

## CD36 Genotype and Long-Chain Fatty Acid Uptake in the Heart

Taigo Kintaka, MD; Takao Tanaka, MD; Makoto Imai, MD;  
Itaru Adachi, MD\*; Isamu Narabayashi, MD\*; Yasushi Kitaura, MD

Homozygous or compound heterozygous mutation of the *CD36* gene (*CD36*<sup>-/-</sup>) in humans results in severe defects of the uptake of long-chain fatty acids (LCFAs) in the heart. Because the effect of a single mutation of this gene (*CD36*<sup>+/-</sup>) on the LCFA uptake is not known, it was evaluated in 29 subjects with the *CD36* wild-type gene (WT) (6 healthy subjects, 10 patients with heart disease), *CD36*<sup>+/-</sup> (4 healthy subjects, 5 patients) and *CD36*<sup>-/-</sup> (4 patients). The *CD36* genotype was identified in the coding region of genomic DNA, and the expression of *CD36* protein was examined by flow cytometry after staining with monoclonal anti-*CD36* antibody. The LCFA uptake in the heart was assessed as the radioactivity accumulation ratio of heart to mediastinum after intravenous administration of iodine-123 15-(*p*-iodophenyl)-3-*R*, *S*-methylpentadecanoic acid (H/M ratio). The H/M ratios in WT, *CD36*<sup>+/-</sup> and *CD36*<sup>-/-</sup> were 2.28±0.10, 1.90±0.06 and 1.40±0.11, respectively (*p*<0.0001, among groups). The H/M ratio between healthy subjects and patients with heart disease for WT and *CD36*<sup>+/-</sup> did not differ significantly (ie, those of WT and *CD36*<sup>+/-</sup> in healthy subjects and patients were 2.29±0.08 vs 2.27±0.12 and 1.90±0.07 vs 1.89±0.05, respectively). Not only *CD36*<sup>-/-</sup> but also *CD36*<sup>+/-</sup> resulted in a significant reduction of the LCFA uptake in the heart independent of heart disease, suggesting genotype dependency and that *CD36* might be a fundamental determinant of myocardial LCFA uptake. (Circ J 2002; 66: 819–825)

**Key Words:** *CD36*; Genotype; Long-chain fatty acid uptake; Myocardium; Scintigraphy

**A**lthough the heart preferentially uses long-chain fatty acids (LCFAs) as its main energy substrate, alteration in LCFA utilization is well known in pathological hearts<sup>1</sup> and in such cases, assessment of the LCFA metabolism is of clinical importance in determining etiology and developing therapeutic strategies.

LCFA metabolism in the heart has been clinically evaluated by scintigraphy using iodine-123-15-(*p*-iodophenyl)-3-*R*, *S*-methylpentadecanoic acid (BMIPP), a radioactive LCFA analog. Regional defects of myocardial LCFA uptake are often revealed in patients with coronary artery disease<sup>2–5</sup> and have been interpreted as follows: (1) dissociation between BMIPP and flow-tracer activity (ie, reduced BMIPP uptake but less reduced flow-tracer accumulation) may indicate ischemic but still viable myocardium (ie, the ‘mismatch of blood flow and metabolism’); or (2) diminished accumulation of BMIPP together with reduced flow-tracer radioactivity related to necrotic or fibrotic tissue.

However, despite the increase of flow tracer accumulation in the heart, defects of BMIPP uptake have been noticed in patients with hypertrophic cardiomyopathy and interestingly, an almost negative depiction of the heart by BMIPP scintigraphy has been occasionally observed in patients without discernible defects of coronary perfusion<sup>6–8</sup>. The underlying mechanism of this discrepancy (ie, decreased uptake of LCFA in the heart despite rather increased uptake of flow-tracer) has not been fully elucidated.

The first step of cellular LCFA metabolism is a transverse process of LCFA in the plasma membrane. Although this process is still in dispute, several lines of evidence suggest a protein-mediated process in addition to a simple diffusive process. Among the putative proteins involved, *CD36* is a potential candidate<sup>8–11</sup>. In fact, *CD36* knock-out mice have severe defects of LCFA uptake in the heart<sup>10</sup> and homozygous and compound heterozygous mutation of the *CD36* gene (hereafter referred to as *CD36*<sup>-/-</sup>) in humans also causes severe defects of myocardial LCFA uptake, with an almost negative depiction of the heart by BMIPP scintigraphy<sup>8</sup>.

