Influence of cigarette smoking and inflammatory gene polymorphisms on glycated hemoglobin in the Japanese general population

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Objective. Inflammation is closely involved in the development of type 2 diabetes, and cigarette smoking acts as potent inducer of inflammation. We therefore investigated interactions between inflammation-related gene polymorphisms and cigarette smoking on glycated hemoglobin (HbA1c) in the Japanese general population. Methods. We conducted a cross-sectional study using data collected from 2619 Japanese (1274 males and 1345 females) aged 40–69 years of age who participated in baseline survey of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study (2005–2008). Eight polymorphisms in seven genes (interleukin [IL]-1β, IL-2, IL-4, IL-8, IL-10, IL-13 and tumor necrosis factor-α) were determined using the Invader assay. The interactions of smoking status and gene polymorphisms on HbA1c levels were analyzed using multiple linear and logistic regression models and analysis of covariance with adjustment for potential confounders.

Results. Among the eight polymorphisms, only one significant interaction was detected for IL-1β T-31C (P < 0.0001). Among the subjects carrying TT genotype, current heavy smokers (>20 cigarettes/day) had higher HbA1c (5.83 [95% confidence interval 5.67–5.99] %) versus all other smoking status groups (never 5.49 [5.41–5.56] %, former 5.54 [5.43–5.65] %, current moderate [≤20 cigarettes/day] 5.50 [5.30–5.68] %), whereas such differences were not observed in the subjects with C allele. The logistic regression analyses regarding high-normal HbA1c levels showed a similar pattern of results.

Conclusion. Smoking status did not interact with any other inflammation-related polymorphisms except for IL-1β T-31C. Heavy smokers harboring the TT genotype of IL-1β T-31C polymorphism show a greater adverse effect of smoking on HbA1c levels among Japanese middle-aged subjects.

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1. Introduction

Inflammation plays a critical role in the pathogenesis of type 2 diabetes (Shoelson et al., 2006). The elevation of plasma inflammatory cytokines (IL-1β and IL-6) precedes the development of type 2 diabetes.
diabetes (Spranger et al., 2003). Inflammation occurs not only systemically, but also locally within insulin-sensitive tissues (e.g., liver and skeletal muscle) and the pancreas. Inflammatory cytokines act locally to interfere with insulin signaling at sites where they are produced, and local inflammation can induce β cell dysfunction and subsequent insulin deficiency in the pancreatic islets of subjects with type 2 diabetes mellitus (Shoeleson et al., 2006; Esser et al., 2014).

Cigarette smoking is an environmental factor that potently induces an inflammatory response. It is known that smokers have higher levels of circulating inflammatory cytokines, such as IL-6, IL-8 and TNF-α, than nonsmokers (Iho et al., 2003; Bermudez et al., 2002; Petrescu et al., 2010). In an in vitro study, cigarette smoke condensate treatment increased the expression levels of the IL-1β and IL-8 genes in normal human bronchial epithelial cells, in which the degree of IL-1β gene induction was greatest among the six cytokines examined (Hellermann et al., 2002). The chemical species derived from smoking, such as nicotine and cotinine, are detected in various biological samples including saliva, hair, blood and urine (Matsumoto et al., 2013), thus indicating that the toxic chemical species derived from cigarette smoke are absorbed from the lung capillaries and spread throughout the whole body to all organs, including insulin-sensitive tissues and the pancreas.

Polymorphisms in inflammatory genes have been shown to be involved in the modulation of the circulating inflammatory protein levels, such as C-reactive protein and IL-6 (Pankow et al., 2001; Jerrard-Dunne et al., 2004; Oberbach et al., 2008). Furthermore, it has been suggested that the degree of inflammatory response to cigarette smoking may differ according to individual’s genotype for inflammatory genes (Jerrard-Dunne et al., 2004). Although both external (i.e., cigarette smoking) and internal (i.e., inflammatory-related gene polymorphisms) factors regulate inflammation have been shown to be associated with the development of type 2 diabetes and/or insulin resistance, respectively (Kawakami et al., 1997; Nakanishi et al., 2000; Wannamethee et al., 2001; Sairenchi et al., 2004; Willi et al., 2007; Fernandez-Real et al., 2000; Kubaszk et al., 2003; Ayyub et al., 2007; Banerjee and Saxena, 2012), the interactions between inflammatory polymorphisms and smoking on the glycemic control remain to be elucidated. The glycated hemoglobin (HbA1c) level reflects the mean plasma glucose level over the preceding 2–3 months (Rohlfling et al., 2002), and this clinical index is commonly used to obtain a diagnosis of diabetes.

