Plasma Resistin Is Associated With Single Nucleotide Polymorphisms of a Possible Resistin Receptor, the Decorin Gene, in the General Japanese Population

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I nsulin resistance is a feature of type 2 diabetes (T2DM). Resistin, which antagonizes insulin action, is an adipokine secreted from adipocytes in mice (1,2). The overexpression of the resistin gene (RETN) in the liver causes insulin resistance via elevated plasma levels of resistin in mice (3), whereas mice lacking RETN show decreased fasting plasma glucose (4). Serum resistin is increased in obese diabetic mice. The relationship between serum resistin and insulin resistance, T2DM, or adiposity in humans is controversial (5). Some studies found no changes in circulating resistin in obesity, insulin resistance, or T2DM, but others reported a significant relationship between circulating resistin and these conditions (6,7). In humans, resistin has been reported to be expressed mainly in macrophages and monocytes.

We previously reported that the G/G genotype of a single nucleotide polymorphism (SNP) at −420 (rs1862513), which is located in the promoter region of human RETN, was associated with T2DM susceptibility (8). In the general Japanese population, subjects with the G/G genotype of rs1862513 had the highest plasma resistin, followed by those with the C/G and C/C genotypes (9). Rs1862513 explains ~26% of the total variance in plasma resistin. The G/G genotype of rs1862513 in RETN increases T2DM susceptibility by enhancing its promoter activity (8–10). At SNP −358 (rs3219175), A is required for G at rs1862513 to confer the highest plasma resistin in the general Japanese population (11). These SNPs in the promoter region of RETN could affect plasma resistin as cis factors.

Decorin is an extracellular matrix protein belonging to a family of small leucine-rich proteoglycans. The core decorin protein is attached to a dermatan or chondroitin glycosaminoglycan chain (12). Decorin is a component of connective tissue that binds to type I collagen and affects matrix assembly. Furthermore, decorin has been shown to bind transforming growth factor-β, epidermal growth factor, and the insulin-like growth factor-1 receptor (13,14). The human decorin gene (DCN) was mapped to 12q23, spans more than 38 kb, and contains 8 exons with large introns (15). A fragment of decorin, produced by proteolytic cleavage, lacking the glycosaminoglycan attachment site, and therefore devoid of carbohydrate chains, recently was identified as a receptor for resistin in mice (16). If decorin or its isoform is also a receptor for resistin in humans, polymorphisms of DCN could affect plasma resistin.

In view of this, to determine the association between DCN SNPs and circulating resistin, we cross-sectionally analyzed 2,078 Japanese subjects. Plasma resistin was associated with tag SNPs in the vicinity of DCN.

RESEARCH DESIGN AND METHODS

Subjects. In this cross-sectional study, 2,078 community-dwelling Japanese subjects were recruited during a community-based annual medical check-up. We previously analyzed rs1862513 in RETN and plasma resistin in these subjects (9). The clinical characteristics of the subjects are shown in Table 1. The study was approved by the ethics committee of the Ehime University Graduate School of Medicine, and informed consent was obtained from all subjects.

SNP typing. We selected six SNPs—rs7139228, rs7956537, rs7308752, rs516115, rs3138167, and rs545666—for genotyping as tag SNPs in the 10 kb region around DCN, using HapMap2 Ref24, CHB+JPT. These SNPs were analyzed by a TaqMan probe assay (Applied Biosystems Co., Ltd., Foster City, CA). From the 1Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; the 2Department of Anti-Aging and Genetics, Ehime Proteo-Medicine Research Center, Ehime University, Ehime, Japan; the 3Translational Research Center, Ehime University Hospital, Ehime, Japan; the 4Doctoral Program in Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; the 5Department of Community Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; and the 6Department of Geriatric Medicine, Ehime University Graduate School of Medicine, Ehime, Japan.

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Comparing major alleles (A) and minor alleles (B) of SNPs in BMI were included as independent variables. We performed the analysis by hypotheses were rejected at a level of significance.

rs1862513 genotype (C/C, C/G, and G/G) and rs7139228 (G/G, G/A, and A/A) in ANOVA and multiple regression analysis. The interaction between the rs1862513 genotype, C/C, C/G, and G/G were denoted by two dummy variables (c1, c2) and set as (0, 0), (1, 0), and (0, 1), A/A = 0, A/B = 1, and B/B = 2. The rs1862513 genotype, C/C, C/G, and G/G were associated with plasma resistin (unstandardized regression coefficient (β) = −0.76, P = 0.040; β = −0.50, P = 0.016; and β = −0.47, P = 0.011, respectively) (Table 2). After multiple test correction using the Benjamini-Hochberg correction with the FDR set to 0.05, rs516115 and rs7308752 remained significant for association with plasma resistin.