In addition to the effects of *CD36*<sup>-/-</sup> on the LCFA uptake in the heart, *CD36*<sup>+/-</sup> resulted in a lack of *CD36* expression in platelets and monocytes, referred to as type I *CD36* deficiency<sup>12</sup>. We recently found a strong association between the genotype in the coding region of *CD36* and the expression level of *CD36*. Heterozygous mutations in the coding region of this gene (hereafter referred to as *CD36*<sup>+/-</sup>) resulted in the reduced expression of *CD36* in monocytes, approximately half that observed in the wild-type gene (hereafter referred to as WT)<sup>13</sup>. Accordingly, we hypothesized that not only *CD36*<sup>-/-</sup> but also *CD36*<sup>+/-</sup> might lead to the defects of LCFA uptake in the heart.

The effects of *CD36*<sup>-/-</sup> on the LCFA uptake in the heart have been characterized, so far, only qualitatively; that is, an almost negative depiction of the heart by BMIPP scintigraphy. The quantitative evaluation of LCFA uptake in the heart has been analyzed in a few studies using the radioactivity accumulation ratio of heart to mediastinum (H/M) after intravenous administration of BMIPP<sup>14,15</sup>. Knowing the mutational effects on the LCFA uptake in the heart should further clarify the role of *CD36*. In this study, we investigated the quantitative LCFA uptake in the heart

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Third Division, Department of Internal Medicine and \*Department of Radiology, Osaka Medical College, Osaka, Japan

Mailing address: Takao Tanaka, MD, PhD, Third Division, Department of Internal Medicine, Osaka Medical College, Takatsuki, Osaka 569-8686, Japan. E-mail: in3024@poh.osaka-med.ac.jp

**Table 1** Characteristics of Subjects

Case no.	Age (years)	Sex	Cardiac condition	Mutation		Monocytes	Platelets	H/M
D1	44	F	HCM	CD36 <sup>-/-</sup>	C478T+Ins1159A	0.00	0.4	1.24
D2	46	M	PVC	CD36 <sup>-/-</sup>	Homo (C478T)	0.01	0.2	1.44
D3	67	M	DCM	CD36 <sup>-/-</sup>	C478T+Del539AC	0.01	0.06	1.45
D4	34	M	HCM	CD36 <sup>-/-</sup>	C478T+Del539AC	0.00	0.1	1.48
SP1	64	M	HCM	CD36 <sup>+/-</sup>	C478T	16.5	13.2	1.85
SP2	39	F	DCM	CD36 <sup>+/-</sup>	C478T	12.4	15.7	1.86
SP3	65	M	HT	CD36 <sup>+/-</sup>	C478T	19.0	20.5	1.88
†SP4	61	F	VSA	CD36 <sup>+/-</sup>	C478T	11.4	0.3	1.88
SP5	39	F	HCM	CD36 <sup>+/-</sup>	C478T	17.3	15.0	1.97
SN1	27	M	Norm.	CD36 <sup>+/-</sup>	C478T	13.1	10.3	1.83
SN2	36	M	Norm.	CD36 <sup>+/-</sup>	Del539AC	11.5	15.5	1.86
SN3	39	M	Norm.	CD36 <sup>+/-</sup>	C478T	13.7	12.8	1.95
SN4	35	M	Norm.	CD36 <sup>+/-</sup>	C478T	18.0	11.4	1.96
WP1	55	M	HCM	WT	None	30.7	24.9	2.18
WP2	64	F	HCM	WT	None	31.6	15.6	2.18
WP3	64	M	VSA	WT	None	24.7	18.1	2.18
WP4	59	M	HT	WT	None	22.1	11.0	2.25
WP5	61	M	HT	WT	None	32.1	15.2	2.30
WP6	75	M	HT	WT	None	45.9	37.1	2.39
WP7	78	M	CAD	WT	None	30.5	26.1	2.51
WP8	52	F	HCM	WT	None	22.3	26.4	2.35
WN1	35	M	Norm.	WT	None	33.3	20.6	2.17
WN2	59	M	Norm.	WT	None	39.6	13.6	2.24
WN3	37	M	Norm.	WT	None	32.3	25.8	2.28
WN4	28	M	Norm.	WT	None	34.8	20.0	2.31
WN5	51	M	Norm.	WT	None	40.7	28.8	2.35
WN6	43	M	Norm.	WT	None	23.2	6.5	2.41
†WP9	53	F	DCM	WT	None	25.4	0.1	2.13
†WP10	72	M	VSA	WT	None	27.9	0.4	2.25

