Frequency of the G/G Genotype of Resistin Single Nucleotide Polymorphism at −420 Appears to Be Increased in Younger-Onset Type 2 Diabetes

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OBJECTIVE—Resistin is an adipocyte-secreted cytokine associated with insulin resistance in mice. We previously reported that the G/G genotype of a resistin single nucleotide polymorphism (SNP) at −420 increases type 2 diabetes susceptibility by enhancing its promoter activity. The aim of the present study was to determine the relevance of SNP −120 in a large number of subjects.

RESEARCH DESIGN AND METHODS— We examined 2,610 type 2 diabetic case and 2,502 control subjects. The relation between SNP −420 and the age of type 2 diabetes onset was further analyzed by adding 237 type 2 diabetic subjects with age of onset ≤40 years.

RESULTS—When analyzed without considering subject age, the SNP −420 genotype was not associated with type 2 diabetes. Since we reported that the onset of type 2 diabetes was earlier in G/G genotype, we analyzed the data using a trend test for age intervals of 10 years. The frequency of G/G genotype differed among age grades in type 2 diabetes ($P = 0.037$) and appeared to be higher in younger grades. In type 2 diabetes, G/G genotype was more frequent in subjects aged <40 years than in those aged ≥40 years (G/G vs. C/C, $P = 0.003$). In a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the trend test showed that the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0379$). In control subjects, the frequency of C/G genotype showed an increasing linear trend with increasing age ($P = 0.010$).

CONCLUSIONS—The G/G genotype frequency of resistin SNP −420 appears to be increased in younger-onset type 2 diabetic subjects. ***Diabetes*** **56:2834–2838, 2007**

One characteristic of type 2 diabetes is insulin resistance in insulin target tissues (1). Type 2 diabetes is a probable polygenic disease, and its major genetic factors have yet to be identified (2). Single nucleotide polymorphisms (SNPs) such as peroxisome proliferator–activated receptor (PPAR)γ, KCNJ11, and TCF7L2 have been reported to be associated with type 2 diabetes (3). We reported that SNP at −420 in the resistin gene (*RETN*) (rs1862513) is associated with type 2 diabetes (4).

In mice, resistin is secreted from adipocytes and antagonizes insulin action both in vitro and in vivo (5,6). Serum resistin is increased in obese diabetic mice and is reduced by PPARγ ligands (6). Transgenic mice overexpressing *retn* in the liver have high serum resistin and are insulin resistant (7). The $retn^{-/-}$ mice show lower fasting blood glucose (8). Therefore, the role of resistin as an adipocyte-secreted cytokine inducing insulin resistance appears to be established in rodents.

In humans, *RETN* is rarely expressed in adipose tissues and is expressed at high levels in monocytes or macrophages, in contrast to its dominant expression in adipose tissues in mice (9,10). Macrophages infiltrating into adipose tissues could account for the observed insulin resistance in obese mice, suggesting a possible role of resistin in insulin resistance in humans (11,12). The role of *RETN* in human type 2 diabetes or obesity has been controversial in studies of the association of SNPs or serum resistin (4,13–16). The discrepancy among previous reports may be resolved by considering the SNP −420 genotype or by analyzing a larger number of samples.

We reported that the G/G genotype of *RETN* promoter SNP −420 is associated with type 2 diabetes susceptibility (4). Sp1 and Sp3 transcription factors specifically bind to the DNA element including −420G, resulting in an enhanced promoter activity. *RETN* mRNA in monocytes is positively associated with its simultaneous serum levels and is highest in subjects with G/G genotype (17). Serum resistin is higher in type 2 diabetic subjects than in control subjects and highest in subjects with G/G genotype, followed by C/G and C/C. Therefore, the specific recognition of −420G by Sp1/3 appears to increase *RETN* promoter

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PPAR, peroxisome proliferator–activated receptor; SNP, single nucleotide polymorphism.

