

The 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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Abstract

Objective: Resistin is an adipocyte-secreted hormone associated with insulin resistance in mice. We reported that the G/G genotype of a resistin single nucleotide polymorphism (SNP) at -420 increases type 2 diabetes (T2DM) susceptibility by enhancing its promoter activity.

Research Design and Methods: To determine the relevance of SNP-420 in a large number of subjects, we examined 2610 T2DM cases and 2502 controls. The relation between SNP-420 and the age of T2DM onset was further analyzed by adding 237 T2DM subjects with the age of onset 40 or younger.

Results: When analyzed without considering subject age, the SNP-420 genotype was not associated with T2DM. Since we reported that the onset of T2DM was earlier in G/G genotype, we analyzed the data using trend test for age intervals of 10 years. The frequency of G/G genotype differed among age grades in T2DM ($P=0.037$), and appeared to be higher in younger grades. In T2DM, G/G genotype was more frequent in subjects younger than 40 years than in those who were 40 or older (G/G vs C/C, $P=0.003$). In a total of 2430 T2DM subjects with the age of onset younger than 60, the trend test showed that the G/G genotype had an increasing linear trend as the age grade of T2DM onset became younger ($P=0.0379$). In controls, 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 T2DM subjects.

One of characteristics of type 2 diabetes mellitus (T2DM) is insulin resistance in insulin target tissues (1). T2DM is a probable polygenic disease, and its major genetic factors remain to be identified (2). Single nucleotide polymorphisms (SNPs) such as peroxisome proliferator activated-receptor γ (PPAR γ), KCNJ11, and TCF7L2 have been reported to be associated with T2DM (3). We [Del] reported that SNP at -420 in the resistin gene (*RETN*) is associated with T2DM (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 PPAR γ ligands (6). Transgenic mice [Del] overexpressing *retn* in the liver have high serum resistin [Del] and are insulin-resistant (7). The *retn* (-/-) mice show lower fasting blood glucose [Del] (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 T2DM or obesity has been controversial in studies of the association of SNPs or serum resistin [Del] (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 [Del] reported that the G/G genotype of *RETN* promoter SNP-420 is

associated with T2DM 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 [Del] is higher in T2DM subjects than controls, 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 activity, which could induce insulin resistance and human T2DM through enhanced monocyte mRNA and serum levels of resistin. Therefore, we analyzed the relevance of *RETN* SNP-420 in a large number of samples. [Del]

Research Design and Methods

Subjects.

We recruited native Japanese subjects of 2610 T2DM cases and 2502 controls from 6 prefectures located in Honshu and Shikoku in Japan. These samples are assumed not to be heterogeneous since Matsumoto et al. showed that the Japanese population is homogenous except for the Ainu from Hokkaido, and the Okinawans from Miyako using genetic markers of human immunoglobulin (Gm) (18). Diabetes mellitus was diagnosed based on the ADA criteria (19). The control subjects were chosen based on either no history of diabetes and HbA_{1c} levels of less than 5.6, or a normal glucose tolerance, as evidenced by a 75g oral glucose tolerance test. To analyze the relation between SNP-420 and the age of T2DM onset, 237 T2DM patients with onset age 40 years or younger were added. The clinical characteristics of a total of the 2610 T2DM cases and 2502 controls (panel 1),

and additional 237 T2DM subjects (panel 2) are summarized in Supplementary Table 1 (available at <http://diabetes.diabetesjournals.org>). The average age of the controls was significantly older than the age of onset of T2DM in panel 1 (Student's t test, $P < 0.0001$). Of subjects in [del] panel 1, we typed SNP-420 in 397 T2DM patients and 406 control subjects as panel 1 and 2, and 154 cases and 143 controls as panel 3 in the previous paper (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 the 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 IDF 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 2610 cases and 2502 controls can be detected with a power >99.6%.

SNP typing

The Taqman analysis was employed 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, the trend test using 10 year age intervals was employed. Student's t test, [Del] ANOVA, or Chi-squared test were used where indicated.

Results and Discussion

We analyzed *RETN* SNP-420 in 2610 T2DM cases and 2502 controls recruited from 6 different prefectures in Japan. SNP-420 was in Hardy-Weinberg equilibrium in both cases and controls. [Del] Neither the allele nor the genotype was associated with T2DM (Table 1). [Del].

