Title

CDH13 Genotype-Dependent Association of High-Molecular Weight Adiponectin With All-Cause Mortality: The J-SHIPP Study.

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CDH13 Genotype–Dependent Association of High–Molecular Weight Adiponectin With All-Cause Mortality: The J-SHIPP Study

OBJECTIVE
Despite its anti-inflammatory and antiatherogenic effects, adiponectin is potentially associated with adverse clinical outcomes, such as all-cause mortality. As plasma adiponectin levels are strongly influenced by single nucleotide polymorphisms in the gene encoding T-cadherin (CDH13), we conducted a longitudinal study to investigate the possible link between the CDH13 genotype, plasma adiponectin levels, and all-cause mortality.

RESEARCH DESIGN AND METHODS
This longitudinal study evaluated 2,020 Japanese subjects. Baseline clinical parameters were obtained from subjects’ personal health records as evaluated at annual medical check-ups. Plasma high–molecular weight adiponectin (HMWA) levels were measured by an ELISA assay, and genotyping was performed by a TaqMan probe assay.

RESULTS
Mean follow-up duration was 6.5 years. Kaplan-Meier analysis showed that HMWA levels were positively associated with mortality ($P < 0.001$). HMWA levels were associated with older age, lower body weight, lower plasma triglyceride and glucose levels, and higher plasma HDL cholesterol. However, the Cox regression analysis showed that the positive association between HMWA and all-cause mortality was independent of these covariates (hazard ratio [HR] 1.92, $P = 0.006$). The $CDH13$ rs4783244 genotype was strongly associated with baseline HMWA levels (per-allele effect size 1.65 $\mu$g/mL, $P < 0.001$). In a separate analysis by the $CDH13$ genotype, the HR for all-cause mortality was linearly increased with the number of G alleles ($P$ value for HMWA–$CDH13$ genotype interaction = 0.023).

CONCLUSIONS
Higher plasma HMWA level was an independent prognostic factor for all-cause mortality in a general population. The $CDH13$ genotype may be a factor that affects not only the plasma level of HMWA but also the prognostic significance of HMWA.

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Adiponectin is an adipocyte-derived secretory protein that has peripheral insulin-sensitizing activity (1). Adiponectin also exerts systemic anti-inflammatory effects via suppression of the growth and proliferation of macrophage progenitors, as well as the inflammation caused by fully differentiated macrophages (1). Further, cross-sectional studies in various populations have reported that a higher plasma adiponectin level is associated with a lower frequency of type 2 diabetes (2), metabolic syndrome (3), and thinner carotid arterial wall thickness (4). The results of these experimental studies and population-based cross-sectional association studies indicate that adiponectin is a cardioprotective factor in humans.

In contrast, several longitudinal epidemiological studies have reported that a higher plasma adiponectin level was associated with an increased incidence of cardiovascular disease (CVD) (5,6) and mortality (7–12). The underlying mechanism of the inverse association between these cross-sectional association studies and longitudinal prospective studies, the “adiponectin paradox,” is unclear and prevents a complete understanding of the biological role of adiponectin. A number of possibilities warrant investigation including reverse causality stemming from a compensatory increase of adiponectin to subclinical CVD risks and adiponectin resistance (13). Recent advances in DNA array technology have enabled the high-throughput analysis of single nucleotide polymorphisms (SNPs). Genome-wide association studies (GWAS) have successfully identified SNPs of the CDH13 gene that have a strong association with plasma adiponectin levels (14,15). The CDH13 gene encodes T-cadherin, a receptor for hexameric and high–molecular-weight forms of adiponectin (HMWA) (16). Although earlier studies (17,18) and recent GWAS in European subjects (19,20) reported a strong susceptibility of SNPs in the ADIPOQ gene that encodes adiponectin, GWAS of adiponectin in East Asian subjects, namely Korean (15) and Japanese (21), identified SNPs in the CDH13 locus, such as rs4783244. Because plasma adiponectin levels in subjects homozygous for the allele that with an association with higher adiponectin levels (affected allele) were 1.5 times higher than those homozygous for the allele without (unaffected allele) (21), genetic variation might be partially involved in the adiponectin paradox.

To clarify our hypothesis, we analyzed the possible interaction of the CDH13 genotype in the association of plasma levels of HMWA and all-cause mortality in Japanese middle-aged to elderly community residents.