The purpose of the current study was to investigate possible gene–environment interactions between several inflammation-related gene polymorphisms and cigarette smoking on the HbA1c levels in the Japanese general population. We therefore conducted the current cross-sectional study to test the hypothesis that the association of cigarette smoking on the HbA1c levels would be modified by polymorphisms of genes that encode inflammatory cytokine proteins.

2. Materials and methods

2.1. Subjects

In the current cross-sectional study, we analyzed data for 4512 Japanese subjects 40–69 years of age who voluntarily participated in the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study during the period of 2005–2008. The study subjects were recruited from 10 different areas throughout Japan. The J-MICC study was launched in 2005 to confirm and detect gene–environment interactions involved in the development of lifestyle-related diseases in the Japanese general population, and the details of this cohort are described in detail elsewhere (Hamajima and J-MICC Study Group, 2007; Wakai et al., 2011). The J-MICC study was conducted in accordance with the ethical guidelines for epidemiological research of the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare of Japan. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committees at the Nagoya University Graduate School of Medicine and other institutions participating in the J-MICC study.

2.2. Questionnaire and measurements

Data on cigarette smoking, alcohol consumption, dietary habits, physical activity, as well as current medications and past disease history, were collected using a self-administered questionnaire. As for the smoking status, the subjects were first asked about their smoking status from the past to the present. Then, the current smokers were requested to report their usual cigarette consumption (cigarettes/day). The smoking status was categorized as never, former, current 1–19, or ≥20 cigarettes/day. In the present study, current smokers who smoked <20 cigarettes/day were described as moderate smokers, while current smokers who smoked ≥20 cigarettes/day were described as heavy smokers. The total energy intake and ethanol consumption were calculated using data obtained via a validated short food frequency questionnaire (FFQ) (Tokudome et al., 2004; Tokudome et al., 2005; Imaeda et al., 2007). Ethanol consumption was categorized as never, former, current 0.1–22.9, 23.0–45.9 or ≥46 g/day. The amount of habitual physical activity more than an intensity corresponding to 3 metabolic equivalents (METs) was assessed as previously described (Hara et al., 2012). A first-degree family history of diabetes was categorized as positive, negative or unknown. Anthropometric measurements and blood sampling were conducted as part of the health checkup or for research purposes at the institutions participating in the J-MICC study (Hamajima and J-MICC Study Group, 2007). Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was determined by dividing body weight in kilograms by the square of height in meters. The HbA1c level was measured using a latex aggregation immunoassay (Japan Diabetes Society [JDS] value). The HbA1c value was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated according to the following formula: HbA1c (NGSP [%]) = 1.02 × HbA1c (JDS [%]) + 0.25% (Kashiwagi et al., 2012). A significantly elevated HbA1c (high-normal HbA1c) level may be a superior clinical index for predicting the development of type 2 diabetes (American Diabetes Association, 2010). An HbA1c level of 5.7% was used as a cut-off value to define a high-normal 5.7% (Heianza et al., 2011).

2.3. Genotyping

One hundred and seven single-nucleotide polymorphisms were genotyped using an Invader assay (Third Wave Technologies, Madison, WI, USA) (Ohnishi et al., 2001) at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, as previously described (Wakai et al., 2011). Among 107 single-nucleotide polymorphisms, we selected eight polymorphisms in seven inflammatory genes for the current analyses (IL-1β: T-31C [rs1143627], IL-2: T-330G [rs2069762], IL-4: T-33C [rs2070874], IL-8: T-251A [rs4073], IL-10: T-819C [rs1800871], IL-13: C-1111T [rs1800925], TNF-α: T-1031C [rs1799964] and TNF-α: C-857T [rs1799724]). The genotype distributions of these eight polymorphisms did not deviate from Hardy–Weinberg equilibrium (P > 0.05) (Wakai et al., 2011). The IL-6 C-634G polymorphism (rs1800796) was not selected because its genotype distribution deviated from Hardy–Weinberg equilibrium (P < 0.05) (Wakai et al., 2011).

