Depleted Leukocyte Mitochondrial DNA Copy Number Correlates With Unfavorable Left Ventricular Volumetric and Spherical Shape Remodeling in Acute Myocardial Infarction After Primary Angioplasty

Ching-Hui Huang, MD, PhD; Chen-Ling Kuo; Ching-San Huang, BSc; Chin-San Liu, MD, PhD; Chia-Chu Chang, MD

Background: Left ventricular (LV) shape influences LV systolic function. It is possible to assess LV shape using 3-D echocardiography sphericity index (SI). Maintaining mitochondrial DNA copy number (MCN) is important for preserving mitochondrial function and LV systolic function after acute myocardial infarction (AMI). Information is limited, however, regarding the relationship between leukocyte MCN and the subsequent change in LV shape after AMI.

Methods and Results: Fifty-five AMI patients undergoing primary angioplasty were recruited. Plasma MCN was measured before primary angioplasty using quantitative polymerase chain reaction. 3-D echocardiography measurement of SI was performed at baseline, and at 1-, 3-, and 6-month follow-up. AMI subjects with MCN lower than the median had a higher 6-month SI and LV volume compared with those with higher MCN. Baseline echocardiographic parameters were similar between the 2 groups. MCN was negatively correlated with 3- and 6-month SI, and 3- and 6-month LV volume. On multiple linear regression analysis, baseline plasma MCN could predict LV SI and LV volume at 6 months after primary angioplasty for AMI, even after adjusting for traditional prognostic factors.

Conclusions: In AMI patients, higher plasma leukocyte MCN at baseline was associated with favorable LV shape and remodeling at 6-month follow-up. Plasma leukocyte MCN may provide a novel prognostic biomarker for LV remodeling after AMI.

Key Words: Acute myocardial infarction; Mitochondrial DNA copy number; Sphericity index

Improved acute coronary care has led to an increase in acute myocardial infarction (AMI) survivors, who are at increased risk of heart failure.1–3 Left ventricular (LV) shape has been shown to change from elliptical to spherical during the remodeling process following AMI.4–7 This increased LV sphericity is associated with significantly increased LV end-diastolic and end-systolic volumes (ESV).8 Although LV volume remains a powerful indicator of cardiovascular prognosis following cardiac injury, change in cardiac shape beyond LV volume has recently gained attention as a predictor of remodeling.9 Moreover, LV shape influences LV systolic function.10,11 In heart disease, changes in cardiac shape occur before functional changes to the heart, and initiate the cascade of heart failure.12 Clinically, LV shape can be assessed using 3-D echocardiography sphericity index (SI).4,5 A higher SI correlates with a more spherical LV shape; and baseline 3-DSI can accurately predict LV remodeling after AMI.4,5 Mitochondria play a central role in energy production through oxidative phosphorylation.13 Mitochondrial DNA (mtDNA) is situated near the inner mitochondrial membrane and electron transport system, making it prone to oxidative stress from the electron transport system.14,15 Expression of mtDNA-encoded genes is largely regulated by mtDNA copy number (MCN). Moreover, maintenance
of MCN is important for the preservation of mitochondrial function.16,17 Accumulating evidence has suggested that mitochondrial dysfunction plays a critical role in the development of heart failure.18–21 In addition, in epidemiological studies lower leukocyte mtDNA content was associated with an increased risk of coronary artery disease and heart failure.22–24 Therefore, we postulated that maintenance of MCN is important for preserving mitochondrial function and LV systolic function after AMI. Limited information is available, however, on the relationship between leukocyte MCN at baseline and the development of subsequent LV remodeling at 6 months after AMI.

A study combining both volumetric and geometric assessments of the LV is important for evaluating cardiac remodeling after AMI. The aim of this study was therefore to evaluate whether baseline peripheral leukocyte MCN is predictive of LV remodeling after primary angioplasty in patients with ST-segment elevation myocardial infarction (STEMI). LV shape was assessed on 3-DSI, and LV volume was analyzed on percent change.

### Methods

**Subjects and Study Protocol**

In this prospective study, we enrolled 55 consecutive patients with de novo acute STEMI who underwent primary percutaneous coronary intervention (PCI) and thrombectomy between January 2010 and January 2011 at Changhua Christian Hospital in Taiwan. We also enrolled 54 age- and sex-matched healthy volunteers as a control group for leukocyte MCN comparison. Enrolled patients and healthy volunteers ranged from 18 to 80 years of age and all provided informed consent. The protocol with approval number 071206 received ethics approval from the Institutional Review Board of Changhua Christian Hospital, Taiwan. Leukocyte MCN was measured from samples of venous blood obtained prior to PCI. Baseline lipid and glucose concentration were measured after fasting for 8 h. Creatine kinase-MB (CK-MB) fraction and troponin I concentration were measured every 4 h until they started to decline, then peak values were recorded. A diagnosis of STEMI was based on the Joint Task Force