TABLE 1
G/G genotype was not associated with type 2 diabetes when age was not considered

Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
n	2,610	2,502	—	—	—	—
CC	1,169	1,080	CC/CG/GG	1.44	0.486	—
CG	1,144	1,123	GG vs. CC	0.87	0.351	0.92 (0.77–1.10)
GG	297	299	CG vs. CC	1.04	0.308	0.94 (0.84–1.06)
			GG vs. CG	0.08	0.784	0.98 (0.81–1.17)
			GG + CG vs. CC	1.37	0.242	0.94 (0.84–1.05)
			GG vs. CG + CC	0.40	0.525	0.95 (0.80–1.12)
G-allele	1,738 (33.3)	1,721 (34.4)	G- vs. C-allele	1.38	0.241	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

activity, which could induce insulin resistance and human type 2 diabetes through enhanced monocyte mRNA and serum levels of resistin. Therefore, we analyzed the relevance of *RETN* SNP −420 in a large number of samples.

RESEARCH DESIGN AND METHODS

We recruited native Japanese subjects—2,610 type 2 diabetic case and 2,502 control subjects—from six prefectures located in Honshu and Shikoku in Japan. These samples are assumed not to be heterogeneous since Matsumoto et al. (18) showed that the Japanese population is homogenous, except for the Ainus from Hokkaido and the Okinawans from Miyako, using genetic markers of human immunoglobulin. Diabetes was diagnosed based on American Diabetes Association criteria (19). The control subjects were chosen based on either no history of diabetes and A1C levels <5.6% or normal glucose tolerance as evidenced by a 75-g oral glucose tolerance test. To analyze the relation between SNP −420 and age of type 2 diabetes onset, 237 type 2 diabetic patients with onset age ≤40 years were added. The clinical characteristics of the 2,610 type 2 diabetic case and 2,502 control subjects and additional 237 type 2 diabetic subjects are summarized in Supplementary Table 1 (available in an online appendix at http://dx.doi.org/10.2337/db06-1157). The average age of the control subjects was significantly older than the age of onset of type 2 diabetes in *panel 1* (Student's t test, $P < 0.0001$). Of subjects in *panel 1*, we typed SNP −420 in 397 type 2 diabetic patients and 406 control subjects as *panels 1* and *2* and 154 case and 143 control subjects as *panel 3* in a previous article (4).

All subjects were informed of the purpose of the study, and informed consent was obtained. The study was approved by the ethics committee of Ehime University (including Chiba Central Medical Center), Ehime Prefectural Hospital, Kobe University, the University of Tokyo, the University of Tokushima, and Kyoto Prefectural University of Medicine.

The statistical power was calculated as follows (20). We assume that S and N are susceptibility and normal alleles, respectively. When the genotype relative risk is assumed to be 1.20 for SN and 1.44 for SS, the population frequency of S is 30% as SNP −420 and the prevalence of diabetes is 6.9% based on the International Diabetes Federation Diabetes e-Atlas (http://www.eatlas.idf.org/About_e_Atlas/); the penetrance for genotypes of SS, SN, and NN were calculated to be 0.088, 0.074, and 0.061, respectively. Under this condition, a significant difference in the allele frequency between 2,610 case and 2,502 control subjects can be detected with a power >99.6%.

SNP typing. Taqman analysis was used for typing SNP −420, as previously described (17,21). When required, PCR direct sequencing was performed, as described previously (4,22).

Statistical analysis. To analyze differences in SNP −420 frequencies among ages, trend testing using 10-year age intervals was used. Student's t test, ANOVA, or χ^2 test was used where indicated.

RESULTS

We analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects recruited from six different prefectures in Japan. SNP −420 was in Hardy-Weinberg equilibrium in both case and control subjects. Neither the allele nor the genotype was associated with type 2 diabetes (Table 1).