2.4. Statistical analysis

In the current analysis, 1893 subjects were excluded due to following reasons: missing data on gene polymorphisms (IL-1β: n = 2, IL-2: n = 6, IL-4: n = 2, IL-8: n = 27, IL-10: n = 11, IL-13: n = 4, TNF-α T-1031C: n = 1, and TNF-α C-857T: n = 2), cigarette smoking status...
The statistical analyses were performed using the SAS software program (version 9.3 for Windows, SAS Institute, Cary, NC, USA). A P value of <0.05 was considered to be statistically significant. Sex differences in baseline characteristics were examined using the t test for continuous variables or chi-square test for categorical variables. Because of the low frequency of current smokers among females, males and females were analyzed together and sex was included as an adjusted factor in the following multivariate analyses. Multiple linear and logistic regression analyses were performed to calculate the regression coefficients (β) and evaluate the associations (P trend) between the cigarette smoking status (as an independent variable) and either the HbA1c level or a high-normal HbA1c value (≥5.7%) (as dependent variables) according to each genotype of the eight polymorphisms. Adjustment was made for the following potential confounding factors: sex (categorical), age (continuous), BMI (continuous), energy intake (continuous), physical activity (continuous), alcohol drinking (categorical), first-degree family history of diabetes mellitus (categorical) and investigation site (categorical). The smoking status was coded as follows: never (0), former (1), current moderate (2) or current heavy (3), treated as a continuous variable. Then, to identify gene polymorphism(s) that can modify the associations between cigarette smoking and the HbA1c level, interactions with gene polymorphisms were assessed by including an interaction term, indicated as “gene polymorphism (categorical) × smoking status (continuous).” The genotypes of the each polymorphism were coded as 0 for homozygous genotype, 1 for heterozygous genotype or 2 for the opposite homozygous genotype.

Given that our current purpose was to identify inflammatory polymorphisms that can modify the association of cigarette smoking with the HbA1c level, and the interaction analysis was statistically significant only for the IL-1β T→31C polymorphism among the eight polymorphisms (Table 2), the subsequent analyses focused on the IL-1β T→31C polymorphism. The significance of univariate correlations between either the IL-1β T→31C polymorphism or smoking status and selected characteristics (e.g., age and BMI) was assessed according to Spearman’s rank correlation coefficient. In the correlation analysis, a family history of diabetes was ordered coded as 0 (negative), 1 (unknown), or 2 (positive). To assess the associations of the IL-1β T→31C polymorphism and smoking status with the HbA1c level, the adjusted mean value of HbA1c and its 95% confidence interval (CI) according to the IL-1β T→31C genotype or smoking status group were computed as least square means using the general linear model procedure with the LSMEANS statement in SAS. In these analyses, adjustment was made for sex and age (model 1), with additional adjustment for BMI, energy intake, physical activity, alcohol drinking, first-degree family history of diabetes mellitus, investigation site, smoking status (for the evaluation of the influence of the IL-1β T→31C polymorphism on the HbA1c level) or IL-1β T→31C polymorphism (for the evaluation of the influence of the smoking status on the HbA1c level) (model 2). Similarly, the odds ratios (ORs) and 95% CIs of the IL-1β T→31C polymorphism and smoking status for a high-normal HbA1c value (≥5.7%) were estimated using logistic regression models adjusted for confounding factors, as described above.

Furthermore, an analysis of covariance (ANCOVA) was used to calculate the adjusted mean HbA1c value (and 95% CI) and compare the levels between the smoking status groups within each IL-1β T→31C genotype. In order to conduct a similar analysis on the risk of a high-normal HbA1c level, the ORs for high-normal HbA1c and their 95% CIs were also calculated using never smokers in each genotype as a reference group.

### Table 1

**Characteristics of study subjects by sex.** The J-MICC Study 2005–2008.

|                | Males (n = 1274) | Females (n = 1345) | P*  |
|----------------|------------------|--------------------|-----|
| **Age (years)**|                  |                    |     |
| 18–29          | 206 (16.2)       | 235 (17.3)         | 0.68 |
| 30–39          | 452 (35.6)       | 376 (27.8)         | 0.84 |
| 40–59          | 358 (28.1)       | 406 (30.2)         | 0.78 |
| ≥60            | 258 (20.2)       | 338 (25.0)         | 0.78 |
| **Height (cm)**| 171 ± 7          | 166 ± 7            | 0.43 |
| **Weight (kg)**| 69 ± 11          | 63 ± 10            | 0.36 |
| **BMI (kg/m²)**|                  |                    |     |
| Normal          | 756 (59.3)       | 785 (58.3)         | 0.36 |
| overweight      | 345 (27.1)       | 327 (24.3)         | 0.21 |
| Obesity         | 94 (7.4)         | 100 (7.5)          | 0.92 |
| **Physical activity (MET h/day)** | | |
| 0–0.5           | 88 (7.0)         | 100 (7.5)          | 0.74 |
| 0.5–1           | 208 (16.7)       | 230 (17.2)         | 0.77 |
| 1–2             | 352 (27.8)       | 316 (23.9)         | 0.34 |
| ≥2              | 626 (49.5)       | 698 (52.8)         | 0.21 |
| **Drinking (g/day)** | | |
| 0              | 1274 (100)       | 1345 (100)         |     |
| 21–40           | 556 (44.0)       | 528 (40.0)         | 0.36 |
| 41+             | 707 (56.0)       | 814 (61.7)         | 0.21 |
| **Smoking status** | | |
| Never           | 598 (47.1)       | 633 (47.0)         | 0.92 |
| Current moderate | 389 (30.5)       | 366 (27.3)         | 0.34 |
| Heavy           | 287 (22.5)       | 346 (26.0)         | 0.21 |

Values are expressed as the means ± standard deviation for continuous variables or number (percentage) for categorical variables. BMI, body mass index; MET, metabolic equivalent; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol.