Abbreviations: Charact., characteristic; Norm., healthy volunteer; PVC, premature ventricular contraction; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; VSA, vasospastic angina; HT, hypertension; CAD, coronary artery disease; CD36<sup>-/-</sup>, homozygous or compound heterozygous mutation of the CD36 gene; CD36<sup>+/-</sup>, heterozygous mutation; WT, wild-type gene; None, no mutation was detected in the coding region of the FAT/CD36 gene; Homo (C478T), homozygous mutation of C478T; C478T+Ins1159A, CD36<sup>-/-</sup> for C478T and Ins1159A; C478T+Del539AC, CD36<sup>-/-</sup> for C478T and Del539AC; H/M, heart to mediastinum radioactivity ratio; †, no expression of CD36 protein in platelets but expression in monocytes.

using BMIPP in subjects with WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup>. WT and CD36<sup>+/-</sup> groups included both patients with heart disease and healthy subjects.

## Methods

### Study Population

The study population consisted of 29 unrelated Japanese subjects (10 healthy male volunteers; age range, 27–59 years and 19 patients with heart disease 12 males, 7 females; age range, 39–72 years) (Table 1).

WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup> were found by flow cytometry with fluorescein isothiocyanate (FITC)-labeled monoclonal antibody (FA6-152, Marseille, France) and the expression of CD36 protein was assessed as mean fluorescent intensity (MFI).<sup>13</sup> Mutations were identified by genomic analysis in the CD36 coding region of genomic DNA.<sup>8</sup> Volunteers were free of any disease, had an unremarkable medical history and were not taking any medication at the time of the investigation. The diagnoses of the patients were based on medical history, physical examination, electrocardiogram, chest radiography, echocardiography, and coronary angiography. Patients were treated at outpatients clinics and were not severely ill (New York Heart Association class I–II).

The investigation conformed to the principles outlined in the Declaration of Helsinki, and written informed consent

was obtained from all the subjects.

### BMIPP Myocardial Scintigraphy and the Heart-to-Mediastinum Radioactivity Ratio (H/M ratio)

Both planar and single photon emission computed tomography (SPECT) imaging of all the subjects were done with a standard acquisition method. After overnight fasting, subjects had an intravenous injection of 111 MBq of iodine-123-15-(p-iodophenyl)-3-R, S-methylpentadecanoic acid (I-123 BMIPP; Nycomed-Amersham, UK) at rest. A three-head SPECT system (GCA-9300A, Toshiba Medical System, Tokyo, Japan) equipped with a low-energy high-resolution collimator was used to acquire anterior planar images with a 512×512 matrix for 5 min at 30 min after injection. Next, SPECT projection data with a 64×64 matrix were obtained from a 360-degree circular orbit in 60 steps (30 s per step) with a 20% window centered on the 160 keV photopeak. The projection data were processed using a GMS-550 WorkStation (Toshiba Medical System). The reconstruction was performed by the filtered back projection technique with a Butterworth filter. Reconstruction slices were set parallel to the short, vertical long, and horizontal long axes.

Radioactivity was counted in the region of interest (ROI), which was manually drawn over the whole heart by a nuclear-cardiologist (I.A.) unaware of the CD36 genotype, from the planar image (Fig 1). To evaluate regional

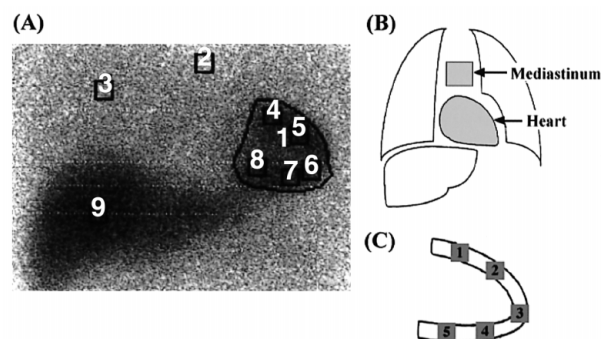


Fig 1. Measurements of BMIPP radioactivity in the whole-heart region, the contour of which was hand-defined, and in the mediastinum region (A), as schematically illustrated in (B). Regional myocardial radioactivity was measured in 5 regions of the heart, as schematically illustrated in (C).

