Echocardiographic Characterization of Cardiomyopathy in Friedreich’s Ataxia With Tissue Doppler Echocardiographically Derived Myocardial Velocity Gradients

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Background—Conventional and tissue Doppler echocardiographically derived myocardial velocity gradients (MVGs) were used to characterize the myocardium in patients with Friedreich’s ataxia (FRDA), and the relationship between MVGs and the mutation in the FRDA gene, a GAA triplet repeat expansion, was investigated.

Methods and Results—We studied 29 patients with FRDA (10 men, mean age 31 ± 9 years) who were homozygous for the GAA expansion in the FRDA gene and were without cardiac symptoms. A comparison was made with a group of 30 age-matched control subjects. In patients with FRDA, interventricular septal thickness (1.17 ± 0.26 versus 0.85 ± 0.13 cm, P < 0.005), posterior left ventricular wall thickness (1.00 ± 0.24 versus 0.88 ± 0.15 cm, P < 0.01), and left atrial diameter (3.3 ± 0.5 versus 2.9 ± 0.3 cm, P = 0.01) were increased compared with control subjects. MVGs were reduced in FRDA during systole (3.1 ± 1.2 versus 4.5 ± 0.5 s⁻¹, P < 0.0001) and in early diastole (4.9 ± 2.7 versus 8.8 ± 1.8 s⁻¹, P < 0.0001) but increased in late diastole (2.0 ± 1.3 versus 1.1 ± 0.9 s⁻¹, P < 0.01). The strongest relationship was seen between age-corrected early diastolic MVGs and the GAA expansion in the smaller allele of the FRDA gene (r = −0.68, P < 0.0001).

Conclusions—MVGs offer a means of further characterizing the myocardial abnormalities in patients with FRDA. Early diastolic MVGs appear to relate most closely to the genetic abnormality and the consequential reduction in frataxin protein. (Circulation. 2000;102:1276-1282.)

Key Words: echocardiography • cardiomyopathy • imaging • myocardium

Friedreich’s ataxia (FRDA) is an inherited neurodegenerative disorder associated with cardiomyopathy and impaired glucose tolerance.¹–⁶ The changes in the left ventricle (LV) consist mainly of cellular hypertrophy, diffuse fibrosis, and focal myocardial necrosis.⁷ Our recent findings⁸ are in accord with other reports¹–⁶ that the LV hypertrophy (concentric, asymmetrical, or both) and thickening of the papillary muscles are variable in FRDA. The most common echocardiographic abnormality is asymmetrical LV hypertrophy and thickening of the papillary muscles, although the range of abnormalities appears to be wide.⁸

The genetic basis for FRDA is a GAA trinucleotide repeat expansion in the first intron of gene X25,⁹ which encodes a 210–amino acid protein, frataxin. In FRDA, it is postulated that the GAA expansion leads to reduced levels of frataxin, resulting in abnormalities of mitochondrial iron transport and antioxidant systems.¹⁰–¹²

We used conventional echocardiography and tissue Doppler echocardiography¹³–¹⁶ to further characterize the myocardial abnormalities associated with FRDA. Tissue Doppler echocardiography facilitates quantification of intramural transmyocardial velocities, and the myocardial velocity gradient (MVG) offers an assessment of structural and functional changes over the cardiac cycle.¹⁷–¹⁹ MVG is independent of cardiac motion²⁰,²¹ and less affected by alterations in preload than Doppler transmitral velocities.²² In addition, MVG in early diastole, particularly when corrected for age, may differentiate between physiological and pathological ventricular hypertrophy.²¹ The aims of the present study were to define cardiac morphology with conventional echocardiography and to use tissue Doppler echocardiographically derived MVGs to investigate the relationship between cardiac phenotype and genotype in FRDA.

Methods
Twenty-nine subjects with FRDA, who were homozygous for the GAA expansion in the FRDA gene, were studied and compared with 30 age-matched healthy subjects. The diagnosis was confirmed by...
the presence of GAA expansions in both alleles of the FRDA gene. The clinical characteristics of the patients and control subjects are given in Table 1. All were in sinus rhythm, and in the patients with FRDA, clinical examination of the heart was normal except for 3 patients who had a soft pansystolic murmur at the apex.

