

Using Imaging Mass Spectrometry to Accurately Diagnose Fabry's Disease

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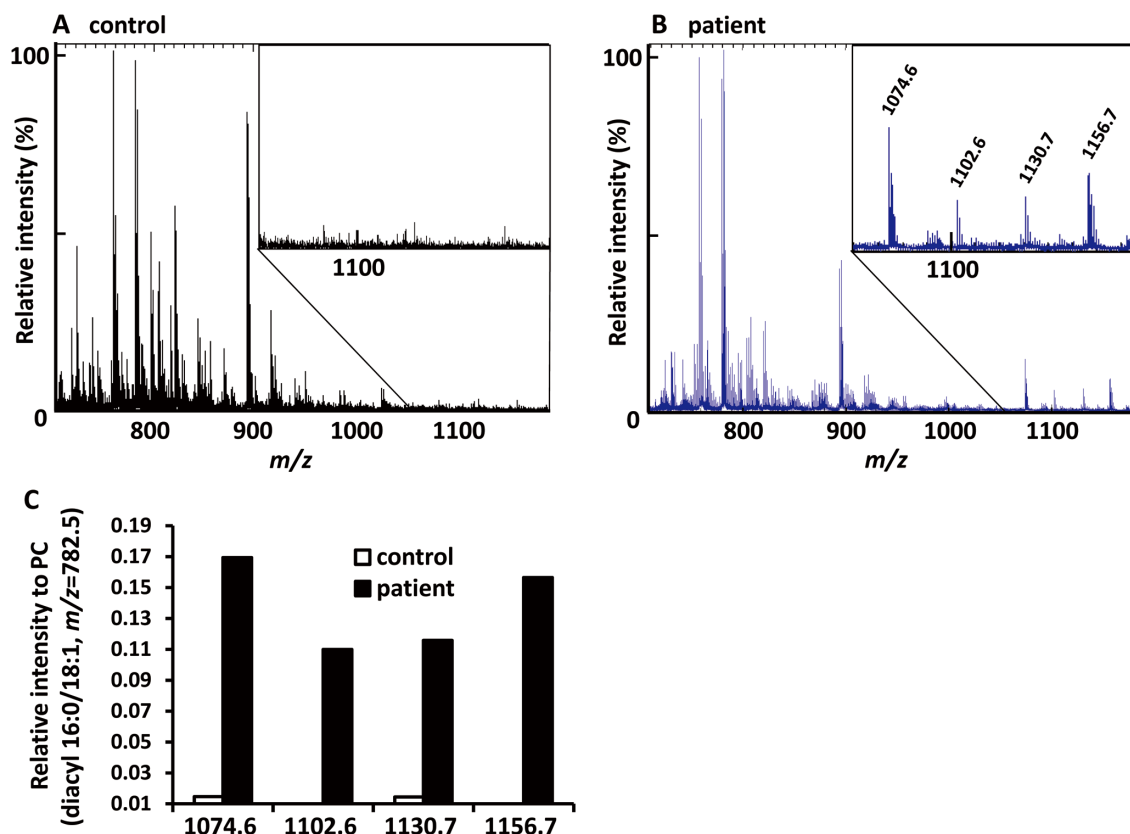


Figure 1. Mass spectrometry of endomyocardial biopsy samples. (A, B) The patient's heart produced unique peaks at mass/charge (m/z) 1,074.6, 1,102.6, 1,130.7 and 1,156.7 that were not observed for the control human heart. These peaks were consistent with globotriaosylceramides (Gb3s) with different fatty acid contents in their ceramide moieties. (C) The signal intensities of these peaks relative to those of m/z 782.5 were much higher in the Fabry's disease patient than in the control patient with congestive heart failure.