**RESEARCH DESIGN AND METHODS**

This longitudinal study enrolled community-dwelling Japanese subjects living in Nomura Town, Ehime Prefecture, a mostly rural town with 11,000 inhabitants. Study subjects were recruited through a community-based annual medical check-up process in 2002 as part of the Shimanami Health Promoting Program (J-SHIPP) (3,22,23), which was a longitudinal epidemiological study evaluating factors relating to CVD, dementia, and death. Among a total of 2,721 residents who agreed to participate in the J-SHIPP study and completed baseline clinical measurements, 2,020 persons for whom an overnight fasting plasma sample was provided (≥11 h) for HMWA measurement (n = 2,084), for whom CDH13 genotype was determined (n = 2,042), and for whom mortality during this study period could be followed up were enrolled in this analysis. All study procedures were approved by the ethics committee of Ehime University Graduate School of Medicine, and all subjects provided signed informed consent prior to participation.

**Baseline Measurements**

Baseline clinical characteristics evaluated at medical checkups, including anthropometric measurements, blood pressure (BP), and basic plasma markers, were obtained from personal health records. Additional characteristics, including medication and history of CVD, were obtained by individual interviews using a structured questionnaire. Hypertension was defined as any or all of the following: systolic BP (SBP) ≥140 mmHg, diastolic BP ≥90 mmHg, and current use of antihypertensive medication. Subjects with a fasting blood glucose level of >126 mg/dL or currently using antihyperglycemic medication were defined as having type 2 diabetes. Renal function was evaluated based on the estimated glomerular filtration rate (eGFR), which was calculated from the plasma creatinine values using the following formula specifically developed by the Japanese Society of Nephrology for the estimation of the glomerular filtration rate in Japanese: 194 × creatinine−1.094 × age−0.287 × 0.739 (if female).

**Follow-up of All-Cause Mortality**

Vital status was ascertained until 30 November 2009 by checking a residential registration certification, which is under local government control. Since the registration of a person’s death is obligatory under the Family Registration Law in Japan, the local government’s vital statistics database enabled the follow-up of all-cause mortality. Further, as the notification of a change of address is obligatory under the residential registration certificate system, we were informed of subjects who moved outside of the cohort area and treated them as a censored case.

**Measurement of Plasma HMWA Levels**

Plasma samples were obtained from each subject after a period of overnight fasting of at least 11 h. Samples were immediately frozen and stored at −80°C until analysis. Plasma concentrations of HMWA were determined using an ELISA assay (Fujirebio, Tokyo, Japan) (3,24), which consisted of HMWA-specific capture antibody (IH7) and HMWA-specific horseradish peroxidase–conjugated detection antibody (POD-IH7). The specificity of this monoclonal antibody has previously been described (25). Standardized HMWA concentrations were determined by human HMWA purified by gelatin-cellulofine column chromatography. Intra- and interassay coefficient variations of the adiponectin assay were 4.4% and 9.7%, respectively.

**Genotyping**

DNA was extracted from peripheral blood using a QIAamp DNA blood kit.
Adiponectin and Mortality

Statistical Analysis
Differences in the numeric variables of baseline data were assessed by ANOVA, and differences in frequency were assessed by a χ² test. Overall survival ratio was calculated by Kaplan-Meier analysis. Covariate-adjusted hazard ratio (HR) for total mortality was analyzed by Cox proportional hazards regression analysis. Combined HR was calculated under a random effects model. A P value < 0.05 was considered statistically significant.

RESULTS
Baseline clinical characteristics of study subjects are shown in Table 1. Mean HMWA level in female subjects (7.62 ± 4.56 μg/mL) was nearly double that in male subjects (4.12 ± 2.93 μg/mL, P < 0.001). Subjects were therefore divided by HMWA quartile within each sex and then combined to avoid potential sex differences (Table 1).

