FTO Gene Polymorphisms and Platelet Counts in a General Japanese Population

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Abstract: The fat mass and obesity-associated (FTO) gene has recently attracted attention as one of the obesity-related genes. Obesity-related gene polymorphisms may be associated with the development of atherothrombosis in relation to platelets. The present study investigated the association between FTO gene polymorphisms (rs1558902, T/A) and hematological parameters, in particular the platelet counts. Anthropometric, hematological, and biochemical parameters, in addition to genotyping by an allele-specific DNA assay, were measured in 209 asymptomatic community-dwelling Japanese subjects (male/female: 80/129; mean age, 65 years; mean [SD] body mass index, 24.0 [3.0] kg/m^2). The subjects with the A-allele (n = 73) showed significantly higher platelet counts than those without the A-allele (mean [SD], 237 [58] vs 217 [57] $\times$ 10^9/L, $P < 0.05$). Even when multiple-adjusted analyses were performed, the platelet counts continued to differ significantly and independently of other variables, including obesity-related parameters such as the index of insulin resistance or high-sensitivity C-reactive protein, between the subjects with and without the A-allele. The FTO gene polymorphisms may be associated with the minor but significant modulation of platelet counts in this population.

Key Words: obesity, fat mass and obesity-associated gene, atherothrombosis, atherosclerosis, insulin resistance

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Obesity is a major risk factor for atherothrombotic diseases such as cardiovascular disease (CVD), thus resulting in increased morbidity and mortality; therefore, better understanding its pathophysiology is crucial for the prevention of CVD.[1] Blood platelets are an essential component for hemostasis and play an important role in atherothrombogenesis.[2] Obesity and related disorders can affect hematological parameters via insulin signals, the alteration of platelet kinetics, and chronic inflammation.[3–6]

Although obesity-related gene polymorphisms may be associated with the development of atherothrombosis in relation to platelets, this topic has not yet been extensively examined. The fat mass and obesity-associated (FTO) gene has recently been identified and attracted attention as one of the obesity-related genes.[7] However, the functions of the FTO gene remain unknown, and multiple roles of FTO have also been suggeted.[7] Thus, the aim of the present study was to investigate the association between common polymorphisms of the FTO gene (rs1558902, T/A) and hematological parameters, in particular the platelet counts, in a general population.

SUBJECTS AND METHODS

A total of 209 community-dwelling Japanese subjects (male/female = 80/129; mean age, 65 years) were recruited during a regular health checkup. Subjects who were basically asymptomatic and not taking any medication were eligible. Excluded were subjects who had features of CVD, severe kidney and liver disease, collagen disorders, or acute infectious diseases such as the common cold. Subjects with blood hemoglobin (Hb) values between 110 and 160 g/L, white blood cell (WBC) counts between 3 and 10 $\times$ 10^9/L, and platelet counts between 100 and 370 $\times$ 10^9/L were included (these values seemed relatively appropriate based on reference intervals[8] and our clinical experience). The study was approved by the ethics committees of the National Hospital Organization Kyoto Medical Center, and each subject gave informed consent.

The smokers were defined as current smokers. In an overnight fasted state, the body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma glucose, serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TGs) were measured by standard methods. Plasma insulin level was measured by an enzyme-linked immunosorbent assay (TOSOH Co, Ltd, Tokyo, Japan). The index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on the plasma glucose and insulin concentrations, as described previously.[9] Serum high-sensitivity C-reactive protein (hsCRP) was measured by an enzyme-linked immunosorbent assay (Assaypro Co, Ltd, St Charles, MO). Complete blood cell analyses including Hb, WBC count, and platelet count were performed with a Sysmex XE-autoanalyzer (Kobe, Japan). The coefficients of variance in these analyses were less than 3%. DNA was extracted from the subjects' buccal mucosa cells obtained using cytobrushes, and genotypes were determined by an intercalater-mediated fluorescent allele-specific polymerase chain reaction method using the forward primer of 5′-TGAGACACTACAGGCATTGTGTC TAGCC-3′ and reverse prime of 5′-TTGCAGCAAAAATCAT ATCAAGTTAGGG -3′ (these primers were originally designed on the basis of the sequence data: Toyobo Gene Analysis Co, Ltd, Tsuruga, Japan).

Data were expressed as means (SD) or medians (interquartile ranges). The genotype and allele frequencies for Hardy-Weinberg equilibrium were examined using the χ^2 test. Differences between the groups were compared using either the unpaired *t* test or the χ^2 test. A general linear model for platelet counts (as a dependent variable) was used to examine the influence of the A-allele (as a fixed variable) with adjustments for multiple variables. Because of the close correlation between the SBP and DBP and between insulin and HOMA-IR, we entered the SBP only and HOMA-IR instead of insulin into the multiadjusted models to avoid statistical

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TABLE 1. Clinical Characteristics Between Subjects With and Without the A-Allele in the FTO Gene Polymorphisms (rs1558902, T/A)

