学 位 論 文 の 要 旨

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学　位　論　文　名　　Cannabinoid Receptor 1 (*CNR1*) *4895C/T* Genetic Polymorphism was Associated With Obesity in Japanese Men

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論 文 内 容 の 要 旨

INTRODUCTION

The endocannabinoid system (ECS) has been well demonstrated to be involved in the regulation food intake process and lipid and glucose metabolisms. ECS overactivation is associated with obesity and obesity-related cardiovascular disease (CVD). Most ECS roles have been discovered by blockade of the receptor, which is the most abundantly expressed ECS receptor. There have been several discrepant reports of the association between *CNR1* gene single nucleotide polymorphisms (SNP) with obesity in Caucasian. Among these SNP, *CNR1 4895C/T* (rs806368) is the only tagged SNP (Minor allele frequency ≥ 10% and R^2 square ≥ 80% for linkage disequilibrium) in Japanese and can therefore be used to study this gene in this population. However, such possible association has yet to be shown in the Japanese population, and studies assessing the relationship of this polymorphism with other CVD risk factors are also scarce. We then investigated the relationship between *CNR1 4895C/T* gene variant and obesity and obesity-related cardiovascular disease (CVD) risk factors in the Japanese population.

MATERIALS AND METHODS

A total of 1452 subjects (678 men, age 56.8 ± 13.4 years and 774 women, age 60.9 ± 11.9 years) were included in this study. Body weight, height and waist circumference (WC) were measured. Body mass index (BMI) was computed as weight (kg) divided by squared height (m^2).

We used cut-off points of BMI ≥ 25kg /m^2, WC ≥ 85 for men and WC ≥ 90cm for women, to define obesity and central obesity status, respectively. Blood pressure was measured twice while the subject was seated. HDL-cholesterol, triglycerides (TG) and LDL-cholesterol were measured by enzymatic methods and glycated hemoglobin (HbA1c) by high-performance liquid chromatography according to the method of Japanese Diabetes Society (JDS). *CNR1 4895C/T* (rs806368) was detected using a TaqMan genotyping assay using an Applied Biosystem 7900HT Fast Real Time PCR system.

TG and HbA1c were log transformed before performing a statistical analysis. Analyses were conducted comparing TT and TC genotypes to CC genotype, due to a dominant effect of T allele. Student's *t*-test was used to assess the differences in parameters by genotype subgroups. Significant associations were further analyzed using analysis of co-variance (ANCOVA) to adjust for the effects of confounding factors. The χ^2 test was used to test for Hardy Weinberg equilibrium and compare the frequency of obesity based on BMI, central obesity based on WC, and prescribed medications between the genotype subgroups. Odds ratio (OR) for obesity and central obesity status adjusted for age and medications was assessed by logistic regression.

RESULTS AND DISCUSSION

The frequency of the C minor allele of *CNR1 4895C/T* polymorphism was 47%. In men, the CC genotype carriers showed significantly greater BMI (TT+TC, 22.8 ± 3.0 kg/m^2 vs CC, 24.0 ± 3.8 kg/m^2; $P<0.001$) and WC values (TT+TC, 81.9 ± 8.6 cm vs CC, 83.8 ± 9.8 cm; $P=0.016$) than T allele carriers. The frequency of obesity was greater for CC genotype carriers than T allele carriers, at 31.8% and 21.5%, respectively ($P=0.010$). In contrast, no association was found with central obesity. CC genotype carriers also showed significantly greater systolic blood pressure (SBP) (TT+TC, 126 ± 16.0 kg/m^2 vs CC, 131 ± 16.1 kg/m^2; $P=0.005$) and diastolic blood pressure (DBP) (TT+TC, 77.2 ± 10.3 kg/m^2 vs CC, 79.1 ± 11.1 kg/m^2; $P=0.049$) values than T allele carriers. The association between *CNR1 4895C/T* genotype groups with BMI and WC were maintained after further adjustment for age and for medications ($P<0.001$ and $P=0.020$, respectively), and with DBP and SBP also remained after adjustment for age and medications ($P=0.003$ and $P=0.035$, respectively). However, subsequent adjustment for BMI or WC weakened the association between *CNR1 4895C/T* genotype groups and SBP ($P=0.046$ and $P=0.012$, respectively). Contrastingly, any association between *CNR1 4895C/T* genotype

groups and DBP disappeared after the abovementioned subsequent adjustment for BMI or WC. Finally, the ORs for obesity and central obesity were investigated between CC genotype carriers and T allele carriers of *CNR1 4895C/T* polymorphism. In men, the CC genotype carriers were more likely to be obese than the T allele carriers even after adjustment for age and medications (OR (CI95%): 1.7 (1.1-2.6); *P*=0.010). There were no significant differences in the odds for central obesity in men, or in the odds of obesity and central obesity in women.

This study has demonstrated that the Japanese carriers of the CC genotype of *CNR1 4895C/T* show greater BMI and WC values, and in men CC genotype carriers are more likely to be obese than the T allele carriers. ECS has been shown to regulate energy balance at the level of both food intake process in the central nervous system (CNS) and the control of lipid and glucose metabolism in peripheral tissues such liver, adipose tissue and skeletal muscle. In the CNS, the ECS, acting via *CNR1*, has been demonstrated to be involved associated with appetite control, including the hypothalamus, limbic forebrain and amygdala, resulting in a hunger-induced increase in food-intake. In the peripheral tissues, *CNR1* stimulation has been shown to increase lipogenesis through activation of sterol element binding protein 1c (SREBP-1c) and therefore of the fatty acid biosynthetic pathway.

While *CNR1 4895C/T* polymorphism showed in this study significant association with WC values, no significant association was found with central obesity based on WC. This difference may be due to the age-difference in WC cut-off points for the definition of central obesity. When comparing the oldest subjects to the youngest, there was a greater amount of visceral fat in the older aged group regardless of BMI. A lower cut-off point might therefore be needed for younger subjects when defining central obesity. Given that we included adults from 25 to 74 years of age in this study, it is plausible that some centrally obese young subjects may have been misclassified.

CONCLUSION

This study supports the association of *CNR1 4895C/T* with obesity in men. Men carriers of CC genotype are more at risk of obesity than T carriers and may require more restricted measures in order to reduce the prevalence of obesity and its related disorders.