### Table 1. AMI Patient Characteristics vs. Baseline Leukocyte MCN

|                      | MCN ≥82/cell (n=28) | MCN <82/cell (n=27) | P-value |
|----------------------|---------------------|---------------------|---------|
| Sex (M/F)            | 24/4                | 23/4                | 0.651   |
| Age (years)          | 58.9±12.4           | 59.1±11.8           | 0.945   |
| CPK (μL)             | 1,904±1,455         | 2,703±2,303         | 0.154   |
| CK-MB (ng/mL)        | 222±186             | 246±194             | 0.659   |
| Troponin I (ng/mL)   | 7.9±20.9            | 2.2±3.8             | 0.266   |
| Creatinine (mg/dL)   | 1.02±0.21           | 1.02±0.43           | 0.927   |
| Cholesterol (mg/dL)  | 187.7±43.5          | 187.6±44.6          | 0.992   |
| HDL-C (mg/dL)        | 40.0±9.9            | 41.9±9.1            | 0.493   |
| LDL-C (mg/dL)        | 129.4±34.8          | 134.9±42.5          | 0.617   |
| Triglyceride (mg/dL) | 110.8±167.3         | 64.0±65.6           | 0.198   |
| Fasting glucose (mg/dL) | 133.0±43.4        | 171.0±115.2         | 0.142   |
| HbA1c (%)            | 6.1±1.0             | 6.5±2.0             | 0.349   |
| BMI (kg/m²)          | 25.8±3.6            | 24.9±3.6            | 0.408   |

**Risk factors**

- Hypertension: 25 (89%) vs. 19 (70%), P=0.148
- Diabetes mellitus: 6 (21%) vs. 9 (33%), P=0.485
- Dyslipidemia: 25 (89%) vs. 26 (96%), P=0.323
- Smoking: 18 (64%) vs. 18 (67%), P=0.913

**Medications**

- β-blocker: 17 (60%) vs. 19 (70%), P=0.295
- ACEI/ARB: 28 (100%) vs. 25 (92%), P=0.161
- Antiplatelet agents: 28 (100%) vs. 27 (100%)
- Statin: 24 (86%) vs. 23 (85%), P=0.953

**Infarct-related coronary artery**

- LAD: 19 (68%) vs. 17 (63%), P=0.270
- LCX: 3 (11%) vs. 3 (11%)
- RCA: 6 (21%) vs. 7 (26%)

**Post-PCI TIMI flow grade**

- 2.96±0.19 vs. 2.96±0.20, P=0.978

**Myocardial blush grade**

- 2.00±0.80 vs. 2.20±0.18, P=0.381

**D2B time (min)**

- 81.4±31.5 vs. 86.2±42.9, P=0.688

Data given as mean±SD or n (%). *P<0.05, Student’s t-test. ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CK-MB, creatine phosphokinase-MB; CPK, creatine phosphokinese; D2B, door to balloon; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; LDL-C, low-density lipoprotein cholesterol; MCN, mitochondrial DNA copy number; PCI, percutaneous coronary intervention; RCA, right coronary artery; TIMI, Thrombolysis in Myocardial Infarction.
for the Universal Definition of Myocardial Infarction as reported by Thygesen et al and in our previous studies. Briefly, STEMI was diagnosed if all of the following were present: (1) symptoms of cardiac ischemia; (2) ST segment elevation 0.2 mV in ≥2 contiguous electrocardiography leads; and (3) serum troponin I or CK-MB in excess of the 99th percentile reference limit within 24 h of pain onset. The culprit vessel was identified based on clinical data and on electrocardiography and angiography. All patients were placed on 100 mg aspirin and 300 mg clopidogrel prior to PCI. After PCI, patients received standard acute coronary care including aspirin, clopidogrel, β-blockers, statins, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers as appropriate. Patients also received smoking cessation advice and lifestyle counseling as per protocol. Echocardiography was performed within the first 2 days after primary PCI and followed up at 1 month, 3 months and 6 months. Echocardiography was carried out by a qualified cardiologist who was blinded to leukocyte MCN.

**Plasma Biochemistry**

**Plasma Lipid Profiles** Plasma total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride concentrations were measured using a Beckman Unicel DxC 800 chemistry analyzer (Beckman Coulter, USA).