### 3. Results

#### 3.1. Characteristics of the study subjects

The characteristics of the study participants are shown in Table 1. Age was similar between males and females, while both BMI and energy intake were statistically significantly higher in males than females (P < 0.01). Cigarette smoking and alcohol drinking were more prevalent among males than females (P < 0.01). The percentages of participants who had a first-degree family history of diabetes were similar between the sex groups, while the HbA1c levels were significantly higher in males than females (P < 0.01).

#### 3.2. Interaction of inflammatory gene polymorphisms and cigarette smoking on the HbA1c level and a high-normal HbA1c value

Among the eight gene polymorphisms investigated, only one statistically significant interaction was found for the IL-1β T→31C polymorphism (P interaction < 0.0001), in which the P value was statistically significant even after using Bonferroni correction (Bonferroni-corrected P < 0.0063) (Table 2). None of the interactions between the other seven polymorphisms and cigarette smoking on the HbA1c level were statistically significant (IL-2: P = 0.66, IL-4: P = 0.78, IL-8: P = 0.08, IL-10: P = 0.09, IL-13: P = 0.50, TNF-α T→31C: P = 0.74, and TNF-α C→857T: P = 0.74). A similar pattern of results was obtained from the logistic regression analyses with respect to the risk of a high-normal HbA1c value, although the P value was marginal for the interaction between the IL-1β T→31C

(n = 5), HbA1c (n = 1763), taking type 2 diabetes medications (n = 193) or having a dietary energy intake extraordinarily greater than 4000 kcal/day (n = 2). Consequently, 2619 subjects in seven investigation areas remained for the current analysis. Among these 2619 participants, there were subjects with missing data regarding alcohol consumption (20 males and 22 females), physical activity (9 males and 14 females), height (1 male), weight (1 male) and BMI (1 male).

HbA1c, the ORs for high-normal HbA1c, and their 95% CIs were also calculated using never smokers in each genotype as a reference group.
The current smokers had the highest OR for a high-normal HbA1c value according to the IL-1β -31C polymorphism and smoking status on the HbA1c levels.

Table 4 shows the adjusted mean of the HbA1c level and ORs for a high-normal HbA1c value according to the IL-1β -31C polymorphism or smoking status. The IL-1β -31C polymorphism was not significantly associated with the HbA1c levels, whereas the smoking status was significantly and positively associated with the HbA1c levels following adjustment for all confounding factors. The current heavy smokers had the highest OR for a high-normal HbA1c value (1.30 [95% CI 0.90–1.89]) in comparison to never smokers, although the difference between these ORs did not reach statistical significance.

Table 2

| Polymorphism | Genotype | HbA1cab (±SE) | P trend | P interactionb | HbA1cc (±SE) | P trend | P interactionb |
|--------------|----------|---------------|---------|----------------|---------------|---------|----------------|
| IL-1β -31C  | CC (n = 584) | -0.0040 0.0254 0.88 | <0.0001 | 0.01 0.13 0.96 | 0.06 | 0.001 | 0.09 0.81 |
|             | CT (n = 1274) | -0.0004 0.0166 0.98 | -0.02 | 0.24 0.10 0.02 |
|             | TT (n = 761) | 0.0963 0.0292 0.001 | 0.10 0.09 0.28 | 0.54 |
| IL-1β -330G | TT (n = 1166) | 0.0191 0.0214 0.37 | 0.66 | 0.12 0.09 0.18 |
|             | TG (n = 1172) | 0.0363 0.0189 0.05 | -0.21 | 0.20 0.29 |
|             | GC (n = 281) | 0.0533 0.0306 0.08 | 0.02 0.09 0.84 | 0.96 |
| IL-1β -33G  | TT (n = 1181) | 0.0305 0.0197 0.12 | 0.78 | 0.11 0.09 0.24 |
|             | TC (n = 1162) | 0.0193 0.0206 0.35 | 0.11 | 0.19 0.38 |
|             | CC (n = 276) | 0.0450 0.0318 0.16 | 0.17 | 0.19 0.38 |
| IL-1β -251A | AA (n = 1205) | 0.0456 0.0197 0.02 | 0.08 | 0.09 0.09 0.29 | 0.97 |
|             | AT (n = 1150) | 0.0124 0.0186 0.51 | 0.07 | 0.09 0.42 |
|             | TT (n = 264) | 0.0768 0.0484 0.11 | 0.10 | 0.19 0.61 |

HbA1c, glycated hemoglobin; IL, interleukin; TNF, tumor necrosis factor; SE, standard error.

a Indicates regression coefficient of multiple regression between smoking (independent variable) and either HbA1c or high-normal HbA1c (dependent variables).

b Interaction with smoking status.