Since we previously reported that the onset of type 2 diabetes was earlier in subjects with the G/G genotype (4), we examined the allele frequencies and genotype distribution of SNP −420 as a function of subject age. A trend test for 10-year intervals revealed that the G-allele frequency differed significantly among age grades in type 2 diabetic subjects ($P = 0.022$); the G-allele appears to be more frequent in younger type 2 diabetic subjects, especially those aged <40 years, although the increasing trend was not linear ($P = 0.458$) (Fig. 1). In contrast, this increase was not evident in control subjects.

The trend test also revealed that the frequency of the G/G genotype differed significantly among age grades in type 2 diabetic subjects ($P = 0.037$). The G/G genotype also appears to be more frequent in younger type 2 diabetic subjects, especially those below the age of 40 years, although the increasing trend was not linear ($P = 0.265$) (Fig. 2). In constrast, no difference was found in the frequency of the G/G genotype among age grades in control subjects ($P = 0.440$). There appeared to be no differences between male and female subjects (data not shown). Therefore, in type 2 diabetes, the frequency of both the G-allele and the G/G genotype appears to be higher in younger subjects.

Since the G-allele and G/G genotype frequency appear to be high in younger type 2 diabetic subjects, especially those aged <40 years, we compared the allele and genotype frequencies of SNP −420 between type 2 diabetic subjects aged <40 years and those aged ≥40 years (Table 2). The frequencies of either the G-allele or the G/G genotype were higher in the younger group (G-allele for younger group 43.0% vs. older group 33.0%, $P = 0.008$; odds ratio [OR] of G/G to C/C 2.47, $P = 0.003$). When both case and control subjects aged <40 years were analyzed, the frequencies of both the G-allele and the G/G genotype were higher in type 2 diabetic subjects (G-allele in type 2 diabetic subjects 43.0% vs. control subjects 33.3%, $P = 0.016$; OR of G/G to C/C 2.28, $P = 0.012$). Therefore, the G/G genotype at SNP −420 appeared to be associated with type 2 diabetes in younger subjects.

Finally, to examine the relation between SNP −420 and the age of type 2 diabetes onset, we added 237 type 2 diabetic subjects with onset age ≤40 years. To adjust the effect of aging on the increasing frequency of the G-allele, we analyzed a total of 2,430 type 2 diabetic subjects with age of onset <60 years. The trend test revealed that G-allele and G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0492$ and $P = 0.0379$, respectively).

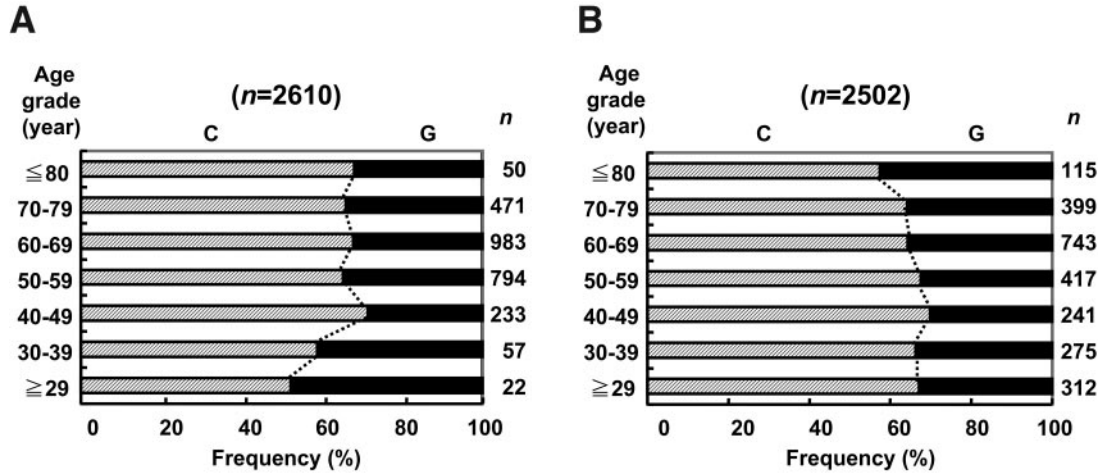

FIG. 1. The frequency of the G-allele of SNP −420 appears to be increased in younger type 2 diabetic subjects and showed an increasing linear trend in older control subjects. The allele frequencies of resistin SNP −420 stratified for 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G-allele differed among age grades in type 2 diabetic subjects (P = 0.022), although the trend was not linear (P = 0.458). In control subjects, the frequency of the G-allele showed an increasing linear trend with increase in age (P = 0.008).