In the patients, the mean±SD age of onset of ataxic symptoms was 11±5 years (range 5 to 28 years), with a mean duration of neurological symptoms of 16±8 years at the time of the study. Seventeen (59%) patients were dependent on others for the activities of daily living. None of the patients or control subjects were receiving any cardiac medication, and the study was approved by the hospital ethical committee.

### Analysis of the GAA Expansion

The size of the GAA triplet repeat was determined with long-range polymerase chain reaction (PCR) techniques with genomic DNA extracted from peripheral blood leukocytes. The amplifiers 5'-GGGATTGGTTGCCAGTGCTTAAAAGTTAG-3' and 5'-GATCTAAGGACCATCATCAGGCGACCACCTGCGC-3' were used to generate a product of 457 bp plus the number of base pairs that composed the GAA triplet repeat expansion. The ELONGASE enzyme mix (Taq and Pyrococcus species polymerases; Gibco BRL) was used for amplification (35 cycles; 94°C for 30 seconds, 60°C for 30 seconds, and 68°C for 120 seconds). The PCR products were separated by electrophoresis through a 1% agarose gel, and the size of the GAA expansion was determined by ethidium bromide staining. The results were confirmed with the Expand high-fidelity PCR system (Taq and Pwo polymerases; Boehringer-Mannheim) with 10 cycles of 94°C for 15 seconds, 60°C for 30 seconds, and 68°C for 120 seconds. The PCR products were separated by electrophoresis through a 1% agarose gel, and the size of the expansion was determined by ethidium bromide staining. The results were confirmed with the Expand high-fidelity PCR system (Taq and Pwo polymerases; Boehringer-Mannheim) with 10 cycles of 94°C for 15 seconds, 60°C for 30 seconds, and 68°C for 120 seconds. The PCR products were separated by electrophoresis through a 1% agarose gel, and the size of the expansion was determined by ethidium bromide staining.

### Tissue Doppler Echocardiography

The tissue Doppler echocardiographic data from the patients with FRDA and age-matched normal subjects were assessed by unpaired Student’s t tests. The potential influence of patient sex, age, heart rate, systolic and diastolic blood pressures, and conventional echocardiographic variables (including myocardial wall thickness and transmitral Doppler blood inflow indices) on MVG measurements were analyzed with stepwise multivariate regression analysis. Finally, simple linear regression was performed to investigate the relationship between the size of the GAA expansion and cardiac phenotype. The significance level, α, was set at 0.05.

### Results

#### Conventional Echocardiography

The conventional echocardiographic data from the patients with FRDA and age-matched normal volunteers are presented in Table 2. The interventricular septum and the posterior LV wall were thicker in the patients with FRDA than in the control subjects (38% and 14%, respectively; Table 2). The ventricular hypertrophy was predominantly asymmetrical, with the septal thickness exceeding the control mean±2 SDs in 45% of patients with FRDA. The LV cavity was smaller and the left atrial diameter was larger in patients with FRDA than in control subjects. There were no significant differences between patients with FRDA and age-matched control subjects in LV mass and ejection fraction.

| TABLE 1. Clinical Data for Patients With FRDA and Age-Matched Control Subjects |
|---------------------------------|----------------|-----------------|-----------------|
|                                | Patients With FRDA | Control Subjects | P (unpaired t test) |
|                                | (n=29)           | (n=30)          |                  |
| Sex, M/F                        | 10/19            | 13/17           |                  |
| Age, y                          | 31±9             | 31±9            | NS               |
| Resting heart rate, bpm         | 77±13            | 68±10           | NS               |
| Systolic blood pressure, mm Hg  | 116±9            | 121±7           | NS               |
| Diastolic blood pressure, mm Hg | 72±4             | 67±7            | NS               |

### Statistical Methods

The results are expressed as mean±SD. The differences between patients with FRDA and age-matched normal subjects were assessed by unpaired Student’s t tests. The potential influence of patient sex, age, heart rate, systolic and diastolic blood pressures, and conventional echocardiographic variables (including myocardial wall thickness and transmitral Doppler blood inflow indices) on MVG measurements were analyzed with stepwise multivariate regression analysis. Finally, simple linear regression was performed to investigate the relationship between the size of the GAA expansion and cardiac phenotype. The significance level, α, was set at 0.05.
Transmitral Doppler blood flow indices demonstrated that only the E/A ratio was reduced (by −21%, \( P<0.005 \)) in patients with FRDA; all other LV Doppler inflow indices, including E-wave deceleration time and IVRT, were similar to control values.