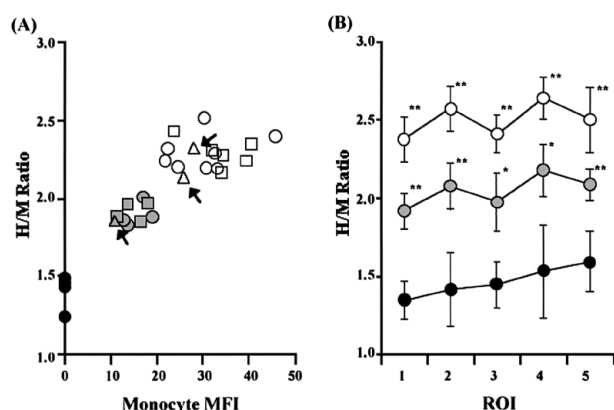


Fig 3. Whole-heart H/M ratio and monocyte MFI (A), and regional H/M ratio (B). (A) Relation between the whole-heart H/M ratio (vertical axis) and monocyte MFI (horizontal axis) in each individual. The filled, hatched and open symbols represent subjects with homozygous or compound heterozygous mutation of the *CD36* gene (*CD36*<sup>-/-</sup>), heterozygous mutation (*CD36*<sup>+/-</sup>) and wild-type gene (WT), respectively. Squares and circles represent healthy subjects and patients with heart disease, respectively. Subjects lacking *CD36* protein expression in platelets but present in monocytes (type II *CD36* deficiency) are indicated as open and hatched triangles with arrows. (B) The H/M ratio in the 5 regions of the heart. Closed, hatched, and open circles represent subjects with *CD36*<sup>-/-</sup>, *CD36*<sup>+/-</sup> and WT, respectively. \*\**p*<0.0001 in *CD36*<sup>-/-</sup> vs *CD36*<sup>+/-</sup> and *CD36*<sup>+/-</sup> vs WT, respectively; \**p*<0.005 in *CD36*<sup>-/-</sup> vs *CD36*<sup>+/-</sup>.

LCFA uptake, 5 ROIs (3×3 pixels in size) were placed on the myocardial image obtained from the planar image (Fig 1). The ROI was also placed over the mediastinum and used as the background activity. The H/M ratio was calculated as a fraction of the mean counts per pixel in the heart divided by those in the mediastinum.

#### Statistical Analysis

Data are presented as mean±standard deviation (SD). The data were compared by one way analysis of variance (ANOVA) with the Scheffe's test. A value of *p*<0.05 was considered significant.

## Results

#### Characteristics of the Subjects (Table 1)

There were 16 subjects with the WT: 6 healthy subjects

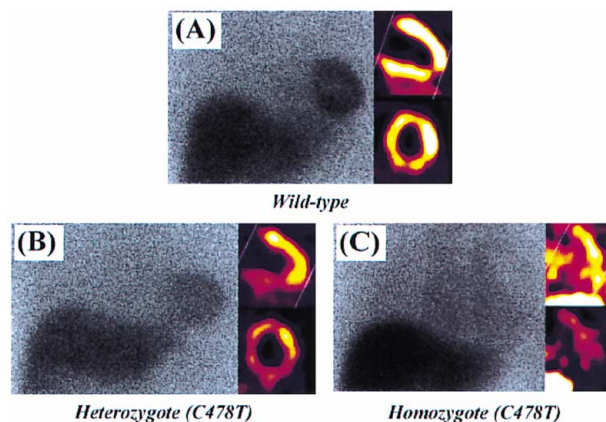


Fig 2. Representative scintigrams in subjects with the wild-type *CD36* gene, heterozygous mutation of C478T, and homozygous mutation of C478T.