The SNPs rs7308752 and rs516115 in DCN were associated with plasma resistin independent of age, sex, BMI, and rs1862513 in RETN. To examine isolated effects of the genotypes of DCN on plasma resistin, a multiple regression analysis was performed using plasma resistin as a dependent variable and the genotypes of these SNPs, with adjustments for age, sex, BMI, and rs1862513 as independent variables. The rs7308752 (A/A), rs7308752 (G/G), or rs516115 (C/C) genotypes were inversely associated with plasma resistin (unstandardized regression coefficient (β) = −0.76, P = 0.040; β = −0.50, P = 0.016; and β = −0.47, P = 0.011, respectively) (Table 2). After multiple test correction using the Benjamini-Hochberg correction with the FDR set to 0.05, rs516115 and rs7308752 remained significant for association with plasma resistin. When the FDR was set to 0.1, rs7139228 was associated with plasma resistin.

We next examined the effect of a combination of rs7139228 (GG vs. GA+AA) and rs1862513 on plasma resistin. Of the SNPs analyzed, rs7139228 was chosen because it seemed to be most strongly associated with plasma resistin (β = −0.76). Plasma resistin seemed to be highest in subjects with the G/G genotype of rs7139228 and G/G genotype of rs1862513 (Fig. 2). The effect of the two SNPs seemed to be additive, although no synergistic interaction was observed (P = 0.308). To assess the interaction between rs1862513 and each of the six SNPs in DCN on plasma resistin was assessed by ANCOVA. All analyses were performed with the JMP 7.0 software program (SAS Institute, Cary, NC). Null hypotheses were rejected at a level of significance of P < 0.05.

RESULTS

The SNPs rs7139228, rs7956537, rs516115, and rs3138167 in DCN were associated with plasma resistin in the general Japanese population. To determine whether plasma resistin is associated with SNPs in DCN in the general Japanese population, we examined the relationship between plasma resistin and genotypes of the six selected tag SNPs. Based on a confidence interval analysis, these six SNPs analyzed were in the same LD block (Fig. 1). Plasma resistin was associated with the following genotypes (Table 2): rs7139228 (GG, 11.7 ± 6.7, GA, 10.4 ± 5.5, and AA, 9.2 ± 3.5 ng/mL; P = 0.015), rs7956537 (TT, 11.7 ± 6.7, TG, 10.4 ± 5.5, and GG, 9.4 ± 3.4 ng/mL; P = 0.020), rs516115 (TT, 12.0 ± 7.1, TC, 11.1 ± 6.3, and CC, 11.0 ± 5.8 ng/mL; P = 0.010), and rs3138167 (CC, 11.8 ± 6.9, CT, 10.8 ± 5.8, and TT, 10.6 ± 6.8 ng/mL; P = 0.006). After multiple test correction using the Benjamini-Hochberg correction with the FDR set to 0.05, rs516115, rs7956537, and rs3138167 remained significant for association with plasma resistin.

The SNPs rs7308752 and rs516115 in DCN were associated with plasma resistin independent of age, sex, BMI, and rs1862513 in RETN. To examine isolated effects of the genotypes of DCN on plasma resistin, a multiple regression analysis was performed using plasma resistin as a dependent variable and the genotypes of these SNPs, with adjustments for age, sex, BMI, and rs1862513 as independent variables. The rs7308752 (A/A), rs7308752 (G/G), or rs516115 (C/C) genotypes were inversely associated with plasma resistin (unstandardized regression coefficient (β) = −0.76, P = 0.040; β = −0.50, P = 0.016; and β = −0.47, P = 0.011, respectively) (Table 2). After multiple test correction using the Benjamini-Hochberg correction with the FDR set to 0.05, rs516115 and rs7308752 remained significant for association with plasma resistin. When the FDR was set to 0.1, rs7139228 was associated with plasma resistin.

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**TABLE 2**

Associations between the decorin SNPs and plasma resistin in the general Japanese population SNP