P < 0.05.

3.3. Associations of the IL-1β -31C polymorphism and cigarette smoking on a high-normal HbA1c value (P interaction = 0.056).

Table 3 shows the univariate associations of the IL-1β -31C polymorphism and smoking status with the six selected characteristics. The T allele of the IL-1β -31C polymorphism was significantly and positively correlated with BMI and alcohol drinking. The current smokers had higher BMI values, higher energy intake and higher percentages of current drinkers and subjects with a first-degree family history of diabetes.
3.5. Stratified analyses according to the IL-1β T-31C genotypes

As shown in Fig. 1A, the adjusted HbA1c levels in the subjects carrying the C allele (CC or CT) were similar among the four different smoking status groups (CC: never 5.52 [95% CI 5.46–5.57] %, former 5.42 [5.32–5.52] %, current moderate 5.41 [5.24–5.58] %, current heavy 5.53 [5.39–5.67] %; CT: never 5.49 [5.45–5.53] %, former 5.51 [5.45–5.57] %, current moderate 5.49 [5.39–5.60] %, and current heavy 5.49 [5.40–5.58] %). In contrast, among the individuals carrying the TT genotype, the HbA1c levels were significantly higher in heavy smokers (5.83 [5.67–5.99] %) than in all other smoking status groups (never 5.49 [5.41–5.56] %, former 5.54 [5.43–5.65] %, and current moderate 5.50 [5.30–5.69] %). A similar pattern of results was observed regarding a high-normal HbA1c value (Fig. 1B). The high-normal HbA1c risk (adjusted OR) was significantly higher in the subjects carrying the TT genotype (never 1.00 [reference], former 1.21 [0.71–2.08], current moderate 1.35 [0.63–2.87] and current heavy 2.16 [1.14–4.10]), whereas the ORs were similar among the participants with the C allele (CC: never 1.00 [reference], former 0.85 [0.44–1.64], current moderate 0.39 [0.11–1.41] and current heavy 1.22 [0.57–2.64]; CT: never 1.00 [reference], former 1.46 [0.96–2.22], current moderate 1.18 [0.61–2.27] and current heavy 0.88 [0.48–1.62]).

3.6. Separate analyses according to sex

Since the smoking rate in females was low in the current subjects, additional subanalyses on the interaction between cigarette smoking and IL-1β T-31C polymorphism on HbA1c values were performed for males and females. While interactions between the IL-1β T-31C polymorphism and cigarette smoking on the HbA1c levels were not significant in females alone (HbA1c: P interaction = 0.80; high-normal HbA1c: P interaction = 0.65), significant interactions were observed when analyzed in males alone (HbA1c: P interaction = 0.0016; high-normal HbA1c: P interaction = 0.0796). As shown in Table 5, adjusted HbA1c and high-normal HbA1c values in male subjects showed a similar pattern to that for males and females combined.

4. Discussion

In the current study, we investigated whether the influence of cigarette smoking on the HbA1c levels varied according to polymorphisms of inflammatory genes in a middle-aged Japanese population. Of the eight gene polymorphisms examined, only one statistically significant interaction was detected for the IL-1β T-31C polymorphism (P interaction = 0.0001). The association between cigarette smoking and the HbA1c levels in subjects carrying C allele (CT or CC genotype) did not reach statistical significance, whereas the smoking status was positively associated with higher levels of HbA1c in the individuals harboring the TT genotype (P trend = 0.001). A similar pattern of results was observed regarding the risk of high-normal HbA1c. Therefore, the current results suggest that heavy smokers with the TT genotype of the IL-1β T-31C polymorphism may show a greater adverse effect of smoking on the HbA1c levels among middle-aged Japanese subjects.

As mentioned in the introduction, the IL-1β gene expression response to cigarette smoking condensate was highest compared with several inflammation-related genes (Hellermann et al., 2002), therefore we presumed that its high reactivity to smoking could be a reason for the sole significant interaction detected in the current study. IL-1β is a major proinflammatory cytokine that is known to be produced from endothelial or epithelial cells and macrophages and plays a central role in the regulation of inflammatory responses (Banerjee and Saxena, 2012). IL-1β adversely affects peripheral insulin sensitivity; however, it is especially well known to be closely involved in the impairment of β-cells in pancreatic islets (Banerjee and Saxena, 2012). There are clinical reports showing that blocking the biological activity of IL-1β (via the administration of IL-1-receptor antagonist or IL-1β-specific antibody) improves both the HbA1c level and insulin secretion, but not peripheral insulin sensitivity (Larsen et al., 2007; Donath and Shoelson, 2011; Sloan-Lancaster et al., 2013).