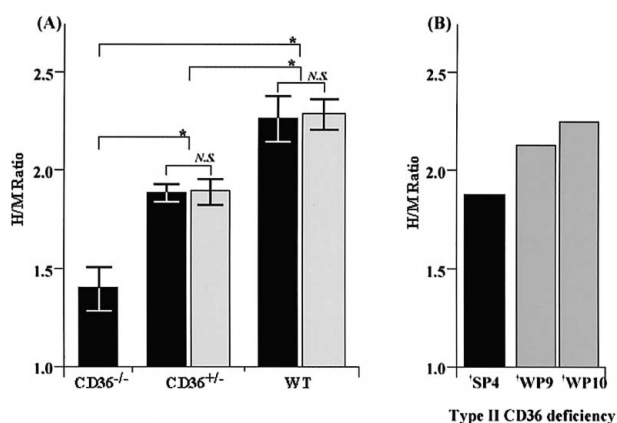


Fig 4. The H/M ratio in healthy subjects and patients with heart disease (A) and in patients with type II *CD36* deficiency (B). (A) Whole-heart H/M ratio in patients with heart disease and in healthy subjects with *CD36*<sup>-/-</sup>, *CD36*<sup>+/-</sup> and WT. Closed and shaded bars represent patients with heart disease and healthy subjects, respectively. Patients with *CD36*<sup>-/-</sup>: *n*=4; patients with *CD36*<sup>+/-</sup>: *n*=5; healthy subjects with *CD36*<sup>+/-</sup>: *n*=4; patients with WT: *n*=8; healthy subjects with WT: *n*=6. \**p*<0.0001 among groups, NS, not significant. (B) Whole-heart H/M ratio in patients with type II *CD36* deficiency. The closed bar is SP4 in Table 1 and the shaded bars are WP9 and WP10, respectively, in Table 1. SP4 was *CD36*<sup>+/-</sup> and WP9 and WP10 were WT.

and 10 patients with heart disease, including vasospastic angina, hypertension, coronary artery disease, dilated cardiomyopathy and hypertrophic cardiomyopathy. There were 9 subjects with *CD36*<sup>+/-</sup>: 8 for C478T and 1 for Del539AC; 4 were healthy subjects and 5 were patients, including vasospastic angina, hypertension, dilated cardiomyopathy and hypertrophic cardiomyopathy. There were 4 patients with *CD36*<sup>-/-</sup>: 1 homozygous mutation (*CD36*<sup>-/-</sup>) for C478T, 1 compound heterozygous mutation (*CD36*<sup>-/-</sup>) for C478T and Ins1159A, and 2 *CD36*<sup>-/-</sup> for C478T and Del539AC.

#### H/M Ratio

Representative SPECTs from subjects with WT, *CD36*<sup>+/-</sup> and *CD36*<sup>-/-</sup> are shown in Fig 2. The relation between the whole-heart H/M ratio (vertical axis) and monocyte MFI (horizontal axis) is shown in Fig 3A. The whole-heart H/M

**Table 2** Mutations, H/M Ratio and Expression of CD36 Protein in Monocytes and Platelets

Mutation	n	H/M ratio (mean±SD)	Monocyte MFI (mean±SD)	Platelet MFI (mean±SD)
CD36 <sup>-/-</sup>	4	1.40±0.11	0.01±0.01	0.19±0.15
CD36 <sup>+/-</sup>	9	1.90±0.06*	14.8±2.94*	12.7±5.53*
WT	16	2.28±0.10*	31.7±6.47*	18.1±10.3 <sup>NS</sup>

CD36<sup>-/-</sup>, homozygous or compound heterozygous mutation of the CD36 gene; CD36<sup>+/-</sup>, heterozygous mutation; WT, wild-type gene; H/M ratio, whole-heart to mediastinum radioactivity ratio; MFI, mean fluorescence intensity; SD, standard deviation.

\**p*<0.0001 CD36<sup>-/-</sup> vs CD36<sup>+/-</sup>, and CD36<sup>+/-</sup> vs WT, respectively. NS, No significant difference between CD36<sup>+/-</sup> and WT (*p*=0.16).

ratio representing LCFA uptake in the heart showed a good correlation with monocyte MFI representing expression level of CD36 in monocytes. The whole-heart H/M ratios in subjects with WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup> were 2.28±0.10, 1.90±0.06, and 1.40±0.11 (*p*<0.0001 among groups) (Table 2). Although the H/M ratios in the basal, mid-ventricular and apical regions demonstrated a significant and homogeneous reduction in subjects with CD36<sup>+/-</sup> and CD36<sup>-/-</sup> compared with the WT, those the respective genotypes stayed within the same range of magnitude (Fig 3B). The whole-heart H/M ratio between healthy subjects and patients with heart disease did not significantly differ in either WT (2.29±0.08 in healthy subjects and 2.27±0.12 in patients) or CD36<sup>+/-</sup> (1.90±0.07 in healthy subjects and 1.89±0.05 in patients) (Fig 4).