DISCUSSION

We report here that the G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects but not in total subjects by analyzing 2,610 type 2 diabetic case and 2,502 control subjects. Differences in G-allele frequencies among age grades in case and control subjects—namely, an increasing linear trend in control subjects in older grades—and an apparent increase in type 2 diabetic cases aged <40 years could result in no association between the SNP −420 genotype and type 2 diabetes in the total subjects. The association of SNP −420 with type 2 diabetes has been controversial, suggesting that a variety of factors could affect the results (4,13,14,16). This discrepancy may be resolved by considering age grades and increasing the number of samples, as suggested by the present study.

We have shown that the G/G genotype frequency was increased in younger type 2 diabetic subjects, in whom genetic factors are thought to have stronger effects on disease susceptibility. Conversely, this finding means that the G/G genotype frequency was decreased with increasing age. It is possible that resistin may become less of a significant risk factor as age increases or that type 2 diabetic patients with the G/G genotype may not live longer. It should be noted that P values observed were marginal and that the sample size, especially that of type 2 diabetic subjects with younger age of onset, was limited in this study. A larger number of samples should be analyzed for replication. When stratified by seven grades (2-kg/m^2 intervals) of BMI, no apparent linear trend of G-allele or G/G genotype was observed in control or type 2 diabetic subjects (data not shown). This supports that the trends in the age stratification are relevant although the effect of possible heterogeneity among areas cannot be completely excluded.

In contrast to type 2 diabetic subjects, a trend test revealed that in control subjects, the G-allele frequency had an increasing linear trend as the age grade became

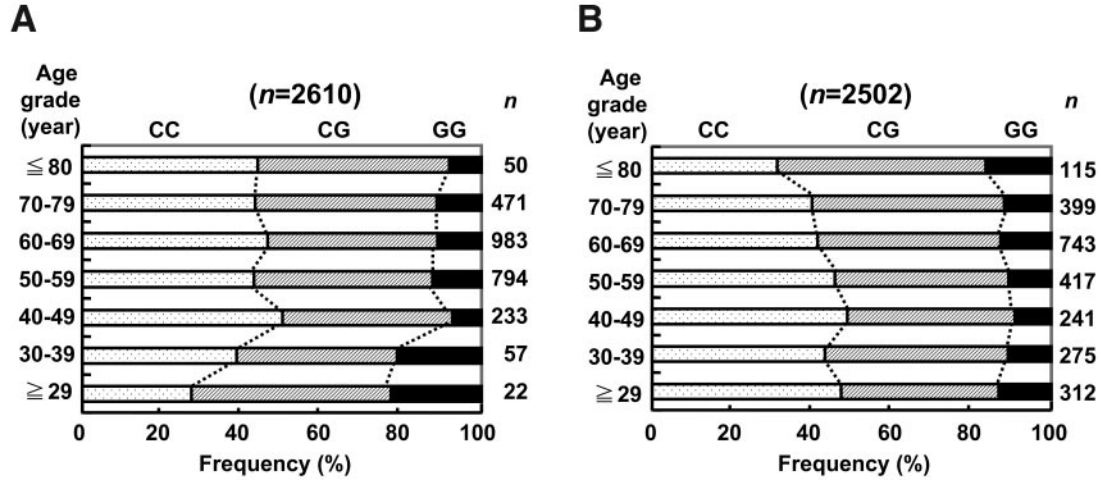

FIG. 2. The frequency of G/G genotype of SNP −420 appears to be increased in younger type 2 diabetic subjects, whereas that of C/G genotype showed an increasing linear trend in older control subjects. The genotype frequencies of resistin SNP −420 stratified by 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G/G genotype differed among age grades in type 2 diabetic subjects (P = 0.037), though the trend was not linear (P = 0.265). In control subjects, the frequency of the G/G genotype did not differ among age grades (P = 0.440). The frequency of the C/G genotype showed an increasing linear trend with an increase in age in control subjects (P = 0.010), whereas that of the C/C genotype showed an decreasing linear trend (P = 0.002).