IL-1β mRNA is primarily synthesized as an inactive precursor IL-1β (called pro-IL-1β) and the immature protein is then digested by the proteolytic enzyme caspase 1 to produce a biologically active mature form of IL-1β (Dinarello, 2011). The IL-1β T-31C is a TATA-box polymorphism that profoundly affects DNA-protein interactions in vitro; the −31T allele has been shown to have a 5-fold enhanced binding activity with the transcription initiation factor by way of preserving TATA-box (El-Omar et al., 2000). Similarly, another study showed that the −31T allele is associated with a 10-fold increase in the transcriptional activity compared with the −31C allele (Chakravorty et al., 2006). Therefore, although the current subjects carrying the TT genotype might have had higher IL-1β production and may be susceptible to high HbA1c levels, as shown in Table 4, an increase in the T allele alone was not

**Table 4**

| IL-1β (T-31C) | Smoking status | Current | Current |
|---------------|---------------|---------|---------|
| CC | CT | TT | β | P trend |
| Moderate (<20 cigarettes/day) | Heavy (≥20 cigarettes/day) | Moderate (<20 cigarettes/day) | Heavy (≥20 cigarettes/day) |
| Adjusted means of BMI | | | | |
| Model 1 | 23.1 (22.9–23.4) | 23.1 (22.9–23.1) | 22.3 (21.3–23.5) | 0.120 | 0.15 |
| Model 2 | 23.1 (22.9–23.4) | 23.1 (22.9–23.3) | 22.3 (21.3–23.5) | 0.080 | 0.34 |
| Adjusted means of HbA1c | | | | |
| Model 1 | 5.49 (5.45–5.54) | 5.50 (5.47–5.54) | 5.53 (5.49–5.57) | 0.022 | 0.16 |
| Model 2 | 5.50 (5.45–5.54) | 5.50 (5.47–5.53) | 5.53 (5.49–5.57) | 0.016 | 0.29 |

Values are expressed as the adjusted means or odds ratios (95% confidence interval). BMI, body mass index; HbA1c, glycated hemoglobin; IL-1, interleukin.

a Regression coefficients for the associations between either IL-1β (T–31C) polymorphism or cigarette smoking status and HbA1c levels.

b Model 1: adjusted for sex and age.

c Model 2: adjusted for Model 1 and further adjusted for BMI (for the association between either IL-1β (T–31C) polymorphism or cigarette smoking status and HbA1c levels).

d Regression coefficients for the associations between either IL-1β (T–31C) polymorphism or cigarette smoking status and HbA1c levels.

P < 0.05.
Fig. 1. Influence of cigarette smoking on the HbA1c levels according to the IL-1β T-31C genotypes in middle-aged Japanese subjects. Plots represent adjusted means of HbA1c (A) or ORs for high-normal HbA1c (B) and their 95% CIs. The J-MICC Study 2005–2008.

Table 5
Influence of cigarette smoking on the HbA1c levels according to the IL-1β T-31C genotypes in male subjects. The J-MICC Study 2005–2008.

| IL-1β (T-31C) genotypes | Smoking status |  |  |  |  |
|-------------------------|----------------|------------------|-----------------|------------------|------------------|
|                         | Never          | Former           | Current moderate | Current heavy    | P trend          |
| CC (n = 263)            | 5.55 (5.43–5.66) | 5.47 (5.38–5.56) | 5.50 (5.29–5.72) | 5.52 (5.38–5.66) | –0.0053 0.86     |
| CT (n = 612)            | 5.49 (5.42–5.56) | 5.52 (5.46–5.57) | 5.54 (5.42–5.66) | 5.46 (5.40–5.57) | 0.0001 1.00      |
| TT (n = 399)            | 5.48 (5.31–5.56) | 5.59 (5.45–5.72) | 5.54 (5.23–5.85) | 5.90 (5.69–6.10) | 0.1279 <0.01*    |

Adjusted odds ratios for high-normal HbA1c

| IL-1β (T-31C) genotypes | Smoking status |  |  |  |  |
|-------------------------|----------------|------------------|-----------------|------------------|------------------|
|                         | Never          | Former           | Current moderate | Current heavy    | P trend          |
| CC (n = 263)            | 1.00 (reference) | 1.17 (0.53–2.58) | 0.41 (0.08–2.22) | 1.19 (0.45–3.18) | 0.0068 0.97      |
| CT (n = 612)            | 1.00 (reference) | 1.53 (0.92–2.54) | 1.80 (0.82–3.96) | 0.95 (0.48–1.91) | 0.0004 0.95      |
| TT (n = 399)            | 1.00 (reference) | 1.75 (0.94–3.28) | 2.04 (0.79–5.32) | 2.61 (1.23–5.55) | 0.2982 0.013*    |

Values are expressed as the adjusted means or odds ratios (95% confidence interval). HbA1c, glycated hemoglobin; IL, interleukin. Adjusted for sex, age, body mass index, energy intake, physical activity, alcohol drinking, family history of diabetes mellitus and investigation site.