### Table 2. Echocardiography Parameters vs. Baseline Leukocyte MCN

| Parameter                        | MCN ≥82 (n=28) | MCN <82 (n=27) | P-value |
|----------------------------------|----------------|----------------|---------|
| **3-D Sphericity index**         |                |                |         |
| Baseline                         | 0.335±0.061    | 0.358±0.059    | 0.200   |
| 1 month                          | 0.319±0.049    | 0.379±0.066    | 0.001** |
| 3 months                         | 0.324±0.055    | 0.372±0.075    | 0.021*  |
| 6 months                         | 0.328±0.061    | 0.382±0.056    | 0.005** |
| **LV volume and EF**            |                |                |         |
| LVEDV (mL)                       | 77.2±25.3      | 75.7±15.6      | 0.825   |
| 1 month                          | 74.3±21.6      | 83.3±21.8      | 0.182   |
| 3 months                         | 74.5±15.9      | 85.2±21.4      | 0.068   |
| 6 months                         | 73.0±15.8      | 86.1±20.9      | 0.025** |
| LVEESV (mL)                      | 31.6±25.1      | 29.6±12.4      | 0.743   |
| 1 month                          | 25.6±12.2      | 30.2±17.4      | 0.320   |
| 3 months                         | 26.4±9.0       | 36.5±17.9      | 0.030*  |
| 6 months                         | 25.3±6.2       | 35.6±20.7      | 0.044*  |
| LVEF (%)                         | 60.5±10.5      | 59.4±11.5      | 0.747   |
| 1 month                          | 65.4±8.5       | 61.7±12.9      | 0.272   |
| 3 months                         | 64.9±7.2       | 58.3±14.2      | 0.071   |
| 6 months                         | 64.5±7.1       | 59.2±15.6      | 0.176   |
| Percentage change in LVEF at 6 months† | 9.9±25.5  | −0.4±18.1       | 0.140   |
| WMSI                             | 1.24±0.21      | 1.32±0.20      | 0.271   |
| 1 month                          | 1.20±0.22      | 1.25±0.22      | 0.470   |
| 3 months                         | 1.14±0.16      | 1.23±0.20      | 0.102   |
| 6 months                         | 1.10±0.12      | 1.23±0.27      | 0.047*  |

(Table 2 continued the next page.)
Society of Echocardiography guidelines. Primary measurements of mitral inflow included the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, E/A ratio, and the deceleration time of early filling velocity. TDI was performed in the apical view to acquire mitral annular velocities. Early diastolic annular velocity was expressed as e', and late diastolic velocity as a'. The ratio of the mitral inflow E velocity to tissue Doppler e' (E/e' ratio) was calculated. The average E/e' ratio obtained from the septal and lateral sides of the mitral annulus was used to predict LV filling pressure.

Real time 3-D echocardiography was obtained using an iE33 xMATRIX echocardiography system (Philips Medical Systems, Andover, MA, USA). 3-DSI was calculated by dividing the end-diastolic volume (EDV) by the volume of a sphere, the diameter of which was derived from the major end-diastolic LV long axis (D). The LV long axis (D) was obtained from the 3-D echocardiographic dataset as the longest distance between the center of the mitral annulus and the endocardial apex. A spherical volume in mL was calculated using the formula: \( \frac{4}{3} \pi r^3 \), of which D is the diameter (cm). 3-DSI was calculated as EDV/(\( \frac{4}{3} \pi \times \frac{D^3}{2} \)), as described by Mannaerts et al. Higher SI indicates a more spherical LV shape.

**Statistical Analysis**

Data are given as mean±SD. Differences between the AMI group and the control group were evaluated using Student’s t-test. The Jonckheere-Terpstra test for trend was used to analyze the association between baseline leukocyte MCN, 6-month 3-DSI and 6-month LVEDV. The Jonckheere-Terpstra test is similar to the Kruskal-Wallis test but is applied to samples with a priori ordering. A multivariate linear regression model was used to evaluate independent associations between possible predictors and 3-DSI at 6 months. Factors associated with leukocyte MCN and 6-month 3-DSI after AMI were evaluated using a univariate correlation model. Receiver operating characteristic curves were constructed and the area under the curve.
Circulation Journal Vol. 81, December 2017

MCN and SI in AMI

Patients with adverse LV remodeling had significantly lower baseline MCN compared with patients without adverse LV remodeling. In addition, patients with adverse LV remodeling also had higher SI at 3 and at 6 months after STEMI than those without adverse LV remodeling (Table 3).

Six-Month SI Is Inversely Proportional to MCN in AMI
AMI patients were divided into 4 subgroups according to MCN quartile: group 1, MCN ≤59/cell (n=14); group 2, MCN 60.0–82.0/cell (n=14); group 3, MCN 83.0–139.9/cell (n=14); group 4, MCN ≥140.0/cell (n=13). On trend analysis, SI at 6 months was inversely proportional to baseline MCN (Jonckheere-Terpstra test, P=0.027, Figure 1A).

Adverse LV Remodeling
Adverse LV remodeling was defined as a change in LVEDV >10% at 3 months and at 6 months after STEMl. AMI patients with adverse LV remodeling had significantly lower baseline LVEDV at 6 months after STEMI compared with patients without adverse LV remodeling. In addition, patients with adverse LV remodeling also had higher SI at 3 and at 6 months after STEMI than those without adverse LV remodeling (Table 3).

Six Month LVEDV Is Inversely Proportional to MCN in AMI
LVEDV at 6 months was inversely proportional to baseline MCN, according to the AMI MCN quartile groups (Jonckheere-Terpstra test, P=0.006; Figure 1B).

Baseline MCN Predicts Post-AMI LV Spherical Remodeling at 6 Months
On multiple linear regression analysis of variables associated with LV 3-DSI at 6 months after AMI, baseline leukocyte MCN could predict LV SI (i.e., increased spherical remodeling). LV SI was predicted even when traditional prognostic factors such as LVEF, LV WMSI, cardiac enzyme levels, presence of mitral regurgitation, and infarct location were taken into account (Table 5). Cardiac enzyme CKP was positively associated with 6-month SI (P=0.051).