**MVG Measurements**

MVG measurements were different in all analyzed phases of the cardiac cycle in patients with FRDA compared with age-matched normal subjects (Figures 1 and 2). In systole and early diastole (during rapid ventricular filling), MVGs were reduced in patients with FRDA (systole 3.1±1.2 versus 4.6±0.5 s\(^{-1}\), \( P<0.0001 \); early diastole 4.9±2.7 versus 8.8±1.7 s\(^{-1}\), \( P<0.0001 \)). In contrast, in late diastole during atrial contraction, the MVG was higher in patients with FRDA than in control subjects (2.0±1.3 versus 1.2±0.9 s\(^{-1}\), \( P<0.01 \), Figure 1).

**Multivariate Analysis of MVGs**

MVG measurements taken in diastole during rapid ventricular filling and atrial contraction were age related in both patients with FRDA and normal subjects but otherwise were independent of other clinical and echocardiographic variables, including heart rate, LV wall thickness, and transmitral Doppler blood inflow indices.

![Figure 1. MVGs in patients with FRDA and control subjects in systole and early and late diastole (mean±SD are represented by horizontal and vertical lines, respectively).](image)

Because tissue Doppler echocardiographically derived indices are age related,\(^{1,26}\) the relationship between the thickness of the LV posterior wall and age-corrected MVGs in early diastole is presented in Figure 3. In 21 (72%) of the patients with FRDA, early diastolic MVG was <8.8 s\(^{-1}\) (control mean−2 SD) in the absence of hypertrophy of the LV posterior wall (<1.25 cm). However, in 8 (28%) of the patients with FRDA, early diastolic MVGs were within normal values. In comparison with the other 21 subjects with FRDA, these 8 subjects had a smaller GAA triplet repeat expansion (467±153 versus 760±225; \( P<0.001 \)) and less myocardial hypertrophy (interventricular septal thickness 0.99±0.08 versus 1.25±0.27 cm; \( P<0.001 \)) and tended to be older (35±9 versus 29±8 years; \( P=0.155 \)). Other clinical and echocardiographic measurements, including LV posterior wall thickness, LV size, and transmitral Doppler blood inflow indices, were similar in the 2 subgroups. In patients with FRDA, an age-corrected early diastolic MVG of <8.8 s\(^{-1}\) was consistently present when the size of the GAA expansion in the smaller allele of the FRDA gene was >600 repeats.

**Relationship Between Genotype and Echocardiographic Phenotype in FRDA**

**Echocardiography**

A relationship was demonstrated between the number of GAA triplet repeats in the smaller frataxin allele and diastolic interventricular septal thickness (\( r=0.55, P<0.005 \), Figure 4A), but the relationship between the frataxin mutation and the thickness of the posterior LV wall was weaker and of borderline significance (\( r=0.35, P=0.06 \), Figure 4B). A relationship was not found between the GAA triplet repeat expansion and other conventional echocardiographic variables, such as LV dimension, left atrial size, or transmitral Doppler blood flow indices.

**MVG Measurements**

Age-corrected MVGs were inversely related to the size of the GAA triplet repeat expansion in all phases of the cardiac cycle.