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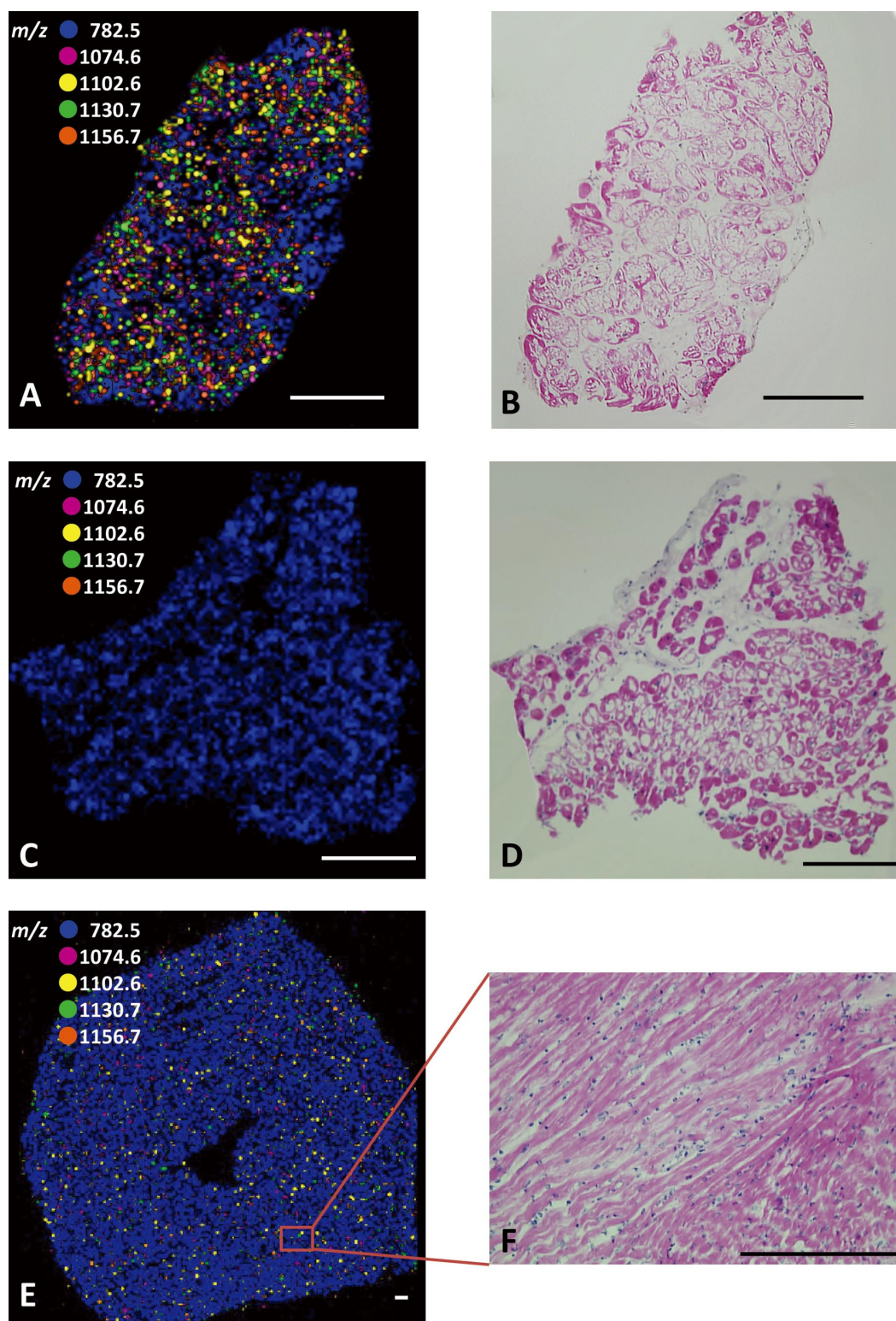


Figure 2. Imaging mass spectrometry (IMS) and histology of the samples. We constructed a figure by plotting the positions of the mass/charge 782.5, 1,074.6, 1,102.6, 1,130.7 and 1,156.7 peaks. When we compared the (A) IMS with (B) a hematoxylin and eosin-stained section, the peaks of globotriaosylceramides (Gb3s) were more densely packed in cardiomyocytes with vacuolar degeneration, and the Gb3s existed together in some parts and separately in others. (C,D) In contrast, Gb3 was not detected in a control sample from a patient with secondary myocardial degenerative changes with aortic regurgitation. (E,F) Gb3s were also detected on IMS in the heart of a mouse with Fabry's disease even though there was no evidence of vacuolar changes in the myocardium on light microscopy. Bar, 200 μ m.

Fabry's disease is an X-linked hereditary lysosomal storage disorder caused by abnormalities in the α -galactosidase (GAL) A gene (*GLA*), which leads to reduced α -GAL A activity and the subsequent accumulation of globotriaosylceramide (Gb3).¹ Fabry's disease can be diagnosed using several techniques, including measuring α -GAL A activity in leukocytes or plasma, analyzing genotype, and examining the histopathology of biopsied specimens. In some cases, however, it is difficult to definitively diagnose patients using these methods because (1) there may be residual enzymatic activity in patients with variants of Fabry's disease or in patients who are heterozygous; (2) the *GLA* gene has high genetic heterogeneity with >400 mutations and novel mutations that must be checked to determine whether the mutation truly results in Fabry's disease; and (3) vacuolar degeneration or lamellar inclusion bodies observed on light or electron microscopy are not specific for Gb3.² Therefore, it would be advantageous to develop a comprehensive and accurate diagnostic technique for Fabry's disease.