#### H/M Ratio and Expression of CD36 Protein

The H/M ratio in each individual was strongly associated with the monocyte MFI (Fig 3A), but not with platelet MFI in either WT or CD36<sup>+/-</sup>. Platelet MFI in both WT and CD36<sup>+/-</sup> was distributed in a broad range (0.1–37.1 in WT and 0.3–20.5 in CD36<sup>+/-</sup>, Table 1) and no significant difference of platelet MFI was observed between the 2 genotypes (*p*=0.16, Table 2). However, the H/M ratio in both WT and CD36<sup>+/-</sup> subjects was within the same range of magnitude (2.17–2.51 in WT and 1.83–1.97 in CD36<sup>+/-</sup>, Table 1) and significant difference of the H/M ratio was observed between them (*p*<0.0001, Table 2). Consequently, we speculated that the LCFA uptake in the heart and the expression of CD36 in monocytes were regulated by the same or closely related factor(s), but was not the case for the expression of CD36 in platelets.

A phenotype lacking CD36 expression in platelets, but present in monocytes, has been reported and is referred to as type II CD36 deficiency!<sup>6</sup> We had 3 cases in the present study (SP4, WP9 and WP10 in Table 1) and we investigated their CD36 genotype, H/M ratio, and expression of CD36 in platelets and monocytes.

Case SP4 was CD36<sup>+/-</sup> for C478T, but the other 2 cases (WP9 and WP10) did not demonstrate any mutation in the coding region of CD36. The H/M ratio in SP4 was 1.88 and 2.13 and 2.25, respectively, for WP9 and WP10 (Fig 4). The monocyte MFI from Case SP4 was 19.0, and 25.4 and 27.9 for Cases WP9 and WP10, respectively. This finding also suggests that the LCFA uptake in the heart and the expression of CD36 in monocytes, but not in platelets, strongly depend on the CD36 genotype.

## Discussion

We studied the effects of mutations of the CD36 gene on the LCFA uptake in the heart and demonstrated the following.

(1) Mutations of this gene, not only CD36<sup>-/-</sup> but also CD36<sup>+/-</sup>, resulted in a significant reduction of the LCFA uptake in the heart compared with the WT.

(2) The uptake of LCFA in the heart was strongly associated with the CD36 genotype independent of heart disease.

(3) The type II CD36-deficient phenotype is heterogeneous and did not always accompany a defect in myocardial LCFA uptake.

#### LCFA Uptake in the Heart and CD36 Genotype

The cardiac image on the BMIPP scintigram was almost invisible in patients with CD36<sup>-/-</sup>, but was clearly visible for those subjects with either CD36<sup>-/-</sup> or WT and did not differ qualitatively between them (Fig 2). However, the H/M ratio, a semi-quantitative assessment of the BMIPP uptake in the heart, revealed significant differences among the genotypes. The H/M ratio was significantly decreased in CD36<sup>-/-</sup> (1.40; 61.4% of WT) and CD36<sup>+/-</sup> (1.90; 83.3% of WT) compared with WT (2.28) (Table 2). CD36<sup>-/-</sup> resulted in a null level of CD36 expression in monocytes and platelets and an almost negative depiction of the heart on BMIPP scintigraphy.<sup>8</sup> Accordingly, we expected complete loss of myocardial radioactivity accumulation in such patients, but contrary to this expectation, substantial radioactivity was observed in their hearts, consistent with the myocardial BMIPP radioactivity observed in CD36 knock-out mice (50–80% of control!).<sup>10</sup> Hence, the myocardial radioactivity observed in both patients and mice implies additional myocardial LCFA uptake processes (eg, simple diffusion and/or other protein-mediated process).<sup>17–20</sup> Assuming that the difference between the H/M ratio in a given genotype and that in CD36<sup>-/-</sup> represents the fraction of CD36-mediated LCFA uptake in the heart, those fractions in WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup> were 0.88, 0.5 and 0, respectively, suggesting a gene–dosage-dependent effect of CD36 on LCFA uptake in the heart. As well, the expression of CD36 in monocytes suggests a gene–dosage-dependency; that is, the monocyte MFI in WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup> was 31.7±6.47, 14.8±2.94 and 0, respectively (Table 2). We could not analyze CD36 expression in cardiomyocytes, but we consider it likely that functional CD36 protein is not expressed in cardiomyocytes of the CD36<sup>-/-</sup> genotype and are expressed at half the level of the WT in the CD36<sup>+/-</sup> subjects, which may account for the differences in myocardial BMIPP uptake among the 3 genotypes.