Table 1—Clinical parameters of HMWA quartiles

<table>
<thead>
<tr>
<th></th>
<th>Total subjects</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2,020</td>
<td>501</td>
<td>507</td>
<td>507</td>
<td>505</td>
<td></td>
</tr>
<tr>
<td>HMWA (μg/mL)</td>
<td>6.09 ± 4.33</td>
<td>2.34 ± 1.07</td>
<td>4.21 ± 1.51</td>
<td>6.36 ± 2.05</td>
<td>11.43 ± 4.60</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.4 ± 12.3</td>
<td>57.9 ± 11.9</td>
<td>60.9 ± 12.4</td>
<td>63.3 ± 11.7</td>
<td>67.6 ± 11.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male %)</td>
<td>44.2</td>
<td>44.3</td>
<td>43.8</td>
<td>43.4</td>
<td>44.2</td>
<td>0.998</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 3.2</td>
<td>24.6 ± 3.2</td>
<td>23.8 ± 3.2</td>
<td>23.3 ± 2.9</td>
<td>22.2 ± 3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>139 ± 22</td>
<td>140 ± 22</td>
<td>136 ± 21</td>
<td>139 ± 23</td>
<td>142 ± 22</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>203 ± 35</td>
<td>205 ± 36</td>
<td>204 ± 35</td>
<td>201 ± 33</td>
<td>203 ± 34</td>
<td>0.302</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>62 ± 16</td>
<td>56 ± 14</td>
<td>60 ± 14</td>
<td>63 ± 15</td>
<td>68 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>113 ± 74</td>
<td>139 ± 92</td>
<td>115 ± 69</td>
<td>107 ± 64</td>
<td>91 ± 58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>80 ± 17</td>
<td>82 ± 18</td>
<td>80 ± 17</td>
<td>81 ± 17</td>
<td>78 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>98 ± 21</td>
<td>102 ± 24</td>
<td>98 ± 23</td>
<td>98 ± 22</td>
<td>95 ± 15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihyperglycemic medication (%)</td>
<td>3.4</td>
<td>3.8</td>
<td>2.8</td>
<td>4.1</td>
<td>3.0</td>
<td>0.575</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>7.4</td>
<td>10.6</td>
<td>6.9</td>
<td>6.7</td>
<td>5.4</td>
<td>0.011</td>
</tr>
<tr>
<td>CDH13 genotype (G allele %)</td>
<td>65.8</td>
<td>51.7</td>
<td>65.3</td>
<td>68.3</td>
<td>77.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated. Quartile of HMWA was calculated within each sex and then combined to avoid potential sex differences. Statistical significance in numeric variables was assessed by ANOVA, while differences in frequency were assessed by a χ² test. Trends in numeric variables among the HMWA quartiles were assessed by regression analysis adjusted for age and BMI. The eGFR was calculated from plasma creatinine values using the following formula: 194 × creatinine⁻⁰.⁰⁹⁴ × age⁻⁰.₂⁵⁷ × 0.⁷³⁹ (if female). Type 2 diabetes was defined as a fasting plasma glucose level >126 mg/dL or the current use of medication to lower blood glucose levels. G-allele frequency of the CDH13 rs4783244 genotype is shown.

The G allele of the CDH13 rs4783244 genotype was strongly associated with baseline plasma HMWA level (Fig. 2). Although obese subjects, defined as having a BMI >25 kg/m², showed significantly lower HMWA levels in both sexes (male obese 3.25 ± 2.22, nonobese 4.49 ± 3.12, P < 0.001; female 6.37 ± 3.68, 8.18 ± 4.86, P < 0.001), the association between CDH13 genotype and HMWA was independent of obesity status (Fig. 2). Per-allele effect size of the genotype calculated by a regression analysis adjusted for age, sex, and BMI was 1.65 μg/mL (P < 0.001).

Nevertheless, the prognostic significance of HMWA on total mortality was independent of the CDH13 genotype (Table 2 [model 4]). In a separate analysis by the genotype, however, HR for all-cause mortality was linearly increased with the number of G alleles (Fig. 1B), and the association between HMWA and poor prognosis was seen only in the GG genotype carriers. The term of interaction between the CHD13 genotype and plasma HMWA level was identified as a significant factor for all-cause mortality (P = 0.023) in the Cox regression analysis adjusted for the same covariates as are adjusted for in model 3 of Table 2. These results
indicate that the poor prognostic significance of HMWA was genotype dependent.

CONCLUSIONS

In this longitudinal study in a general population, we observed that higher plasma HMWA levels were significantly associated with all-cause mortality. Further, the association between HMWA and mortality showed a high degree of variation among CDH13 rs4783244 genotypes. To our knowledge, this is the first study showing a genotype-dependent association of HMWA with all-cause mortality.

Our results confirmed the increased risk of all-cause mortality in subjects with a higher baseline HMWA level. Several previous studies reported a similar relationship in general populations (5, 8–10), as well as in patients with type 2 diabetes (7), coronary artery disease (6,11), and chronic heart failure (12). Higher plasma HMWA in a general population is a complex phenotype that may represent a clustering of subclinical risk statuses, namely, reduced renal and cardiac function, obesity, insulin resistance and metabolic disorders, atherosclerosis, systemic inflammation, weight loss in elderly, and anemia. However, these studies were conducted mainly in European subjects (7,9–12) or in European and African American subjects (5,6,8), with no positive results being reported from East Asian subjects. In contrast, it has been reported that the Japanese population has distinct anthropometric characteristics, including a lower frequency of obesity compared with developed Western countries (26), and considerably lower plasma adiponectin levels (27), even in comparison with Japanese Americans living in Hawaii (28). In populations with lower HMWA levels, our results demonstrate the cross-validity of the prognostic significance of HMWA.

