mL; P=0.82). At 12 months, cardiac events had occurred in 3 of 14 fibrosis-positive and none of 15 fibrosis-negative patients (P=0.1); their baseline PICP levels were similar.

We conclude that PICP, a serum marker of collagen synthesis, is elevated in Friedreich ataxia and indicates baseline abnormal LV geometry and subsequent dilation. Cardiac magnetic resonance and PICP warrant consideration as complementary biomarkers in therapeutic trials of Friedreich ataxia cardiomyopathy. ***(Tex Heart Inst J 2016; 43(4):305-10)***

Friedreich ataxia (FA), an autosomal recessive disorder with an incidence of 1 in 30,000 to 50,000 persons, is caused by DNA triple-repeats guanine-adenine-adenine (GAA) of the frataxin gene (*FRDA*) on chromosome 9, or rarely from point mutations.[1] The genetic abnormality produces deficiency in the inner mitochondrial protein frataxin and leads to mitochondrial iron accumulation. The increased sensitivity to oxidative stress consequent to mitochondrial dysfunction produces neurologic symptoms and cardiomyopathy.[2,3] The left ventricle (LV) in patients with FA cardiomyopathy might exhibit hypertrophy, dilation, or concentric remodeling; arrhythmias and heart failure contribute to death.[1,4,5] Cardiac magnetic resonance (CMR) affords direct, in vivo evaluation of LV remodeling and fibrosis that can develop before cardiomyopathy in FA becomes clinically apparent.[6]

The myocardial extracellular matrix plays a key role in determining myocardial architecture. The matrix's constitution depends on the balance between the production and degradation of collagen; disequilibrium leads to cardiac remodeling.[7] Collagen type I is synthesized as procollagen with a small amino terminal and a larger procollagen I carboxyterminal propeptide (PICP). The cleavage and shedding of PICP as a soluble peptide is a hallmark of collagen production in the extracellular matrix. A stoichiometric ratio of 1:1 exists between PICP and the number of collagen molecules produced. Therefore, the PICP level has been used as a surrogate for collagen production.[7,8] Given that collagen production occurs in all forms of LV remodeling, we sought to measure PICP in patients with FA in 2 contexts: GAA expansion, by means of genetic testing; and LV remodeling and fibrosis, by means of CMR analysis.

Patients and Methods

We identified 30 patients who had FA and no history of cardiomyopathy, heart failure, arrhythmias, or systolic dysfunction. All had been referred for cardiac evaluation from 2007 through 2011 at a single academic medical center, including 26 subjects enrolled from a previously completed prospective study of subclinical myocardial disease.[6] One subject was excluded after CMR examination revealed advanced LV dysfunction (LV ejection fraction, 0.15) and extensive scarring. In 27 patients, FA had been diagnosed by means of genetic testing: 25 had GAA triplet expansions (minimum GAA repeat length was recorded), and 2 had point mutations. The 2 other patients were diagnosed with FA on the basis of classic phenotypic findings and typical patterns of inheritance. For comparative serum PICP measurement, we enrolled 29 control subjects of similar age who had no known cardiovascular disease, diabetes mellitus, or hypertension and who were considered otherwise healthy. Table I shows the characteristics of the groups. Friedreich ataxia patients who had abnormal renal function (calculated glomerular filtration rate, <30 mL/min/m^2), allergies to gadolinium-based contrast medium or adenosine, severe claustrophobia, ferromagnetic foreign bodies, or nonmagnetic resonance-compatible devices were excluded from this study. Monitoring at 12 months in FA patients included repeat imaging, serologic testing, and documentation of cardiac symptoms, hospitalizations, and other clinical events during that period. All participants provided written informed consent to participate in this Institutional Review Board-approved investigation.

PICP Measurement. The PICP levels in the peripheral blood were quantified by means of sandwich enzyme-linked immunosorbent assay (ELISA) techniques. Serum was collected at the time of enrollment. Sandwich ELISA kit #MK101 (Takara Korea Biomedical Inc.; Seoul, Republic of Korea) was performed with 96-well plates pre-coated with capture antibody that can detect PICP in the range of 10 to 640 ng/mL with a sensitivity of 10 ng/mL. A calibration curve for PICP concentration was obtained in each run by using the standard provided in the kit. The intra-assay precision coefficient of variation was 4.5% to 7.4%, and the inter-assay coefficient of variation was 4.3% to 6.3%. Before assay, frozen samples were brought to room temperature slowly and were gently mixed. A 100-µL aliquot of 1:20 diluted serum was incubated in each well for 2 hours, followed by washing and one-hour incubation with peroxidase-conjugated primary antibody. The reaction was stopped by using 1N sulphuric acid and was developed by using the substrate solution (hydrogen peroxide and tetramethylbenzidine). The amount of PICP in each sample was determined by measuring the absorbance at 450 nm with use of an ELISA plate-reader. Each sample was analyzed in duplicate; the average absorbance was used to estimate PICP concentration from the calibration curve.