#### LCFA Uptake in the Heart and Heart Disease

Modifications of LCFA utilization in the heart are well known in pathological hearts<sup>1</sup> and the reduced myocardial radioactivity observed in the present study thus might be a consequence of disease-induced pathophysiology. We could not evaluate LCFA uptake in any healthy subject with CD36<sup>-/-</sup>, but were able to do so in 4 with CD36<sup>+/-</sup>.

The H/M ratio between healthy subjects and patients with heart disease in both the WT and CD36<sup>+/-</sup> genotypes were as follows.

(1) The whole-heart H/M ratio in both CD36<sup>+/-</sup> and WT genotypes did not differ significantly between healthy subjects and patients with heart disease (Fig 4).

(2) The whole-heart H/M ratio in WT was comparable to the reported values in normal subjects.<sup>14,15</sup>

(3) The reduction of myocardial BMIPP uptake in CD36<sup>-/-</sup> (61.4% of WT) was consistent with that observed in CD36 knock-out mice (50–80% of control).<sup>10</sup>

(4) The whole-heart H/M ratio was rather strongly associated with the CD36 genotype independent of heart disease; that is, WT > CD36<sup>+/-</sup> > CD36<sup>-/-</sup>.

Overall, we consider that the defects of BMIPP uptake in the heart are unlikely to be a secondary consequence of heart disease. However, the number of patients in this study was small and none were severely ill (New York Heart Association class I–II). Further studies in a sufficient number of patients with a broad range of severity of heart disease are still necessary.

#### *LCFA Uptake in the Heart and Expression of CD36 Protein*

The present study included 3 patients with type II CD36 deficiency, which is a lack of CD36 in platelets but not in monocytes. The expression of CD36 in the monocytes of 2 patients (Cases WP9 and WP10) was comparable to that in the WT, but was halved in Case SP4. We identified CD36<sup>+/-</sup> for C478T in Case SP4, but did not detect any mutation in Cases WP9 and WP10. The whole-heart H/M ratio and the expression of CD36 in the monocytes of Cases WP9 and WP10 were comparable to those in WT, and those in Case SP4 were comparable to those in CD36<sup>+/-</sup>.

We recently found that type II CD36 deficiency has 2 subgroups.<sup>13</sup>

(1) Platelet-restricted CD36 expression disorder(s) with the WT coding region of *CD36*. In this group, CD36 expression is lacking in platelets, but the expression in monocytes is comparable to the WT (eg, Cases WP9 and WP10).

(2) Complex disorders; that is, platelet-restricted CD36 expression disorder(s) together with CD36<sup>+/-</sup> in the coding region of *CD36*. In this group, CD36 expression is also lacking in platelets, but in monocytes the expression is comparable to CD36<sup>+/-</sup>, at approximately half that of the WT (eg, Case SP4).

Despite the broad range of platelet MFI in subjects with either WT or CD36<sup>+/-</sup>, the H/M ratio stayed in a narrow range of magnitude in both groups. Furthermore, the H/M ratio in patients with type II CD36 deficiency was associated with the genotype in the coding region of *CD36* (Fig 4). Taking all the results together, we consider that the genotype in the coding region of *CD36* strongly affects the LCFA uptake in the heart and the expression of CD36 in monocytes, but not in platelets. However, further studies in a large number of subjects will be necessary to clarify this.

#### *LCFA Analog Uptake in the Heart and Factors Influencing Radioactive Uptake*

In the present study, the radioactivity in the heart was measured at 30 min after intravenous administration of BMIPP. This time point was regarded as representing the LCFA uptake in the heart, because plasma radioactivity during this time period decreased sharply and then increased slowly thereafter probably because of metabo-

lites of BMIPP.<sup>21</sup> However, several factors still modify the LCFA uptake in the heart.

Among them, plasma concentrations of energy substrates such as glucose and LCFA are known to affect the myocardial extraction of radioactive LCFA analogs.<sup>22</sup> In the present study, the concentrations of plasma glucose, insulin, and free fatty acid under fasting conditions and the results of a 75-g oral glucose tolerance test did not significantly differ among the subjects with WT, CD36<sup>+/-</sup> and CD36<sup>-/-</sup> (data not shown). Thus, we consider plasma substrate concentrations had little effect on the myocardial BMIPP uptake, but further studies in large mass populations will be necessary to verify this.