<table>
<thead>
<tr>
<th>Position</th>
<th>Allele (major/minor)</th>
<th>Genotype frequency</th>
<th>Plasma resistin (ng/mL)</th>
<th>Regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>rs7139228</td>
<td>9151311</td>
<td>G/A</td>
<td>11.7 ± 6.7</td>
<td>10.4 ± 5.5</td>
</tr>
<tr>
<td>rs7956637</td>
<td>91526402</td>
<td>T/G</td>
<td>11.7 ± 6.7</td>
<td>10.4 ± 5.5</td>
</tr>
<tr>
<td>rs7308752</td>
<td>91527181</td>
<td>A/G</td>
<td>11.7 ± 6.9</td>
<td>11.1 ± 6.2</td>
</tr>
<tr>
<td>rs516115</td>
<td>91557292</td>
<td>T/C</td>
<td>12.0 ± 7.1</td>
<td>11.1 ± 6.3</td>
</tr>
<tr>
<td>rs3138167</td>
<td>91572415</td>
<td>C/T</td>
<td>11.8 ± 6.9</td>
<td>10.8 ± 5.8</td>
</tr>
<tr>
<td>rs545666</td>
<td>91580359</td>
<td>T/C</td>
<td>11.5 ± 6.8</td>
<td>11.4 ± 6.3</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD. ANOVA was used for statistical analysis. Multiple regression analysis involving plasma resistin (nanograms per milliliter) as a dependent variable, with age, sex (male = 0, female = 1), BMI, and genotypes of $DCN$ and $RETN$ SNPs as independent variables, was performed as described in RESEARCH DESIGN AND METHODS. $b$, unstandardized regression coefficient of plasma resistin. Boldface indicates statistical significance. SE, standard error.

In this study involving 2,078 subjects from the general Japanese population, plasma resistin was associated with four tag SNPs located in the vicinity of $DCN$ (rs7139228, rs79566402, rs516115, and rs3138617) in the same LD block. A multiple regression analysis adjusted for age, sex, and BMI indicated that plasma resistin was associated with two tag SNPs (rs7308752 and rs516115) independent of rs1862513. No synergistic interaction was found between the SNPs around $DCN$ and rs1862513.

Some of the tag SNPs around $DCN$ were associated with plasma resistin in the general Japanese population. Although the genotype of rs1862513 is tightly associated with plasma resistin, the effect of the SNPs around $DCN$ on plasma resistin was independent of rs1862513. We previously reported that plasma resistin also is associated with T2DM susceptibility SNPs, namely, THADA (rs7578597) and PPARG Pro12Ala (rs1801282) via a trans-acting effect (19,20). Plasma resistin is associated with rs1862513 and rs3219175, both of which are located in the promoter region of $RETN$, via a cis-acting effect (11,20). Plasma resistin seems to be regulated by cis- and trans-acting SNPs, which merits further investigation as an acceptable model for protein quantitative trait loci.

It has been suggested that the relevance of resistin in humans could be different from that in mice. Human resistin is expressed predominantly in monocytes and macrophages, and expression in adipose tissue is derived from inflammatory cells (21). It has been shown that resistin induces the production of inflammatory cytokines in human macrophages and that inflammatory stimuli induce expression of the resistin gene (22). Mice with humanized resistin, in which human resistin is expressed in macrophages, show adipose tissue inflammation and insulin resistance (23). Inflammation is now increasingly recognized to be involved in the pathogenesis of insulin resistance and T2DM, and human resistin could link inflammation and insulin resistance.

Decorin is recognized as a secreted multifunctional proteoglycan involved in cell adhesion, migration, and proliferation. Decorin also binds to C1q as a regulator, resulting in the regulation of inflammatory responses. Decorin and C1q were reported to be involved in adipose tissue inflammation, which could lead to insulin resistance (24). Most recently, it was reported that a proteolytic isoform of decorin ($\Delta$DCN) was expressed on adipocyte stromal cell surfaces and serves as a functional resistin receptor in mice (10). The findings of the current study...
indicate the existence of a significant association between tag SNPs around DCN and plasma resistin. The possibility that human decorin is a human resistin receptor should be pursued.

In this study, plasma decorin was not associated with each genotype of the six tag SNPs in DCN in the general Japanese population. These SNPs are located in introns of DCN or intergenic regions and potentially could affect DCN expression. Whether decorin located on the cell surface but not in plasma might be associated with these SNPs merits further investigation.

It also was reported that decorin is expressed in adipose tissue in humans and that plasma decorin was elevated in subjects with T2DM (25). In a previous study, we reported that plasma resistin was higher in subjects with T2DM (10). Although the association between plasma resistin and plasma decorin was expected, no association was found in this study (data not shown). Possible isoform and multimer formation of decorin in the plasma should be analyzed.

In summary, plasma resistin was associated with tag SNPs around DCN in the general Japanese population. How plasma resistin is affected by these SNPs, and whether decorin or its isoform is a receptor for resistin in humans, remains unknown. Further studies will be required to clarify these points.

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

H.On. designed the experiments, researched data, contributed to discussion, and wrote the manuscript. Y.Tab. designed the experiments, researched data, and contributed to discussion. R.Kawam. researched data and contributed to discussion. J.O. researched data. W.N. and Y.Tal. contributed to discussion. M.O. and T.N. researched data. R.Kawam., K.K., and T.M. reviewed the manuscript. H.Os. designed the experiments, contributed to discussion, and reviewed and edited the manuscript. H.On., Y.Tab., and H.Os. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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