Mass spectrometry of biological specimens using matrix-assisted laser desorption/ionization (MALDI) can profile biological molecules based on their molecular masses.^{3,4} Furthermore, the emerging method of imaging mass spectrometry (IMS) can analyze specimens as small as biopsy samples and visualize the distribution of microscopically observed substances without losing spatial information.⁵ Therefore we used IMS to diagnose Fabry's disease, and examined the efficacy of this tool using 2 samples. One sample was an endomyocardial biopsy (EMB) specimen from a 69-year-old man who had been diagnosed with Fabry's disease based on vacuolar degeneration and lamellar inclusion bodies within cardiac tissue on light and electron microscopy, reduced α -GAL A activity in leukocytes, and a genetically proven N215S mutation, which is commonly observed in patients with the cardiac variant of Fabry's disease. The second sample was heart tissue excised from a 45-week-old male mouse in a model of Fabry's disease with an α -GAL A deficiency.⁶

The specimens were frozen and cut into 8- μ m-thick sections. The thinly sliced sections were thaw-mounted on glass slides coated with indium tin oxide. 2,5-Dihydroxybenzoic acid matrix solutions (50 mg/ml) containing 70% methanol and 0.1% trifluoroacetic acid were prepared and sprayed over the tissue surface. IMS was performed using a recently developed MALDI quadrupole ion trap time-of-flight (QIT/TOF)-type instrument (Shimadzu, Kyoto, Japan).⁷ The data were acquired in the positive ion mode, and signals between mass/charge (m/z) 600 and 1,200 were recorded. The spatial resolution was set at 10 μ m. Image reconstruction was performed using BioMap software (Novartis, Basel, Switzerland). Variations in the ionization efficiency in the IMS results were standardized by equalizing the ion intensity of a major endogenous lipid, phosphatidylcholine (diacyl 16:0/18:1), at m/z 782.5 for each peak in the spectrum. The normalized ion intensities were compared to those of a control EMB sample from a patient with congestive heart failure due to aortic regurgitation. To identify the molecular species, the m/z obtained on IMS was sent to the Human Metabolome Database (<http://www.hmdb.ca>).

On IMS of the EMB sample from the patient with Fabry's disease, extra peaks were seen at m/z 1,074.6, 1,102.6, 1,130.7 and 1,156.7 that were not observed in the control EMB sample (Figures 1A,B). The signal intensities of these unique peaks relative to that of m/z 782.5 were much higher than in the control (Figure 1C). Using information from the database, we determined that these peaks were consistent with Gb3 molecules that contain different fatty acid components in the

ceramide moiety, including C18:0, C20:0, C22:0 and C24:1, respectively. We constructed a figure by determining the location of these peaks within the EMB sample. This showed that the distribution of Gb3s was consistent with that of cardiomyocytes, especially in areas that were affected by vacuolar degeneration, and that the Gb3 types existed together in some parts and separately in others (Figures 2A,B). In contrast, as shown in Figures 2C,D, Gb3 was not detected in the control EMB sample. When we analyzed the heart from a mouse with Fabry's disease, we also detected Gb3s in the cardiac tissue even though there was no evidence of vacuolation in the cardiomyocytes on light microscopy (Figures 2E,F). Gb3 was not detected in the control mouse heart (data not shown).

A recent study reported that mass spectrometry of Gb3 in urine samples is useful to diagnose Fabry's disease.⁸ Urine analysis, however, is not helpful in cases involving the cardiac variant of the disease, in which renal manifestations are not observed until the advanced stages of disease. Nakao et al reported that cardiac hypertrophy is caused by cardiac Fabry's disease in 3% of patients over the age of 40.^{9,10} It is important to distinguish primary hypertrophic cardiomyopathy from secondary cardiac hypertrophy because several causes of cardiac hypertrophy can be effectively treated.

We could detect Gb3s not only in a human heart from a Fabry's disease patient but also in a heart from a mouse model of Fabry's disease without discernible degenerative changes on light microscopy. Although the significance of each type of Gb3 distribution is unknown, it is possible that these distribution patterns could help distinguish variations in the disease phenotype or assess the effectiveness of enzyme replacement therapy, which may also help elucidate the basis for the disease. This is an issue that requires further studies.

The current study presents novel findings suggesting that IMS is useful to diagnose Fabry's disease with cardiac manifestations, especially in questionable cases. Because IMS can directly analyze the molecular weight of each existing component, IMS has a higher specificity than electron microscopy or enzyme activity assays when Fabry's disease is suspected based on light microscopy. The present results indicate that IMS is a new tool that can be used to accurately diagnose not only Fabry's disease, but also other unknown storage diseases.

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