Since we previously reported that the onset of T2DM 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 T2DM ($P = 0.022$). In T2DM, the G allele appears to be more frequent in younger subjects, especially those below the age 40, although the increasing trend was not linear ($P = 0.458$) (Fig. 1). In contrast, this increase was not evident in controls.

The trend test also revealed that the frequency of the G/G genotype differed significantly among age grades in T2DM ($P = 0.037$). The G/G genotype also appears to be more frequent in younger T2DM subjects, especially those below the age of 40, although the increasing trend was not linear ($P = 0.265$) (Fig. 2). In contrast, no difference was found in the frequency of the G/G genotype among age grades in controls ($P = 0.440$). There appeared to be no differences between males and females (data not shown). Therefore, the frequency of both the G allele and the G/G

genotype appears to be higher in younger subjects in T2DM.

Since the G allele and G/G genotype frequency appears to be high in younger T2DM subjects, especially those younger than 40, we compared the allele and genotype frequencies of SNP-420 between subjects younger than 40 years and those who were 40 years of age or older in T2DM (Table 2). The frequencies of either the G allele or the G/G genotype were higher in the younger group (G allele, 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 cases and controls both younger than 40 years were analyzed, the frequencies of both the G allele and the G/G genotype were higher in T2DM (G allele, T2DM 43.0 vs control 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 T2DM in younger subjects.

In contrast to T2DM subjects, a trend test revealed that, in controls, the G allele frequency had an increasing linear trend as the age grade became older ($P=0.008$) (see right panels in Figs. 1 and 2). 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 gender 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 non-diabetic controls. 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 *C. Elegans* and *Drosophila* (24; 25).

Finally, to examine the relation between SNP-420 and the age of T2DM onset, we added 237 T2DM subjects with onset age 40 or younger. To adjust the effect of aging on the increasing frequency of the G allele, we analyzed a total of 2430 T2DM subjects with the age of onset younger than 60. The trend test revealed that G allele and G/G genotype had an increasing linear trend as the age grade of T2DM onset became younger ($P=0.0492$, and 0.0379, respectively).

We report here that the G/G genotype at SNP-420 was associated with T2DM in younger subjects but not in total subjects by analyzing 2610 T2DM cases and 2502 controls. Differences in G allele frequencies among age grades in cases and controls, namely an increasing linear trend in controls in older grades, and an apparent increase in T2DM cases younger than 40, could result in no association between the SNP-420 genotype and T2DM in the total subjects. The association of SNP-420 with T2DM 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 T2DM, 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 T2DM patients with the G/G genotype may not live longer. It should be noted that P values observed were marginal, and the sample size,

especially that of T2DM subjects with younger age of onset, was limited in this study. A larger number of samples should be analyzed for replication. When stratified by 7 grades (2 kg/m² interval) of BMI, no apparent linear trend of G allele or G/G genotype was observed in controls or T2DM (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.

Recently, we reported that plasma resistin was correlated with insulin resistance in 2078 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, gender, and BMI. The 26% of total variance of plasma resistin could be explained by SNP-420, suggesting that not only SNP-420, but the other genetic and environmental factors could affect plasma resistin levels. The direct association between T2DM and SNP-420 may be more difficult to be detected.

In summary, we analyzed *RETN* SNP-420 in 2610 T2DM cases and 2502 [Del] controls. Although the SNP-420

was not associated with T2DM when analyzed without considering subject age, the G/G genotype frequencies appears to be higher in younger subjects with T2DM. [Del] When 237 T2DM subjects with the age of onset 40 or younger were added, in a total of 2430 T2DM subjects with the age of onset younger than 60, the G/G genotype had an increasing linear trend as the age grade of T2DM onset became younger. Therefore, the [Del] G/G genotype frequency was increased in younger T2DM subjects. In contrast, the C/G genotype showed an increasing linear trend as the age grade became older in controls. It is not clear how resistin induces T2DM in younger subjects, or is beneficial for longer life. Further studies will be required to clarify these points.

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Table 1. The G/G genotype was not associated with T2DM when age was not considered.