We also found an inverse association between HMWA and all-cause mortality between CDH13 TT and CDH13 GG genotype carriers, with the HR of the CDH13 TT genotype failing to reach statistical significance, presumably due to the smaller subsample size. Although the CDH13 gene is known to encode T-cadherin, a receptor for HMWA, the functionality of each CDH13 rs4783244 genotype or other unidentified responsible SNPs in linkage disequilibrium have not been clarified. However, T-cadherin knockout mice were reported to show dramatically increased plasma adiponectin levels due to impaired linkage between adiponectin and target cells (29). It is therefore possible that the G allele of the CDH13 genotype causes secondary hyperadiponectinemia by altering the amount or functionality of T-cadherin. This genetically dependent adiponectin resistance and consequent reduction in adiponectin’s anti-inflammatory and antiatherogenic effects may explain the increased mortality ratio in subjects with higher HMWA levels. Because differences in HMWA levels for each CDH13 rs4783244 genotype were similar between nonobese and obese subjects (Fig. 2), the production of adiponectin and formation of high–molecular weight isoforms might not be a factor in the genotype-specific mortality ratio.

According to the HapMap database (http://hapmap.ncbi.nlm.nih.gov/), frequency of the CDH13 rs4783244 genotype is similar across all ethnic groups. However, GWAS in European subjects (19,20) identified only the
ADIPOQ gene as susceptible loci for plasma adiponectin levels. Although more large-scale GWAS might identify the CDH13 gene as another susceptible loci, it is probable that genetic polymorphisms in the ADIPOQ gene have a larger effect than those in the CDH13 genotype in European populations regardless of the type of adiponectin complex (30). Because the ADIPOQ gene encodes adiponectin, our “genetic adiponectin resistance” hypothesis involving the CDH13 gene as a means of explanation might not be easily extrapolated to other populations. However, our results provide novel supporting evidence that adiponectin resistance is a key mechanism of the adiponectin paradox, which is lacking in other proposed mechanisms such as renal dysfunction and decreased adiponectin clearance, weight loss, or sarcopenia in the elderly and compensatory rise in adiponectin due to subclinical risks (reverse causality) (13). Our present results also excluded the possibility of confounding effects of decreased renal function.

Several limitations of this study warrant mention. First, we could not evaluate cause-specific mortality, particularly cardiovascular mortality. Results of long-term analysis for cardiovascular mortality would strengthen our findings. Second, we did not analyze other molecular isoforms of adiponectin. As T-cadherin is a receptor for HMWA and the HMW isoform is considered an active form, further investigations regarding isofrom specificity will be necessary. Third, this study contained ~2,000 study subjects. Replication studies, particularly in East Asian populations, with a larger sample size are therefore needed to confirm our present findings.

In summary, our longitudinal study demonstrated that the prognostic significance of HMWA is genotype specific. Our findings may provide the insight necessary to help solve the adiponectin paradox and further highlight the importance of genetic factors in understanding the contribution of adiponectin levels to adverse clinical outcomes.

**Table 2—HR of plasma HMWA levels on total mortality**

<table>
<thead>
<tr>
<th>Model</th>
<th>Factors adjusted for</th>
<th>10 µg/mL (HR (95% CI), P)</th>
<th>4th quartile (HR (95% CI), P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age, sex</td>
<td>2.03 (1.34–2.96), 0.001</td>
<td>1.78 (1.19–2.68), 0.006</td>
</tr>
<tr>
<td>2</td>
<td>Age, sex, BMI</td>
<td>1.85 (1.20–2.76), 0.006</td>
<td>1.63 (1.08–2.48), 0.021</td>
</tr>
<tr>
<td>3</td>
<td>Age, sex, BMI, SBP, TC, HDLC, glucose, CVD, eGFR, current smoking, current medication</td>
<td>1.91 (1.20–2.96), 0.007</td>
<td>1.59 (1.03–2.46), 0.035</td>
</tr>
<tr>
<td>4</td>
<td>Age, sex, BMI, SBP, TC, HDLC, glucose, CVD, eGFR, current smoking, current medication, CDH13 genotype</td>
<td>1.83 (1.12–2.91), 0.017</td>
<td>1.51 (0.97–2.37), 0.068</td>
</tr>
</tbody>
</table>

HR was calculated by a Cox regression analysis. CVD indicates previous history of CVDs (namely, stroke and myocardial infarction). Medication includes antihypertensive drugs and antihyperglycemic drugs. HDLC, HDL cholesterol; TC, total cholesterol.

**Figure 2**—Mean HMWA level and CDH13 rs4783244 genotype. Values are means ± SD. Statistical significance was assessed by ANOVA. Number of subjects for each genotype is shown in the column. Obesity was defined as a BMI >25 kg/m². White bars, nonobese; black bars, obese.

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**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** E.U. and Y.T. conducted research data acquisition, statistical analysis, and manuscript writing. R.K. and H.On. conducted research data acquisition. K.K. and H.Os. conducted research data acquisition and contributed to conclusions. T.M. supervised the study. Y.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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