Six-Month Post-AMI SI Predicts Percentage Change in LVEF From Baseline
Percentage change in LVEF at 6 months was calculated by subtracting the LVEF at baseline from the EF at 6 months, and then dividing that by the baseline EF. On multiple

(AUC) was calculated. AUC was used to assess the sensitivity and specificity of leukocyte MCN at baseline for predicting LV sphericity remodeling at 1 month, 3 months, and 6 months. P<0.05 was considered statistically significant. All statistical analysis was performed using SPSS for Windows (version 15.0, SPSS, Chicago, IL, USA). Because this was a pilot study, we had no reference data on leukocyte MCN in patients with STEMI. Therefore, we were not able to estimate the sample size and study power.

Results

Baseline Characteristics and Leukocyte MCN
There were 55 patients in the AMI group (age, 57.4±11.4 years; male, n=47; female, n=8) and 54 age- and sex-matched healthy volunteers (age, 55.3±7.4 years; male, n=44; female, n=10). Baseline plasma leukocyte MCN in the AMI group was significantly lower than in the control group (122.7±109.3 vs. 194.9±119.5/cell, P=0.003; Figure S1; Table S1).

Baseline characteristics vs. median leukocyte MCN
Median baseline leukocyte MCN in the AMI group was 82/cell. We therefore divided AMI patients into 2 groups according to median leukocyte MCN: MCN≥82 and MCN<82. Biochemistry, risk factors, medication history, and coronary angiography-related characteristics are listed in Table 1. Echocardiography data are listed in Table 2. AMI patients with lower MCN (defined as lower than the median) had higher SI at 1-month, 3-month, and 6-month follow-up. They also had a higher 6-month LVEDV and 6-month LVESV than patients with the higher MCN. At 6 months there was no significant difference in EF with regard to baseline MCN level in AMI patients. WMSI at 6 months, however, was significantly higher in the lower MCN group than in the higher MCN group. There were no significant differences in baseline echocardiographic parameters between the 2 subsets within the AMI group.

Six-Month SI Is Inversely Proportional to MCN in AMI
AMI patients were divided into 4 subgroups according to MCN quartile: group 1, MCN ≤59/cell (n=14); group 2, MCN 60.0–82.0/cell (n=14); group 3, MCN 83.0–139.9/cell (n=14); group 4, MCN ≥140.0/cell (n=13). On trend analysis, SI at 6 months was inversely proportional to baseline MCN (Jonckheere-Terpstra test, P=0.027, Figure 1A).

Adverse LV Remodeling
Adverse LV remodeling was defined as a change in LVEDV >10% at 3 months and at 6 months after STEMl. AMI patients with adverse LV remodeling had significantly lower baseline LVEDV at 6 months after STEMl compared with patients without adverse LV remodeling. In addition, patients with adverse LV remodeling also had higher SI at 3 and at 6 months after STEMl than those without adverse LV remodeling (Table 3).

Six Month LVEDV Is Inversely Proportional to MCN in AMI
LVEDV at 6 months was inversely proportional to baseline MCN, according to the AMI MCN quartile groups (Jonckheere-Terpstra test, P=0.006; Figure 1B).

Baseline MCN Predicts Post-AMI LV Spherical Remodeling at 6 Months
On multiple linear regression analysis of variables associated with LV 3-DSI at 6 months after AMI, baseline leukocyte MCN could predict LV SI (i.e., increased spherical remodeling). LV SI was predicted even when traditional prognostic factors such as LVEF, LV WMSI, cardiac enzyme levels, presence of mitral regurgitation, and infarct location were taken into account (Table 5). Cardiac enzyme CKP was positively associated with 6-month SI (P=0.051).

Six-Month Post-AMI SI Predicts Percentage Change in LVEF From Baseline
Percentage change in LVEF at 6 months was calculated by subtracting the LVEF at baseline from the EF at 6 months, and then dividing that by the baseline EF. On multiple
Discussion

Spherical Remodeling and LV Function

The main finding was that baseline peripheral leukocyte MCN was correlated with and predictive of LV sphericity and LVEDV at 6 months after AMI in patients receiving primary angioplasty. This shows that maintenance of the LV elliptical shape is energy related, and that changes to LV shape occur before systolic function decline. During heart disease, changes to LV shape occur before functional changes, and initiate the cascade of heart failure. The human heart normally has an elliptical shape, like a football. With disease progression, however, the heart undergoes remodeling to a spherical shape, and begins to resemble a basketball. The ejection and suction in a normal heart linear regression analysis of variables associated with percentage change in LVEF at 6 months after AMI, 6-month SI could negatively predict percentage change in LVEF from baseline to 6 months after AMI (P=0.025; Table 6). That is, patients with higher 6-month SI had a lower LVEF at 6 months than at baseline. Six-month SI was a better predictor for 6-month LVEF percentage change than infarct size as reflected by CK-MB (P=0.053).

Baseline Leukocyte MCN Is a Predictor of Serial LV Spherical Remodeling After STEMI

STEMI patients were divided into 2 subgroups according to median baseline leukocyte MCN: a lower MCN group and a higher MCN group. Serial LV 3-DSI was evaluated at 1 month, 3 months, and 6 months. AUC for the lower MCN group at baseline for predicting LV 3-DSI at 1 month, 3 months, and 6 months was 0.765 (95% CI: 0.619–0.912, P=0.003); 0.710 (95% CI: 0.555–0.864, P=0.019); and 0.720 (95% CI: 0.568–0.871, P=0.014), respectively (Figure 2).