Left Ventricular Remodeling and Fibrosis by CMR. The FA patients' CMR examination included standard cine imaging. Left ventricular mass and ejection fraction were measured from contiguous short-axis cine images with use of endocardial and epicardial contours drawn at end-systole and end-diastole, summing the volumes from each short-axis slice to obtain LV end-diastolic and end-systolic volumes, and using the specific gravity of myocardium (1.04 g/mL) to compute LV mass. Relative wall thickness (RWT) was computed at end-diastole as follows: RWT = (septal thickness + lateral-wall thickness)/LV internal dimension.

Left ventricular hypertrophy (LVH) was present when LV mass indexed to body surface area exceeded the 95% percentile on the basis of sex (>91 g/m^2 for males, and >77 g/m^2 for females). Concentric remodeling was present if the RWT was >0.44 for males and >0.45 for females. Late post-gadolinium enhancement (LGE) images were acquired 15 min after a total of 0.15 mmol/kg of gadolinium-based contrast medium had been delivered intravenously, and visual rating by observers blinded to other study data was performed to rate LGE images as positive or negative for myocardial enhancement.

Statistical Analysis

Characteristics of the study population were summarized as appropriate to the type of data. Mean ± SE or the median value and interquartile range were reported for continuous variables. Comparisons between the FA patients and control subjects were made with use of independent 2-sample *t* tests or the Wilcoxon rank-sum test (if the normality assumption was violated) for continuous variables; and the Fisher exact test, for categorical variables. Paired *t* tests were used to compare the mean values of continuous clinical and laboratory measurements between baseline and 12-month evaluation, because the normality assumption was not violated for any of the variables. The correlation between continuous variables was computed with use of the Pearson correlation coefficient. Data were analyzed with use of Stata® 11.2 (StataCorp LP; College Station, Texas). *P* values <0.05 were considered statistically significant.

Results

Fifteen FA patients returned for repeat CMR examination. We had serum samples for 14 patients (PICP analysis), and 15 patients underwent echocardiography during follow-up (for RWT and LV analysis).

PICP Levels and Baseline Measures of Left Ventricular Structure and Function. The PICP values were markedly elevated in the FA patients in comparison with the healthy control subjects (1,048 ± 77 vs 614 ± 23

ng/mL; P <0.001) (Table I, Fig. 1). As in our previous cohort,[6] increased triplet expansion correlated with greater RWT (r=0.37, P <0.05) (Table II). However, GAA repeats did not correlate significantly with PICP level (r=0.22, P=0.3). The PICP levels did not differ between the 14 FA patients who had evident myocardial fibrosis by LGE and the 15 FA patients who were LGE-negative (1,067 ± 125 vs 1,030 ± 98 ng/mL; P=0.82).

Serum versus Imaging Measures of Fibrosis and Outcomes. In the 15 FA patients who returned for repeat CMR examination, the serum PICP levels and values of LV structure and function did not change significantly in 12 months (Table III). Higher baseline PICP levels did not correlate with progressive concentric remodeling (r=0.09, P=0.76) but did indicate subsequent LV dilation (r=0.52, P=0.02) (Figs. 2 and 3). At 12 months, 3 patients had experienced cardiac events: one had diastolic heart failure after undergoing surgery for nephrolithiasis, another had chest pain that necessitated emergency evaluation, and the third developed symptomatic bradycardia that necessitated pacemaker implantation. All 3 had had myocardial fibrosis evidenced by LGE positivity at baseline. No FA patient with baseline LGE negativity experienced a cardiac event (P=0.1).