**Table 2. Echocardiographic Data for Patients With FRDA and Age-Matched Control Subjects**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Patients With FRDA (n=29)</th>
<th>Control Subjects (n=30)</th>
<th>( P ) (unpaired ( t ) test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interventricular septum, cm</td>
<td>1.17±0.26</td>
<td>0.85±0.13</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LV posterior wall, cm</td>
<td>1.00±0.24</td>
<td>0.88±0.15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV dimension (diastole), cm</td>
<td>4.5±0.7</td>
<td>4.9±0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Left atrial dimension, cm</td>
<td>3.3±0.5</td>
<td>2.9±0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>LV mass (corrected), g</td>
<td>192±62</td>
<td>171±39</td>
<td>NS</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>59±9</td>
<td>61±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Peak E wave, cm/s</td>
<td>73±9</td>
<td>68±9</td>
<td>NS</td>
</tr>
<tr>
<td>Peak A wave, cm/s</td>
<td>49±11</td>
<td>38±8</td>
<td>NS</td>
</tr>
<tr>
<td>E-wave deceleration time, ms</td>
<td>208±54</td>
<td>181±16</td>
<td>NS</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>75±17</td>
<td>73±11</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 2. Representative examples of LV posterior wall M-mode tissue Doppler images with calculated MVG over entire cardiac cycle. A, A 35-year-old female FRDA patient (GAA expansion 480 repeats) with a normal LV posterior wall (0.80 cm). B, A 35-year-old male FRDA patient (GAA expansion 770 repeats) with a normal LV posterior wall (0.75 cm). C, A 26-year-old female FRDA patient (GAA expansion 990 repeats) with LV posterior wall hypertrophy (1.45 cm). D, A 29-year-old healthy woman with a normal heart. *Peak MVG during predetermined phases of cardiac cycle. S indicates systole; ED, early diastole; and LD, late diastole.
Although MVG measurements correlated with the number of GAA repeats in systole ($r = 0.48$, $P = 0.01$, Figure 5A), early diastole ($r = 0.60$, $P < 0.005$, Figure 5B), and late diastole ($r = 0.48$, $P = 0.01$, Figure 5C), the strongest relationship was found for MVG measurements corrected for age in early diastole ($r = 0.68$, $P < 0.0005$, Figure 5D).

### Discussion

The present study demonstrated that MVGs in systole and during the rapid ventricular filling phase of early diastole are reduced in patients with FRDA who are without cardiac symptoms. Tissue Doppler echocardiography may be useful to further our understanding of the changes in myocardial structure and function in inherited cardiomyopathies.

The precise molecular abnormalities that result in the development of cardiomyopathy in FRDA remain unclear. Retrospective studies have reported a relationship between the number of GAA repeats in the smaller frataxin allele and cardiomyopathy in approximately two thirds of patients homozygous for the GAA expansion.\(^{28,29}\) This is in accord with our experience, in which 46% of subjects homozygous for the GAA expansion had septal hypertrophy but the posterior wall was thickened in only 18%.\(^{8}\) In this prospective study, we confirmed the previous retrospective observation of a relationship between myocardial hypertrophy and the size of the GAA expansion in the FRDA gene\(^{30}\) but also demonstrated that MVG is abnormal in the majority of patients with FRDA. Although the 2-dimensional echocardiographic studies confirmed myocardial hypertrophy in approximately half of the subjects with FRDA, indices of LV systolic and diastolic function were within the normal range.

We used conventional echocardiography and tissue Doppler echocardiography to further characterize the myocardial abnormalities associated with FRDA. Tissue Doppler echocardiography is a recently introduced technique that enables abnormalities of cardiac structure and function to be characterized by using color Doppler information.\(^{17-20,31,32}\) The detection of consecutive phase shifts of the Doppler signal returning from the endocardium and epicardium, throughout the cardiac cycle, renders this technique particularly suited to the study of myocardial disease. A further advantage of this technique is the accurate definition of the endocardial boundary.\(^{25}\) MVG is also subject to less error than conventional echocardiography with respect to translational and rotational motion of the heart,\(^{20}\) changes in loading,\(^{22}\) and the degree of LV hypertrophy.\(^{21}\) Overall, the assessment of myocardial structure and function with this technique offers several potential benefits over conventional echocardiography.\(^{13-15,20,25}\)

In patients with FRDA, systolic and early diastolic MVGs were reduced by 33% and 44%, respectively, compared with healthy control subjects. The inverse relationship between MVG and the number of GAA triplet repeats is presumed to reflect the reduction in frataxin in the myocyte.\(^{11,33}\) In 72% of patients with FRDA, early diastolic MVGs were reduced in excess of 2 SDs below the control mean in the absence of LV posterior wall hypertrophy. In the remaining subjects, early diastolic MVGs were within normal values. These findings confirm that the MVG is independent of myocardial hypertrophy, LV cavity size, and transmitral Doppler blood flow indices.