Table 3. Effect of Adverse LV Remodeling† on MCN and SI

| LVEDV percent change  | LVEDV percent change  | P-value |
|-----------------------|-----------------------|---------|
| >10% at 6 months      | ≤10% at 6 months      |         |
| n                     | 17                    | 38      |
| MCN                   | 58.1±21.1             | 150.7±120.1 | <0.001* |
| SI at 3 months        | 0.381±0.080           | 0.334±0.061 | 0.018* |
| SI at 6 months        | 0.381±0.053           | 0.332±0.068 | 0.008* |

| LVEDV percent change  | LVEDV percent change  | P-value |
|-----------------------|-----------------------|---------|
| >10% at 3 months      | ≤10% at 3 months      |         |
| n                     | 22                    | 33      |
| MCN                   | 79.1±46.6             | 157.2±131.4 | 0.011* |
| SI at 3 months        | 0.374±0.072           | 0.331±0.065 | 0.023* |
| SI at 6 months        | 0.375±0.056           | 0.327±0.068 | 0.006* |

Data given as mean±SD or n. *P<0.05. †Defined as LVEDV percent change >10%. Abbreviations as in Table 2.

Table 4. Multivariate Predictors of LVEDV at 6 Months

| Predictors            | Unstandardized coefficients | Standardized coefficients | P-value |
|-----------------------|----------------------------|---------------------------|---------|
|                       | B  | SE  | β      |       |
| (Constant)            | 71.438 | 6.173 | 0.000  |       |
| MCN                   | −0.054 | 0.023 | −0.305 | 0.025* |
| CPK                   | 0.005 | 0.001 | 0.465  | 0.001* |
| IRA (LAD vs. RCA)     | 5.202 | 5.609 | 0.135  | 0.360  |
| IRA (LCX vs. RCA)     | 3.707 | 8.444 | 0.063  | 0.663  |

R²=0.432; *P<0.05. IRA, infarct-related artery. Other abbreviations as in Tables 1,2.

Table 5. Multivariate Predictors of SI at 6 Months

| Predictors            | Unstandardized coefficients | Standardized coefficients | P-value |
|-----------------------|----------------------------|---------------------------|---------|
|                       | B  | SE  | β      |       |
| (Constant)            | 0.345 | 0.171 | 0.052  |       |
| MCN                   | 0.000 | 0.000 | −0.386 | 0.020* |
| CPK                   | 1.15×10⁻⁵ | 0.000 | 0.339  | 0.051  |
| LVEF                  | 0.001 | 0.001 | 0.221  | 0.347  |
| MR (+)                | 0.013 | 0.016 | 0.119  | 0.420  |
| WMSI                  | −0.064 | 0.085 | −0.201 | 0.457  |
| IRA (LAD vs. RCA)     | 0.003 | 0.024 | 0.021  | 0.909  |
| IRA (LCX vs. RCA)     | 0.004 | 0.036 | 0.018  | 0.922  |

R²=0.33; *P<0.05. MR, mitral regurgitation. Other abbreviations as in Tables 1–3.
are dependent on the integrity of the apical ellipse. The apical loop fibers are normally efficient and cause maximum shortening and lengthening when they are in an oblique orientation. For example, under normal conditions, an oblique apical loop fiber contraction can cause an EF of 60%; but when fibers are in a more transverse orientation, this results in an EF of only 30%. Spherical remodeling during the progression of heart disease is characterized by an enlarged dilated ventricle and loss of the helical apex, resulting in diminished capacity for shortening and lengthening. Structurally, the consequence of heart disease result in the oblique apical loop fibers becoming more transverse, and the fiber orientation of the apical loop resembling the basal loop. Furthermore, if heart disease is associated with an underlying functional fiber abnormality, shortening becomes severely impaired. Several studies have shown that LV shape and geometry are important for restoring cardiac function and improving clinical results. The more dilated the LV, the less it is able to twist. LV twist has an independent linear relationship with LV SI in patients with dilated cardiomyopathy. Ischemic cardiomyopathy patients who received surgical LV reconstruction to restore elliptical shape were able to maintain LV function and had no evidence of adverse remodeling. The surgical goal of LV reconstruction is to change the spherical shape back to an ellipse, and restore the helical apex. This specific reshaping technique can induce apical rotation and LV torsion, which can be maintained over time. The present findings are similar to those of Ambale-Venkatesh et al, who used magnetic resonance imaging to demonstrate that LV shape predicts different types of cardiovascular events in patients without cardiovascular disease. They showed that extreme sphericity was a strong predictor of incident heart failure and atrial fibrillation during long-term follow-up. Also, the addition of sphericity volume index improved heart failure risk prediction models beyond established risk factors. Despite using a different image modality to evaluate LV shape, the present study also highlights the importance of LV shape in disease progression. In the present study, LV shape differed significantly at 6 months after AMI according to lower and higher baseline MCN, regardless of 6-month EF. WMSI at 6 months, however, was significantly higher in the lower MCN group than the higher MCN group (Table 2). Following AMI, extensive regional wall motion abnormalities may be present. This may be compensated for, however, by regional hyperkinesis of the normal segments, and LVEF may appear normal. Therefore, WMSI may more accurately reflect the magnitude of myocardial damage than LVEF. Furthermore, in the present study, we showed that 3-DSI at 6 months can predict percentage change of LVEF at 6 months (Table 6). This highlights the relationship between spherical remodeling and subtle LV systolic function change after AMI.