Coronary artery disease is also known to cause defects of the BMIPP uptake in the heart.<sup>2–5</sup> Myocardial perfusion assessed by thallium-201 scintigraphy did not show any detectable perfusion disorder in the 4 patients with CD36<sup>-/-</sup>, in agreement with previous findings.<sup>8</sup> In addition, the H/M ratio in the 5 regions of the heart decreased homogeneously and significantly in both subjects with CD36<sup>-/-</sup> and CD36<sup>+/-</sup> compared with those with WT. Therefore, a total and/or regional disorder of coronary blood supply is unlikely to be the cause of the whole and homogeneous reduction of the BMIPP uptake in the heart.

#### *Study Limitations*

We did not include healthy subjects with CD36<sup>-/-</sup>, thus we could not compare the H/M ratio between healthy subjects and patients with heart disease for CD36<sup>-/-</sup>. However, this flaw may be overcome by the finding of a significant reduction of myocardial BMIPP uptake in CD36 knock-out mice.<sup>10</sup> The numbers of subjects with CD36<sup>-/-</sup> and CD36<sup>+/-</sup> was relatively small and the effects of these mutations on cardiac morphology and physiology were not fully studied. Thus, we cannot suggest the pathological relevance of this gene mutation.

However, defects of myocardial BMIPP uptake with an increased accumulation of flow-tracer have been observed in 80% of patients with hypertrophic cardiomyopathy.<sup>6</sup> Whether this phenomenon is a primary or secondary consequence of hypertrophic cardiomyopathy is still unclear. We have observed both CD36<sup>-/-</sup> and CD36<sup>+/-</sup> in patients with hypertrophic cardiomyopathy and hypothesize that there is a possible link of this gene mutation with cardiomyopathy.<sup>23,24</sup> In contrast, Nakamura et al observed little influence of CD36 deficiency on the pathophysiology of hypertrophic cardiomyopathy; however, they did not characterize the *CD36* genotype.<sup>25</sup> The type I and II CD36-deficient phenotypes do not always show mutations in the coding region of *CD36* on qualitative flow cytometric analysis,<sup>13</sup> so phenotype typing by this method is likely to overlook subjects with CD36<sup>+/-</sup> because they show a positive expression of CD36 in both platelets and monocytes. Furthermore, in the present study subjects with type II CD36 deficiency were heterogeneous for LCFA uptake in the heart and expression of CD36 in monocytes. Thus, the inconsistency between our finding and that of Nakamura et al may, at least in part, be explained by the difference in the definition of CD36 deficiency; in other words, classification by genotype or phenotype. We believe that the characterization of the genotype in the coding region of *CD36* is of fundamental importance for exploring a possible involvement of CD36 deficiency in heart disease. However, the significance of this deficiency in cardiomyopathy is the subject of further investigation.

CD36 deficiency has also been found in spontaneously hypertensive rats<sup>26</sup> and CD36 knockout mice generated by gene-knockout technology.<sup>27</sup> Observations in CD36 deficient humans and rodents have suggested the possible pathological involvement of CD36 deficiency in cardiomyopathy<sup>8,23,24,28–30</sup> and altered lipid and carbohydrate handling<sup>31,32</sup> in humans, and spontaneous hypertension, hypertriglyceridemia, abdominal obesity, insulin resistance,<sup>26,33,34</sup> cardiac hypertrophy<sup>35</sup> and dyslipidemia<sup>27</sup> in rodents.

Subjects with CD36<sup>+/-</sup> are relatively common in the Japanese population (approximately 10%)<sup>13</sup> and there were 4 healthy subjects without evident cardiac manifestation in the present study. This may challenge the aforementioned possible pathological involvement and our hypothesis, but the LCFA uptake in the heart, assessed by the H/M ratio, was significantly decreased in all 4. Hajri et al recently demonstrated the elimination of cardiac hypertrophy by dietary supplementation with short-chain fatty acids in CD36-deficient rats<sup>35</sup> which suggests possible involvement of dietary and/or environmental factors on the development and/or progression of cardiac hypertrophy in individuals with mutations for the *CD36* gene, and may, at least in part, explain the difference in cardiac phenotype in these individuals. In addition, because of the existence of a residual LCFA uptake observed in CD36<sup>-/-</sup>, cardiac complications might be finally develop after the cumulative repetition of disadvantageous conditions (eg, prolonged starvation, exercise or serious infection).

In summary, the uptake of LCFA in the heart was strongly associated with the *CD36* genotype, independent of heart disease, suggesting a gene–dosage-dependent effect. Together with our previous report<sup>8</sup>, the findings presented here strongly support our hypothesis that the *CD36* gene is a primary determinant of LCFA uptake in the human heart.

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