Discussion

In this study, we report 3 major findings. First, patients with FA have higher levels of PICP, a serum marker of collagen synthesis, than do healthy control subjects. This is consistent with ongoing collagen synthesis but does not indicate where fibrosis develops. Second, PICP levels in FA predict LV dilation over the next year. This suggests that, as the LV dilates, there is replacement fibrosis. Third, evident myocardial fibrosis by LGE on CMR might be a marker of adverse cardiac events. Whereas the relatively low event rate precluded measuring a statistical difference between the LGE-positive and LGE-negative groups, the trend reinforces the findings in other large-scale studies of the very strong adverse prognostic value of abnormal LGE on CMR.[9,10]

Friedreich ataxia is the prevalent cause of hereditary ataxia and accounts for about 50% of cases of hereditary ataxia.[1] Nearly all patients who meet the genetic and neurologic criteria for FA have evidence of cardiac involvement.[1,5,11] Cardiac involvement determines survival prospects.[1,4,12] Identifying early markers of cardiac involvement should enable the more aggressive institution of cardioprotective therapies to delay disease

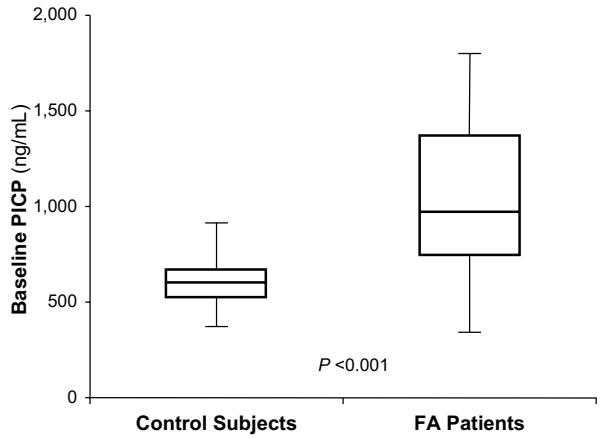

Fig. 1 *Graph shows higher serum procollagen I carboxyterminal propeptide (PICP) levels in patients with Friedreich ataxia (FA) than in healthy control subjects of similar age and the same sex (P <0.001). P <0.05 was considered statistically significant.*

TABLE I. Characteristics of the Study Population

Variable	**FA Patients** (n=29)	**Control Subjects** (n=29)	***P* Value**
Age (yr)	34.2 ± 2.2	32.5 ± 1.1	0.5[a]
Male	9 (31)	15 (52)	0.18[b]
Body weight (lb)	149.1 ± 6.9	162.9 ± 7.1	0.17
Body mass index (kg/m^2)	25.2 (19.9–29.1)	24.8 (21.8–27.3)	0.83
Hypertension	2	0	0.16
Diabetes mellitus	3	0	0.08
Dyslipidemia	1	1	1
Smoking	1	0	0.33
PICP (ng/mL)	1,048 ± 77	614 ± 23	<0.001

FA = Friedreich ataxia; PICP = procollagen I carboxyterminal propeptide

[a]Based on independent 2-sample t test.
[b]Based on Fisher exact test.

Data are presented as mean ± SE, median and interquartile range, or number and percentage. P <0.05 was considered statistically significant.

TABLE II. Baseline Test Results in the FA Patients

Variable	Value
Laboratory values	
Creatinine (mg/dL)	0.74 ± 0.06
Glucose (mg/dL)	85.6 ± 3.4
Total cholesterol (mg/dL)	171 ± 8.1
Triglycerides (mg/dL)	174 ± 63.2
HDL cholesterol (mg/dL)	41 ± 3.2
LDL cholesterol (mg/dL)	105 ± 5.7
ECG characteristics	
Heart rate (beats/min)	73 ± 2.5
PR interval (ms)	153 ± 9.6
QRS interval (ms)	80 ± 1.9
QT interval (ms)	378 ± 12.2
QTc interval (ms)	413 ± 9.2
Axis (°)	77 ± 5
Nonspecific ST-T changes	12
Echo characteristics (n=15)	
RV diameter (cm)	2.7 ± 0.3
Right atrial area (cm^2)	15.4 ± 1.2
Aortic root diameter (cm)	3 ± 0.2
E (m/s)	0.7 ± 0.1
A (m/s)	1.1 ± 0.5
MRI characteristics	
LVEDV (mL)	94.7 ± 4.3
LVEDV index	54.1 ± 2.4
LVESV (mL)	34.4 ± 2.8
LVESV index	19.2 ± 1.3
LV ejection fraction	0.64 ± 0.02
LV mass (g)	108.6 ± 5.5
LV mass index	63.9 ± 3.5
Left atrial area (cm^2)	17.2 ± 0.7
Left atrial area index	10.3 ± 0.5
Relative wall thickness (cm)	0.39 ± 0.02
T2* (ms)	29 ± 1.2
LV geometry	
Normal	21 (72)
Concentric remodeling	7 (24)
Concentric hypertrophy	1 (3)
Eccentric hypertrophy	0
Genetic analysis	
GAA repeats #1	784 ± 90
GAA repeats #2	686 ± 59