**MCN and SI in AMI**

MCN and LV Remodeling

In the present study, baseline MCN was inversely proportional to 6-month SI and 6-month LVEDV (Figure 1). Patients with lower baseline MCN developed more spherical LV remodeling at 6 months after AMI, despite similar baseline LV 3-DSI between the 2 groups. Furthermore, lower baseline MCN could predict 1-, 3-, and 6-month 3-DSI after an AMI event. In addition, lower baseline MCN was associated with adverse LV volumetric remodeling and could predict significantly increased LVEDV at 6 months after STEMI. This suggests that a higher baseline leukocyte MCN is beneficial for maintaining an LV ellip-

### Table 6. Multivariate Predictors of LVEF Percentage Change at 6 Months†

| Predictors | Unstandardized coefficients | Standardized coefficients | P-value |
|------------|-----------------------------|---------------------------|---------|
| (Constant) | 84.547                      | 35.67                     | 0.023   |
| CK-MB mass | −0.038                      | 0.019                     | 0.301   |
| SI at 6 months | −132.208                  | 56.482                    | 0.025*  |
| IRA (LAD vs. RCA) | 3.168                    | 7.51                      | 0.069   |
| IRA (LCX vs. RCA) | 10.552                   | 11.777                    | 0.150   |
| MCN | 0.01                        | 0.034                     | 0.049   |
| Age | −209.073                    | 0.316                     | 0.106   |
| Sex | −19.073                     | 10.18                     | 0.069   |

R²=0.33; *P<0.05. †Calculated by subtracting LVEF at baseline from EF at 6 months, divided by the baseline EF. Abbreviations as in Tables 1,2.
tical shape and preventing an increase in LV volume. Because mitochondria are required for cellular energy, it is reasonable to postulate that maintenance of LV shape is energy dependent.

A previous study by Knez et al showed that peripheral blood mtDNA content (also known as mtDNA copy number) was associated with LV structure and function. They evaluated LV structure and function on echocardiography. In a cross-sectional analysis, they found that subjects with higher blood mtDNA content had smaller LV diameter and volume. They also found that higher mtDNA content was associated with better LV systolic and diastolic function as reflected by an increase in TDI s’ peak and a decrease in E/e’ ratio, respectively. Moreover, participants with higher baseline mtDNA content were less prone to increases in LV diastolic volume and diameter, and in LV mass during the 20-year follow-up period. They concluded that mtDNA content was a significant predictor of longitudinal changes in LV geometry in the general population. The present results were consistent with Knez et al. In the present study, patients with higher baseline MCN had smaller increases in LVEDV and LVESV at 6 months after AMI, compared with the lower baseline MCN group. In addition, the higher baseline MCN group had significant improvement in diastolic function at 6-month follow-up. The peak E-wave velocity change from baseline to 6 months was 76.08±20.33 to 64.09±12.38 cm/s (P=0.005), and the average E/e’ change was 10.01±2.57 to 8.71±2.28, (P=0.033). There was no significant change in diastolic function, however, in the lower baseline MCN group. Baseline 3-DSI did not differ significantly between the lower and higher baseline MCN groups. Moreover, in the lower baseline MCN group, 3-DSI was significantly increased at 1 month, 3 months, and 6 months after AMI. On multiple regression analysis, baseline MCN and infarct size were significant predictors of 3-DSI at 6 months after STEMI, independent of infarct location, baseline systolic function, and the presence of mitral regurgitation (Table 5). In addition, baseline MCN was inversely proportional to 6-month 3-DSI (Figure 1A). We therefore hypothesized that maintenance of elliptical shape (i.e., lower 3-DSI) may preserve LV function and prevent adverse remodeling. Although the mechanism is currently unknown, we postulate that it occurs via an energy-dependent process.

**Mitochondria and LV Function**

The progression of LV dysfunction to heart failure is associated with changes in cardiac energy metabolism. Mitochondria play a vital role in cardiac function, including energy production and programmed cell death. Studies in rodents have shown that manipulation of key proteins involved in mitochondrial replication can trigger mitochondrial dysfunction and cause dilated cardiomyopathy. Mitochondria contain several copies of circular mtDNA molecules. The number of mtDNA copies in a cell correlates with the size and number of mitochondria, which change under different energy demands and oxidative stress, as well as different pathological conditions.