	T2DM (n=2610)	Control (n=2502)		χ^2	P	OR	(95% CI)
CC	1169	1080	CC/CG/GG	1.44	0.486	-	-
CG	1144	1123	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	(0.84-1.04)
			GG vs CG+CC	0.41	0.525	0.95	(0.80-1.12)
G allele	1738	1721	G vs C	1.38	0.241	-	-
(%)	33.3	34.4					

Numbers of subjects or alleles in each category are shown. Chi-square test was used for the statistical analysis. OR, odds ratio calculated by defining “G” as a susceptibility allele.

Table 2. The G/G genotype at SNP-420 was associated with T2DM in younger subjects.

Comparison between subjects younger than 40 years and those who were 40 or older in T2DM							
T2DM (n=2610)							
Age	40 >	40 ≤		χ^2	P	OR	(95% CI)
	(n=79)	(n=2531)					
CC	28	1141	CC/CG/GG	8.96	0.011	-	-
CG	34	1110	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	1670	G vs C	6.96	0.008	-	-
(%)	(43.0)	(33.0)					

Comparison between T2DM and control subjects younger than 40 years

Age	40 >			χ^2	P	OR	(95% CI)
	T2DM (n=79)	Controls (n=587)					
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	391	G vs C	5.84	0.016	-	-
(%)	(43.0)	(33.3)					

Numbers of subjects or alleles in each category are shown. Upper panel represents the comparison between T2DM subjects younger than 40 and those who are 40 or older. Lower panel represents the comparison between T2DM and control subjects both younger than 40.

Chi-square test was used for the statistical analysis. OR, odds ratio calculated by defining “G” as a susceptibility allele.

Figure legends

Figure 1. The frequency of the G allele of SNP-420 appears to be increased in younger T2DM subjects, and showed an increasing linear trend in older controls. The allele frequencies of resistin SNP-420 stratified for 10 year age intervals for T2DM cases (left panel) and controls (right panel) are shown. A trend test revealed that the frequency of the G allele differed among age grades in T2DM ($P=0.022$), although the trend was not linear ($P=0.458$). In controls, the frequency of the G allele showed an increasing linear trend with an increase in age ($P=0.008$).

Figure 2. The frequency of G/G genotype of SNP-420 appears to be increased in younger T2DM subjects, whereas that of C/G genotype showed an increasing linear trend in older controls. The genotype frequencies of resistin SNP-420 stratified by 10 year age intervals for T2DM cases (left panel) and controls (right panel) are shown. A trend test revealed that the frequency of the G/G genotype differed among age grades in T2DM ($P=0.037$) although the trend was not linear ($P=0.265$). In controls, 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 controls ($P=0.010$), whereas that of the C/C genotype showed a decreasing linear trend ($P=0.002$).

Fig.1

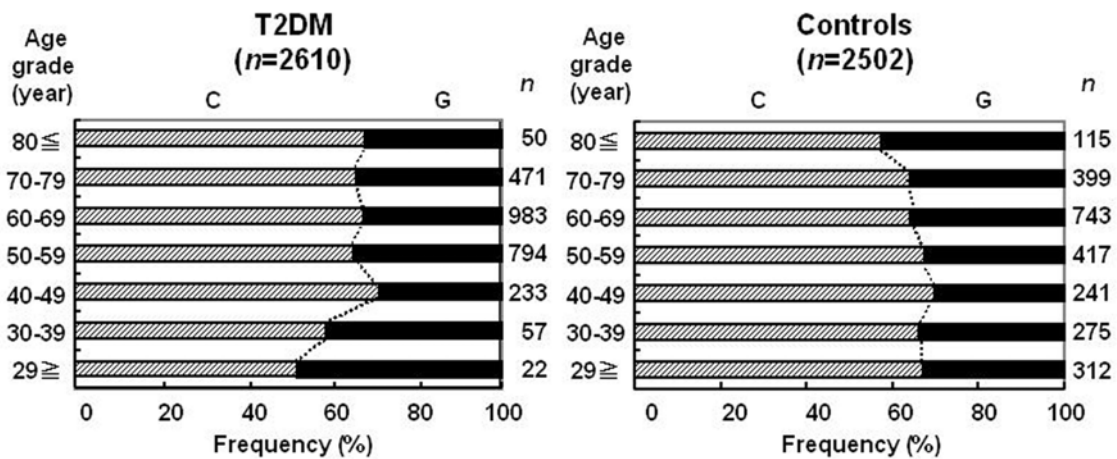


Fig.2