A number of factors influence cardiac diastolic function (eg, myocardial relaxation, elastic recoil, pericardial restraint, and atrial function), and myocardial abnormalities alter not only these factors but also their interrelationship. For example, in familial hypertrophic cardiomyopathy, the force of myocardial contraction starts to decline before the end of ventricular ejection, well in advance of mitral valve opening. Furthermore, myocardial diastolic function, as indicated by an increase in IVRT, is a sensitive index of myocardial ischemia and occurs well before a decline in myocardial systolic contractility, symptoms, and ECG changes are observed.

In FRDA, it has been proposed that the GAA expansion results in a reduction in mitochondrial frataxin, with abnormal iron metabolism and accumulation of free radicals that result in mitochondrial dysfunction.\(^{11,12}\) FRDA shares a number of common features with mitochondrial respiratory chain deficiency (including ataxia, diabetes, and cardiomy-
pathology), and the localization of frataxin to mitochondria supports the hypothesis that mitochondrial dysfunction plays a central role in FRDA. Abnormal respiratory chain complex I, II, and III activity ratios have been observed in the myocardium of FRDA subjects without evidence of derangement of the mitochondrial genome. The yeast counterpart of the FRDA protein is active in iron homeostasis, and disruption of the gene results in mitochondrial iron accumulation. It is proposed that the reduction in frataxin results in the activation of a mitochondrial iron transport system; a deficiency of a Krebs’ cycle enzyme, aconitase; and altered mitochondrial respiratory function through the iron-catalyzed Fenton reaction and oxidative stress.

Oxidative stress also plays a role in coronary artery disease, where ischemia promotes free radical formation, resulting in myocardial and endothelial injury with inactivation of nitric oxide, peroxidation, and altered membrane permeability. The potential protective role of vitamin E as an antioxidant is highlighted by the finding that familial isolated vitamin E deficiency is an autosomal recessive disorder that closely mimics FRDA such that the conditions are indistinguishable before genetic studies and plasma vitamin E measurement. In this study, the results of echocardiography were not reported, but unspecified slight and moderate electrocardiographic abnormalities were present in 4 of the 8 individuals studied, with another who experienced sudden death.

We propose that the abnormal MVG reflects the decreased myocardial contractility and relaxation secondary to abnormal mitochondrial function resulting from reduced levels of frataxin in FRDA. It remains unclear why some patients with profound neurological deficit have no or little evidence of cardiac abnormalities (including normal MVGs), whereas others have both reduced MVG and myocardial hypertrophy and much less neurological disability, although the antioxidant capacity of different organs varies.

**Study Limitations**

The effect of severe ataxia and wheelchair dependence on echocardiographic measurements, including MVG, is unknown. The absence of a relationship between the degree of neurological deficit and MVG suggests that immobility does not influence MVG any more than athleticism. Similarly, the effect on the heart of the considerable effort of maintaining independence despite severe ataxia and the effect of neurodegeneration on cardiac innervation in FRDA remains unknown.

In Doppler echocardiography, “noise” may be observed at the top of the image in the near field, but this does not influence MVG measurements of the posterior wall of the LV, located in the lower third of the image. Furthermore, tissue Doppler echocardiography in the present study was limited to a section of the posterior wall of the LV because the angle dependence of Doppler information restricts accurate measurement of MVG to this region of the LV. We assumed that the abnormalities in this relatively small area of myocardium are representative of those in the entire LV.

**Conclusions**

The cardiomyopathy of FRDA is associated with asymmetrical LV hypertrophy, a decrease in LV cavity size, and a reduction in both systolic and diastolic MVGs. The strongest relationship was found between the size of the smaller GAA triplet repeat expansion and early diastolic MVG and interventricular septal thickness. Tissue Doppler echocardiographically derived MVG technique offers an additional means of assessing structural and functional
changes in inherited myocardial disease. The role of this new echocardiographic technique to assess myocardial dysfunction secondary to oxidative stress merits further study.

Acknowledgments
This work was supported in part by a British Heart Foundation grant (Drs Dutka and Nunez) and by the Special Trustees of the Hammer-smith Hospital. We are grateful to Acuson (UK) Ltd for the loan evaluation of the echocardiographic equipment used in this study. We thank Drs Alan Fleming and Susan Chamberlain for helpful discussions.

References
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_Circulation_. 2000;102:1276-1282
doi: 10.1161/01.CIR.102.11.1276

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/11/1276

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