* Regression coefficients for the associations between cigarette smoking status and HbA1c levels.

* P < 0.05.
significantly associated with higher HbA1c levels. This result does not support the concept that IL-1β plays an important role in controlling the blood glucose level (Spranger et al., 2003; Achuty et al., 2007; Banerjee and Saxena, 2012).

However, the current results showed that overlap of the IL-1β/T-31C TT genotype and heavy smoking (≥20 cigarettes/day) may be unfavorably linked to higher HbA1c levels, as shown in Fig. 1 and Table 5. There is a possible explanation for these results. It has been demonstrated in an animal study that cigarette smoke exposure increases the proteolytic caspase 1 activity as well as release of mature (biologically active) IL-1β (Elton et al., 2011). Therefore, the current heavy smokers carrying the TT genotype might have demonstrated enhanced proteolytic cleavage of immature IL-1β by caspase 1, presumably activated by their heavy smoking, in pancreatic islets, in addition to preserving the increased synthesis of the IL-1β precursor due to the −31T genotype. Hence, we speculate that the coincidence of these two undesirable properties (i.e., the IL-1β/T-31C TT genotype and heavy smoking) may lead to impaired insulin secretion, which can worsen the HbA1c level.

The present result, namely that cigarette smoking was positively associated with higher HbA1c levels only when adjusted for BMI (as shown in model 2 for the HbA1c levels in Table 4), is consistent with a previous report indicating that heavy smoking has a protective effect regarding the future development of type 2 diabetes, mainly through the protection from obesity (Onat et al., 2007). It is well known that smoking has innumerable harmful effects on overall health in addition to a detrimental influence on the blood glucose level; smoking cessation should be highly encouraged among all current smokers, regardless of their distinct IL-1β/T-31C genotype. However, achieving smoking cessation is quite cumbersome, assuming because of the effects of nicotine dependence or addiction and/or insufficient motivation or drive to quit smoking. The current research provides evidence that smoking is especially harmful to individuals bearing the IL-1β/T-31C TT genotype from the perspective of type 2 diabetes prevention, and this information may help individuals with the IL-1β/T-31C TT genotype to avoid starting smoking or possibly consider smoking cessation. Therefore, the present findings are expected to contribute to the development of individualized smoking cessation programs based on a person’s genetic background.

The current study is associated with several limitations. First, since we excluded participants currently using type 2 diabetes medications (n = 193) from the present analyses in order to avoid potential reversal of cause and effect, we were unable to assess the influence on the development of type 2 diabetes. Second, we have no replication data accompanying this study. Confirmation of the present findings by other researchers is expected in the future. Third, although we speculated that the higher levels of HbA1c, noted in the current heavy smokers having the IL-1β/T-31C TT genotype may be attributed to insulin deficiency, we have no data on the circulating insulin concentrations. Additionally, the expression levels of the IL-1β gene in pancreatic β cells were not investigated, as performing a needle biopsy of the pancreas is impracticable in epidemiological studies. Finally, it should be noted that smoking may beneficially affect individuals harboring autoimmune activation (Onat and Can, 2014; Altay et al., 2015; Rasouli et al., 2013), however, we had pertinent no data to identify such individuals in the current population.

5. Conclusion

We found an interaction between the IL-1β T-31C polymorphism and the cigarette smoking status regarding the HbA1c levels, as heavy smokers harboring the TT genotype of IL-1β/T-31C may exhibit a greater adverse effect of cigarette smoking on the HbA1c levels. The smoking status did not interact with any other inflammation-related polymorphisms except IL-1β T-31C. Further studies are needed to confirm the current results in different subject populations and understand the precise mechanisms underlying this interaction.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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References