TABLE 2
G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects

Comparison between type 2 diabetic subjects aged <40 years (n = 79) with those aged ≥40 years (n = 2,531)						
Genotype or allele	<40 years old	≥40 years old	Comparison	χ^2	P	OR (95% CI)
CC	28	1,141	CC/CG/GG	8.96	0.011	—
CG	34	1,110	GG vs. CC	8.82	0.003	2.47 (1.34–4.58)
GG	17	280	CG vs. CC	0.74	0.390	1.25 (0.75–2.07)
			GG vs. CG	5.23	0.022	1.98 (1.09–3.60)
			GG + CG vs. CC	2.88	0.090	1.50 (0.94–2.39)
			GG vs. CG + CC	8.31	0.004	2.20 (1.27–3.82)
G-allele	68 (43.0)	1,670 (33.0)	G- vs. C-allele	6.96	0.008	—
Comparison between type 2 diabetic (n = 79) and control (n = 587) subjects aged <40 years						
Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
CC	28	267	CC/CG/GG	6.27	0.044	—
CG	34	249	GG vs. CC	6.31	0.012	2.28 (1.18–4.40)
GG	17	71	CG vs. CC	0.96	0.327	1.30 (0.77–2.21)
			GG vs. CG	3.02	0.082	1.75 (0.93–3.32)
			GG + CG vs. CC	2.85	0.092	1.52 (0.93–2.48)
			GG vs. CG + CC	5.39	0.020	1.99 (1.10–3.60)
G-allele	68 (43.0)	391 (33.3)	G- vs. C-allele	5.84	0.016	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

older (P = 0.008) (Figs. 1*B* and 2*B*). The C/G genotype showed an increasing linear trend in older age grades (P = 0.010), whereas the C/C genotype showed a decreasing linear trend (P = 0.002). There appeared to be no sex differences (data not shown). These findings suggest that *RETN* may be a longevity gene like adiponectin (23) under certain conditions. We previously reported that serum resistin levels were highest in subjects with G/G genotype, followed by C/G and C/C (4,17). Therefore, moderately elevated serum resistin levels in C/G genotype, by reducing insulin signaling, may be beneficial for a longer life in nondiabetic control subjects. The lower serum resistin levels in C/C genotype may not be sufficient to have this effect. In fact, mutations in the insulin receptor homologous gene are known to result in longevity in *elegans* and *Drosophila* (24,25).

Recently, we reported that plasma resistin was correlated with insulin resistance in 2,078 subjects in the Japanese general population (21). Plasma resistin was highest in subjects with the G/G genotype of SNP −420, followed by C/G and C/C. The effect of SNP −420 on plasma resistin was independent of age, sex, and BMI. The 26% of total variance of plasma resistin could be explained by SNP −420, suggesting that not only SNP −420 but also other genetic and environmental factors could affect plasma resistin levels. The direct association between type 2 diabetes and SNP −420 may be more difficult to detect.

In summary, we analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects. Although SNP −420 was not associated with type 2 diabetes when analyzed without considering subject age, the G/G genotype frequencies appear to be higher in younger subjects with type 2 diabetes. When 237 type 2 diabetic subjects with age of onset ≤40 years were added, in a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger. Therefore, the G/G genotype frequency was increased in younger type 2 diabetic subjects. In contrast, the C/G genotype showed an increasing linear trend as the age grade became older in control subjects. It is not clear how resistin induces type 2 diabetes in younger subjects or whether it is beneficial for longer life. Further studies will be required to clarify these points.

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