Variable	All (n = 209)	Without A-Allele (n = 136)	With A-Allele (n = 73)	*P*
Age, yrs	65 (12)	66 (11)	64 (13)	0.19
Sex (male/female), n	80/129	53/83	27/46	0.78
Smoking, n (%)	28 (13)	19 (14)	9 (12)	0.74
BMI, kg/m^2	24.0 (3.0)	24.0 (3.0)	24.0 (3.0)	0.88
SBP, mm Hg	139.3 (20.4)	139.6 (18.0)	138.8 (24.3)	0.79
DBP, mm Hg	77.4 (11.5)	77.0 (11.0)	78.3 (12.4)	0.43
TC, mM	4.93 (0.88)	4.92 (0.90)	4.96 (0.85)	0.72
TGs, mM	0.99 (0.79–1.37)	1.02 (0.80–1.38)	0.96 (0.76–1.22)	0.32
HDL-C, mM	1.43 (0.36)	1.42 (0.37)	1.45 (0.35)	0.51
Fasting plasma glucose, mM	5.41 (1.33)	5.47 (1.29)	5.29 (1.42)	0.36
Insulin, μU/mL	5.6 (3.8–8.9)	5.6 (3.8–8.8)	5.6 (3.7–9.0)	0.58
HOMA-IR	1.3 (0.9–2.1)	1.3 (0.9–2.1)	1.3 (0.8–2.3)	0.42
hsCRP, μg/mL	0.13 (0.08–0.25)	0.12 (0.08–0.21)	0.13 (0.08–0.30)	0.67
Hemoglobin, g/L	139 (10)	139 (10)	140 (10)	0.50
WBC count, $\times 10^9$/L	5.26 (1.25)	5.32 (1.29)	5.15 (1.18)	0.36
Platelet count, $\times 10^9$/L	224 (58)	217 (57)	237 (58)	0.02*

Values are expressed as means (SD) in parametrically distributed variables or as medians (interquartile range) in nonparametrically distributed variables. Triglycerides, insulin, HOMA-IR, and hsCRP levels were analyzed after log transformation because of their skewed distribution.

P value was based on the comparison between the groups with and without the A-allele using the unpaired *t* test or the χ^2 test. Significance level: $*P \leq 0.05$.

collinearity. The TG, insulin, HOMA-IR, and hsCRP values were log-transformed because of their skewed distributions. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

The distributed number of TT, TA, and AA genotypes was 136, 62, and 11, respectively. The frequency of the A-allele was 20%. This frequency was similar to an earlier Japanese report.[10] These frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 0.43$, $P = 0.81$).

The group of subjects with the A-allele had significantly higher platelet counts than those without the A-allele, whereas between-group differences were not significantly observed in the levels of any of the other variables (Table 1). When basic confounding variables (age, sex, smoking, BMI, SBP, TC, HDL-C, TG, and glucose) were adjusted in a general linear model analysis, the influence of the A-allele on platelet counts remained independently significant (F = 4.2, $P = 0.04$). After being adjusted for the above basic confounders plus hsCRP, the influence of the A-allele on platelet counts continued to be similarly significant (F = 3.9, $P = 0.05$). After being adjusted for the above basic confounders plus HOMA-IR, the influence of the A-allele on platelet counts also continued to be significant (F = 3.9, $P = 0.05$). Moreover, after further adjusting for the above variables plus Hb and WBC count, the influence of the A-allele on platelet counts remained independently significant (F = 6.6, $P = 0.01$).

DISCUSSION

The subjects carrying the A-allele of FTO gene polymorphisms (rs1558902, T/A) exhibited significantly higher platelet counts, independently of multiple variables including obesity-related, chronic inflammatory, and other hematological parameters, than those without the A-allele in a general Japanese population. The unexpected result of the nonsignificant association between the FTO gene polymorphisms and obesity-related parameters in the present study may partly be explained by the fact that the impact of the FTO gene polymorphisms on obesity may not always be strong[7] or may be due to the differences in the populations between studies (our subjects were asymptomatic and not so obese on average). The clinical relevance of the difference in platelet counts between the subjects with and without A-allele should be further established, but even such a minor difference within a reference range of platelet counts has been debatable regarding the etiopathology of diseases such as CVD.[6,11,12] Therefore, the significant association between the FTO gene polymorphisms and platelet counts found in the present study may provide new insights into the areas related to FTO gene research.

The present study design did not determine the causal contribution of the gene polymorphisms to platelet counts, and the precise mechanism of the associations was unclear. Because the functions of the FTO gene remain largely unknown,[7] we can take into account the possibility of linkage disequilibrium in the gene. Originally, the platelet counts are affected by several complex factors (ie, the splenic pool, platelet production rate, and life span).[3,5] In fact, in conflicting studies, a more active[3] or impaired production of platelets[5] was reported in patients with metabolic disorders such as diabetes. Insulin is also known as a key hormone that increases platelet counts,[4] and an inverse association between insulin resistance and the FTO gene expression has been reported.[13] However, although insulin resistance was included in the analyses in the present study, the association between the FTO gene polymorphisms and platelet counts was consistently confirmed.

Collectively, the present study showed that carrying the A-allele of the FTO gene polymorphism (rs1558902, T/A) was significantly associated with the platelet count in a general Japanese population. This suggests that the FTO gene polymorphisms may be associated with the minor but significant modulation of platelet counts, which may provide some insights into the area of FTO gene research. Further studies are warranted to confirm these results and their clinical relevance and to clarify the biologic mechanism responsible for this association.

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