Achuty, B.R., Srivastava, A., Bhattacharya, S., et al., 2007. Genetic association of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with Type 2 diabetes mellitus in North Indians. Clin. Chim. Acta 377, 163–169.
Altay, S., Onat, A., Karadeniz, Y., et al., 2015. Prediction by low plasma HbA1c of mortality, cardiac and noncardiac disease risk: modulation by diabetic status and sex. J. Investig. Med. 63, 821–827.
American Diabetes Association, 2010. Diagnosis and classification of diabetes mellitus. Diabetes Care 33 (Suppl. 1), S62–S95.
Banerjee, M., Saxena, M., 2012. Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. Clin. Chim. Acta 413, 1163–1170.
Bermudez, E.A., Rifai, N., Buring, J.E., et al., 2002. Relation between markers of systemic vascular inflammation and smoking in women. Am. J. Cardiol. 89, 1117–1119.
Chakravorty, M., Ghosh, A., Choudhury, A., et al., 2006. Interaction between IL1B gene promoter polymorphisms in determining susceptibility to Helicobacter pylori associated duodenal ulcer. Hum. Mutat. 27, 411–419.
Dinarello, C.A., 2011. A clinical perspective of IL-1beta as the gatekeeper of inflammation and smoking in women. Am. J. Cardiol. 89, 1117–1119.
Ferrandez-Real, J.M., Broch, M., Vendrell, J., et al., 2000. Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes 49, 517–520.
Hamajima, N., J-MICC Study Group, 2007. The Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) to detect gene–environment interactions for cancer. Asian Pac. J. Cancer Prev. 8, 317–322.
Hara, M., Hijikai, Y., Taguchi, N., et al., 2012. Effect of the PPARG2 Pro12Ala polymorphism and clinical risk factors for diabetes mellitus on HbA1c in the Japanese general population. J. Epidemiol. 22, 523–531.
Heianza, Y., Hara, S., Arase, Y., et al., 2011. HbA1c 5.7–6.4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in Japan (TOPICS 3): a longitudinal cohort study. Lancet 378, 147–155.
Hellermann, G.R., Nagy, S.B., Kong, X., et al., 2002. Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. Respir. Res. 3, 22.
Ihe, S., Tanaka, Y., Takauji, R., et al., 2003. Nicotine induces human neutrophils to produce IL-8 through the generation of peroxynitrite and subsequent activation of NF-κappaB. J. Leukoc. Biol. 74, 942–951.
Imaeda, N., Goto, C., Tokudome, Y., et al., 2007. Reproducibility of a short food frequency questionnaire for Japanese general population. J. Epidemiol. 17, 100–107.
Jerrard-Dunne, P., Sitzer, M., Risley, P., et al., 2004. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res. Clin. Pract. 65, 141–150.
Kashiwagi, A., Kasuga, M., Araki, E., et al., 2012. International clinical harmonization of glycated hemoglobin in Japan: from Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J. Diabetes Investig. 3, 39–40.
Kawakami, N., Takatsuka, N., Shimizu, H., et al., 1997. Effects of smoking on the incidence of non-insulin-dependent diabetes mellitus. Replication and extension in a Japanese cohort of male employees. Am. J. Epidemiol. 145, 105–109.
Kubaszek, A., Pihlajamaki, J., Punnonen, K., et al., 2003. The -174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. Diabetes 52, 558–561.

Larsen, C.M., Faulenbach, M., Vaag, A., et al., 2007. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N. Engl. J. Med. 356, 1517–1526.

Matsumoto, A., Matsumoto, A., Ichiba, M., et al., 2013. Simultaneous measurement of urinary total nicotine and cotinine as biomarkers of active and passive smoking among Japanese individuals. Environ. Health Prev. Med. 18, 244–250.

Nakanishi, N., Nakamura, K., Matsuo, Y., et al., 2000. Cigarette smoking and risk for impaired fasting glucose and type 2 diabetes in middle-aged Japanese men. Ann. Intern. Med. 133, 183–191.

Oberbach, A., Lehmann, S., Kirsch, K., et al., 2008. Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the -174G/C variant in IL-6 gene. Eur. J. Endocrinol. 159, 129–136.

Onat, A., Can, G., 2014. Enhanced proinflammatory state and autoimmune activation: a breakthrough to understanding chronic diseases. Curr. Pharm. Des. 20, 575–584.

Onat, A., Ozhan, H., Esen, A.M., et al., 2007. Prospective epidemiologic evidence of a “protective” effect of smoking on metabolic syndrome and diabetes among Turkish women—without associated overall health benefit. Atherosclerosis 193, 380–388.

Pankow, J.S., Folsom, A.R., Cushman, M., et al., 2001. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis 154, 681–689.

Petrescu, F., Voican, S.C., Silosi, I., 2010. Tumor necrosis factor-alpha serum levels in healthy smokers and nonsmokers. Int. J. Chron. Obstruct. Pulmon. Dis. 5, 217–222.

Rasouli, R., Grill, V., Midtbjell, K., et al., 2013. Smoking is associated with reduced risk of autoimmune diabetes in adults contrasting with increased risk in overweight men with type 2 diabetes: a 22-year follow-up of the HUNT study. Diabetes Care 36, 604–610.