A = late ventricular filling velocity; E = early ventricular filling velocity; ECG = electrocardiographic; Echo = echocardiographic; FA = Friedreich ataxia; GAA = guanine-adenine-adenine; HDL = high-density-lipoprotein; LDL = low-density-lipoprotein; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; MRI = magnetic resonance imaging; RV = right ventricular

progression, although effective treatments remain in development.[5] Our group has previously shown that myocardial fibrosis can occur in FA patients who do not have overt cardiomyopathy.[6] As a measurement criterion for collagen production, PICP holds potential appeal; however, its usefulness in FA cardiomyopathy had not previously been evaluated. In our study, we have identified PICP as a marker of progressive LV dilation. This phenotype increases the risk of heart failure and arrhythmias,[9] the chief clinical complications of FA cardiomyopathy. Given the urgent need for better

TABLE III. Baseline versus 12-Month Follow-Up Results in FA Patients

Variable	Baseline	12 Months	*P* Value*
PICP, ng/mL (n=14)	1,237 ± 100.1	1,078.6 ± 90.6	0.13
Relative wall thickness, cm (n=15)	0.4 ± 0.02	0.39 ± 0.03	0.78
LV ejection fraction (n=15)	0.64 ± 0.02	0.61 ± 0.02	0.09
LV end-diastolic volume, mL (n=15)	96.5 ± 5.4	104.8 ± 3.4	0.12

FA = Friedreich ataxia; LV = left ventricular; PICP = procollagen I carboxyterminal propeptide

*Calculated from paired *t* tests.

Data are presented as mean ± SE. *P* <0.05 was considered statistically significant.

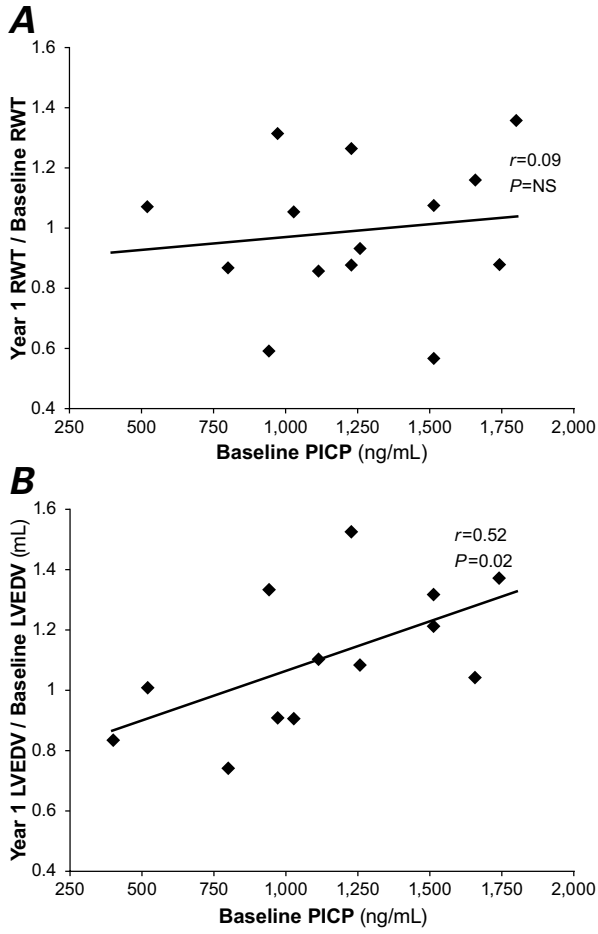

Fig. 2 *Baseline PICP level **A**) did not indicate progressive concentric remodeling (P=NS) but **B**) did indicate left ventricular dilation at 12-month follow-up evaluation (P <0.05) in a cohort of 14 Friedreich ataxia patients with no interval change in cardioprotective medications.*

P <0.05 was considered statistically significant.

LVEDV = left ventricular end-diastolic volume; PICP = procollagen I carboxyterminal propeptide; RWT = relative wall thickness