In recent studies, a decrease in myocardial mtDNA content led to the development of dilated cardiomyopathy in mice. Conversely, preservation of MCN by genetic manipulation of the mitochondrial antioxidant Prx-3, reduced LV cavity dilatation and dysfunction after myocardial infarction in mice. The mitochondrial transcription factor A (TFAM) is essential for maintenance of MCN. Ikeuchi et al created transgenic mice overexpressing human TFAM and found that TFAM overexpression protected against MCN truncation in heart tissue and inhibited LV remodeling after myocardial infarction. In human studies, mtDNA content was decreased by 40% in end-stage heart failure compared with control hearts, suggesting impaired mitochondrial biogenesis. The same authors also observed progressive depletion of mtDNA in the right ventricle of patients with congenital heart disease during the transition from hypertrophy to heart failure. Of note, in that study, a decrease in mtDNA content preceded any changes in mitochondrial enzyme activity or protein levels in cardiomyocytes. Therefore, maintenance of higher MCN is important to preserve mitochondrial function. MCN might be an important early stage biomarker of heart disease progression after STEMI, and the present study has identified an association between mtDNA copy number and cardiac shape and function. Early identification of patients at high risk for adverse LV remodeling after STEMI is important, to facilitate modification of medication and lifestyle interventions.

**Decreased MCN in AMI**

To date, MCN variation in peripheral blood leukocyte has been observed in different human reactive oxygen species-related situations. In the present study, fibrinogen was identified as a negative predictor of MCN after adjusting for possible variables such as oxidative stress, smoking, and lipid profiles (Table S2). This highlights the importance of inflammation in governing mitochondrial dynamics after an AMI event. AMI triggers an acute inflammatory response that activates peripheral leukocytes. The leukocytes orchestrate innate immunity and also serve as messengers that link local inflammation with the systemic inflammation response. We chose peripheral leukocytes to study the relationship between MCN and post-AMI remodeling. The samples were easy to obtain, but, more importantly, leukocytes play an important role in host defenses and act as inflammatory mediators in cardiovascular disease. Furthermore, a previous study demonstrated that peripheral blood MCN was correlated with cardiomyocyte MCN in 10 end-stage heart failure patients who received heart transplants. Taken together, peripheral leukocyte MCN is a promising biomarker for post-MI remodeling and outcome prediction.

**Study Limitations**

There were some limitations in this study. First, this study was observational and therefore we could not provide insights into the underlying mechanisms of action. Second, the sample size was relatively small and a study using a larger sample size is needed to confirm the present observations. Third, we tested only baseline leukocyte MCN. A future study evaluating serial MCN at various times during follow-up is needed to determine whether changes in MCN are associated with clinical changes.

**Conclusions**

In STEMI patients, higher plasma leukocyte MCN at baseline was associated with favorable LV shape and volume remodeling at 6-month follow-up. Plasma leukocyte MCN may provide a novel prognostic and disease progression biomarker for LV remodeling after STEMI.
Disclosures
The authors declare no conflict of interest.

Funding
This work was supported by Changhua Christian Hospital research grant no.101-CCH-ICO-001-2 and no. 99-CCH-IRP-05.

References
1. Ergatoudes C, Thunström E, Rosengren A, Björck L, Boström MCN and SI in AMI 1909

21. Puddu P, Puddu GM, Galletti L, Cravero E, Muscari A. Mitochondria dysfunction as an initiating event in atherogenesis: A plausible hypothesis. Cardiology 2005; 103: 137 – 141.

22. Chen S, Xie X, Wang Y, Gao Y, Xie X, Yang J, et al. Association between leukocyte mitochondrial DNA content and risk of coronary heart disease: A case-control study. Atherosclerosis 2014; 237: 220 – 226.

23. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, del Monte F, Tian R. Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. Circ Res 2010; 106: 1541 – 1548.

24. Karamanlidis G, Bautista-Hernandez V, Fynn-Thompson F, del Nido P, Tian R. Impaired mitochondrial biogenesis precedes heart failure in right ventricular hypertrophy in congenital heart disease. Circ Heart Fail 2011; 4: 707 – 713.

25. Thygensen K, Alpert JS, White HD. Universal definition of myocardial infarction. J Am Coll Cardiol 2007; 50: 2173 – 2195.

26. Huang CH, Chang CC, Huang CS, Kuo CL, Chen CP, Hsieh CH, et al. Using oxidized low-density lipoprotein autoantibodies to predict restenosis after balloon angioplasty in patients with acute myocardial infarction. PLoS One 2013; 8: e74726.

27. Huang CH, Kuo CL, Huang CS, Tseng WM, Lian IB, Chang CC, et al. High plasma coenzyme Q10 concentration is correlated with good left ventricular performance after primary angioplasty in patients with acute myocardial infarction. Medicine 2016; 95: e4501.

28. Liu CS, Tsai CS, Kuo CL, Chen HW, Lii CK, Ma YS, et al. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. Free Radic Res 2003; 37: 1307 – 1317.

29. Huang CH, Su SL, Hsieh MC, Cheng WL, Chang CC, Wu HL, et al. Depleted leukocyte mitochondrial DNA copy number in metabolic syndrome. J Atheroscler Thromb 2011; 18: 867 – 873.

30. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: A report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440 – 1463.

31. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography: American Society of Echocardiography Committee on Standards, Subcommittee on Quantification of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1988; 2: 358 – 367.