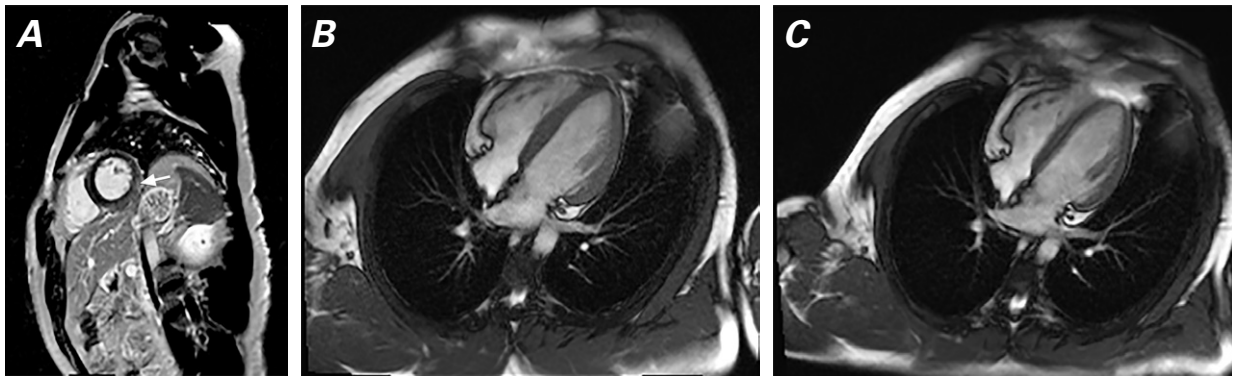

Fig. 3 *Serial cardiac magnetic resonance images of a 21-year-old patient with Friedreich ataxia over a 12-month follow-up period.* ***A****) The short-axis view reveals late post-gadolinium enhancement (arrow) in the inferolateral wall (procollagen I carboxyterminal propeptide = 1,742 ng/mL).* ***B****) Baseline end-diastolic frame from horizontal long-axis cine acquisition shows normal left ventricular end-diastolic volume (LVEDV; 91 mL) and elevated relative wall thickness (0.52 cm).* ***C****) After 12 months, end-diastolic frame from horizontal long-axis cine acquisition shows a decrease in relative wall thickness (0.46 cm); however, LVEDV had significantly increased (125 mL).*

biomarkers of subclinical myocardial disease, this novel link between a baseline biomarker of collagen synthesis and subsequent adverse LV remodeling has potential not only for better risk stratification of individual patients, but also for refining the design of clinical trials of novel cardioprotective regimens.

Hypertensive heart disease typically produces LVH with increased collagen production and deposition,[8] and PICP has shown promise as a measurement criterion for myocardial collagen content in preclinical models of hypertensive heart disease as well as in clinical studies.[8,13] It has also been useful in detecting other conditions marked by LV dilation (such as ischemic cardiomyopathy), and improvement in PICP and related biomarker profiles has been reported after spironolactone therapy for ischemic cardiomyopathy.[7] Concentric remodeling or LVH and fibrosis are hallmarks of the early cardiomyopathy phenotype in FA, so our finding of higher PICP levels with greater concentric remodeling in FA patients makes this serum collagen synthesis value a promising biomarker of subclinical myocardial disease.

Homozygous expansion of the GAA trinucleotide repeat in intron 1 of the *FRDA* gene is the typical genetic abnormality in FA,[2] and this mutation causes a defect in transcription of the frataxin protein.[1] Investigators have shown that an increased number of GAA repeats correlates with earlier onset of presentation of FA, neurologic disease severity, echocardiographic evidence of cardiomyopathy, the onset of diabetes mellitus, and poor survival rates.[3,14] We have previously shown that GAA-repeat length correlates with concentric remodeling; however, the lack of correlation between repeat length and PICP level underscores the fact that increased collagen production and adverse remodeling are distinct processes. Furthermore, serum PICP measurements do not identify the sites of collagen production. In a neuromuscular disorder marked by peripheral-muscle atrophy, the lack of PICP correlation with myocardial fibrosis by LGE on CMR simply suggests a lack of specificity of the serum marker. Of note, elevated PICP levels in patients with sarcoidosis have been correlated with skeletal myopathy.[15]

Study Limitations

It is possible that an imaging measurement of the myocardial extracellular volume fraction, such as T1 mapping before and after contrast administration, would prove to be even more sensitive than LGE on CMR for detecting myocardial fibrosis in FA and would thus warrant further study. In addition, we did not measure additional collagen biomarkers or collagen-degradation products to determine the ratio of collagen production to turnover. Additional longitudinal studies are needed to understand the relative predictive values of LV remodeling versus PICP levels for outcomes in FA cardiomyopathy.

Conclusions

In patients with Friedreich ataxia who carry a substantial genetically mediated risk of cardiomyopathy, the level of PICP—a serum marker of collagen production—is significantly increased and might provide information complementary to that yielded by cardiac imaging in predicting adverse LV remodeling and cardiac events. Longitudinal studies, including serum biomarkers and direct in vivo evaluation of cardiac structure and function, are warranted to better define prognostic significance and to guide clinical trials of novel cardioprotective interventions.

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