32. Naghieh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016; 29: 277 – 314.

33. Bolooki H, DeMarchena E, Mallon SM, Katariya K, Barron M, Bolooki HM, et al. Factors affecting late survival after surgical revascularization for the treatment of heart failure. Nat Rev Cardiol 2012; 9: 703 – 716.

34. Di Mauro M, Iacò AL, Bencivenga S, Clemente D, Marcon S, Nido P, Tian R. Impaired mitochondrial biogenesis precedes heart failure in right ventricular hypertrophy in congenital heart disease. Circ Res 2010; 106: 1241 – 1252.

35. Costa R, Romagna C, Pereira J, Souza-Pinto N. The role of mitochondrial DNA damage in the cytotoxicity of reactive oxygen species. J Bioenerg Biomembr 2011; 43: 25 – 29.

36. Buckberg G. Basic science review: The helix and the heart. Cardiology 2006; 124: 863 – 883.

37. Maffessanti F, Caiani EG, Tamborini G, Muratori M, Sugeng L, Weiner L, et al. Serial changes in left ventricular shape following early mitral valve repair. Am J Cardiol 2010; 106: 836 – 842.

38. Chan DC. Mitochondria: Dynamic organelles in disease, aging, and development. Cell 2006; 125: 1241 – 1252.

39. DeMenchea E, Mallon SM, Katariya K, Barron M, Bolooki HM, et al. Factors affecting late survival after surgical remodeling of left ventricular aneurysms. J Thorac Cardiovasc Surg 2003; 125: 374 – 383.

40. Ambale-Venkatesh B, Yoneyama K, Sharma RK, Ohyama Y, Wu CO, Burke GL, et al. Left ventricular shape predicts different outcomes from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440 – 1463.

41. Montier LLC, Deng JJ, Bai Y. Number matters: Control of mitochondrial DNA copy number. J Genet Genomics 2009; 36: 125 – 139.

42. Bolooki H, DeMarchena E, Mallon SM, Katariya K, Barron M, Bolooki HM, et al. Factors affecting late survival after surgical remodeling of left ventricular aneurysms. J Thorac Cardiovasc Surg 2003; 125: 374 – 383.

43. Thygensen K, Alpert JS, White HD. Universal definition of myocardial infarction. J Am Coll Cardiol 2007; 50: 2173 – 2195.

44. Ide T, Tsutsumi H, Hayashidani S, Kang D, Suematsu N, Nakamura KI, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001; 88: 529 – 533.

45. Knez J, Cauwenberghs N, Thijs L, Winckelmans E, Brguljan-Hitij M, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001; 88: 529 – 533.

46. Thygensen K, Alpert JS, White HD. Universal definition of myocardial infarction. J Am Coll Cardiol 2007; 50: 2173 – 2195.

47. Trusler BA, White HD. Universal definition of myocardial infarction. J Am Coll Cardiol 2007; 50: 2173 – 2195.
42. Marín-García J, Goldenthal MJ. Mitochondrial centrality in heart failure. *Heart Fail Rev* 2008; 13: 137–150.
43. Hansson A, Hance N, Dufour F, Rantanen A, Hultenby K, Clayton DA, et al. A switch in metabolism precedes increased mitochondrial biogenesis in respiratory chain-deficient mouse hearts. *Proc Natl Acad Sci USA* 2004; 101: 3136–3141.
44. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 2004; 429: 417–423.
45. Zhang D, Mott JL, Farrar P, Rye JS, Chang SW, Stevens M, et al. Mitochondrial DNA mutations activate the mitochondrial apoptotic pathway and cause dilated cardiomyopathy. *Cardiovasc Res* 2003; 57: 147–157.
46. Cavelier L, Johannisson A, Gyllensten U. Analysis of mtDNA copy number and composition of single mitochondrial particles using flow cytometry and PCR. *Exp Cell Res* 2000; 259: 79–85.
47. Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 2011; 470: 359–365.
48. Matsushima S, Ide T, Yamato M, Matsuoka H, Hattori F, Ikeuchi M, et al. Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 2006; 113: 1779–1786.
49. Ikeuchi M, Matsuoka H, Kang D, Matsushima S, Ide T, Kubota T, et al. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. *Circulation* 2005; 112: 683–690.
50. Huang J, Tan L, Shen R, Zhang L, Zuo H, Wang DW. Decreased peripheral mitochondrial DNA copy number is associated with the risk of heart failure and long-term outcomes. *Medicine (Baltimore)* 2016; 95: e3323.
51. Gamboa JL, Billings FT, Bojanowski MT, Gilliam LA, Yu C, Roshanravan B, et al. Mitochondrial dysfunction and oxidative stress in patients with chronic kidney disease. *Physiol Rep* 2016; 4: e12780.
52. Libby P, Nahrendorf M, Swirski FK. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease: An expanded “cardiovascular continuum”. *J Am Coll Cardiol* 2016; 67: 1091–1103.

**Supplementary Files**

**Supplementary File 1**

Figure S1. Leukocyte mitochondrial DNA copy number vs. presence of acute myocardial infarction (AMI).

Table S1. Subject characteristics vs. presence of AMI

Table S2. Multivariate predictors of MCN

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-17-0088