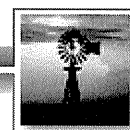


**Figure 1: The rural town, Kofu, Japan - site of the research.**

In 2005, 734 residents of the town of Kofu received a basic medical examination. Their mean age was 66.4 years; mean BMI was 22.7 kg/m<sup>2</sup>; FPG 5.07 mmol/L; HbA1c (NGSP) 5.59%; and fasting triglycerides 1.16 mmol/L (all mean values). Residents who met any of the following criteria underwent an oral glucose tolerance test (OGTT): FPG  $\geq 5.5$  mmol/L or HbA1c (NGSP)  $\geq 5.9\%$ , BMI  $\geq 25$  kg/m<sup>2</sup>, triglycerides  $\geq 1.69$  mmol/L (150 mg/dL), currently being treated for hypertension, or a family history of DM.

The authors performed a multiple regression analysis and receiver operating characteristic (ROC) analysis of the data. It was presumed that non-DM subjects were individuals with normal glucose tolerance and impaired glucose tolerance in OGTT. From a past report<sup>3</sup>, subjects believed to have an extremely low probability of having DM at examination were individuals with FPG  $< 5.5$  mmol/L and HbA1c (NGSP)  $< 5.9\%$ , BMI  $< 25$  kg/m<sup>2</sup>, triglycerides  $< 1.69$  mmol/L, no family history of DM, and no reported treatment for hypertension.

Only 4 subjects were newly diagnosed with DM (FPG  $\geq 7.0$  mmol/L [126 mg/dL]) following the basic medical examination. Of the 220 persons who underwent OGTT, 20 subjects were detected to have DM. Multiple regression analysis indicated that elevated FPG (per 1 mg/dL) and triglycerides ( $\geq 1.69$  mmol/L) were significant risk factors for DM (FPG: OR=1.20, 95%CI:1.13-1.28; triglycerides: OR=3.63, 95%CI: 1.06-12.4) (Table 1). The optimal FPG cut-off for DM was 5.2 mmol/L (94 mg/dL), based on ROC analysis (sensitivity 85%, specificity 78%, area under the curve [AUC] 0.880). Consumption of alcohol was significantly higher ( $>23$  g ethanol  $>3$  times/week) in the DM group compared with the non-DM group (65% vs 33.7%,  $p < 0.005$ ,  $\chi^2$  test). The mean triglycerides level was significantly higher in the group of drinkers compared with non-drinkers (1.22 mmol/L vs 1.13 mmol/L,  $p < 0.05$ , unpaired *t*-test).



**Table 1: Odds ratios for diabetes mellitus by multiple logistic regression analysis**

| Risk factors                        | Odds ratio | P-value | 95% CI    |
|-------------------------------------|------------|---------|-----------|
| Age                                 | 0.97       | 0.233   | 0.92-1.02 |
| Sex (male)                          | 0.78       | 0.667   | 0.25-2.40 |
| Fasting plasma glucose (1mg/dL)     | 1.20       | <0.001  | 1.13-1.28 |
| BMI $\geq 25$ kg/m <sup>2</sup>     | 1.08       | 0.906   | 0.32-3.63 |
| Triglycerides $\geq 1.69$ mmol/L    | 3.63       | 0.040   | 1.06-12.4 |
| Hypertension treatment              | 0.68       | 0.562   | 0.18-2.52 |
| Family history of diabetes mellitus | 1.79       | 0.430   | 0.42-7.65 |

Data are the dependent variable (diabetes mellitus [DM]) vs the explanatory variable (non-DM): DM in 20 persons; non-DM in 548 persons. Adjusted for all explanatory variables.

These results suggest that FPG of  $\geq 6.1$  mmol/L alone is insufficient to detect early-stage DM. The combination of FPG  $\geq 5.2$  mmol/L and triglycerides  $\geq 1.69$  mmol/L was found to be useful as a screening to detect early-stage DM in the group studied. While the suggested FPG criteria may have been too low, it was useful to also consider OGTT results, especially in those with hypertriglyceridemia. It is speculated that moderate alcohol consumption induced diabetes and hypertriglyceridemia in the population studied<sup>4,5</sup>.

The study results should, however, be interpreted with care as it was cross-sectional rather than a cohort study, and the subset of residents who underwent the OGTT was biased. Moreover, the Kofu town residents were an elderly population, so the criteria may not be applicable to other rural areas in Japan. However, it is predicted that the screening criteria established in the present study will contribute to the prevention of stroke in the specific population studied, and may also be useful to consider for other rural residents in the vicinity of the rural town of Kofu.

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## Association of Parental Factors with Student Smoking and Alcohol Use in Japan

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### Summary

A nationwide survey was conducted on smoking and alcohol use among junior and senior high school students and their parents in Japan. The analyses were performed to assess whether parents' smoking or drinking behavior, health knowledge, and attitude toward their children's smoking or drinking behavior influenced their children's behavior using linked datasets of students and parent answers. The number of schools that responded was 24 out of 40 sampled schools. A total of 11,362 questionnaire data sets from students and parents were applied to the analyses. The influence of parental factors including smoking, alcohol use, knowledge, and attitudes were used as the covariates on students' smoking or alcohol use as independent variables. The data were analyzed using a multiple logistic analysis.

The analysis revealed that the parental attitudes of children's smoking or alcohol use were important risk factors as well as parental smoking or drinking behaviors. Conversely, the parental attitude toward warning children of the hazard of smoking or alcohol use was a significant preventive factor for the outcome of their children's behavior.

Key words: adolescent behavior, smoking, alcohol use, parental factors

### Introduction

Smoking and the consumption of alcohol are two important health-related behaviors that are associated with many social and health problems among minors (under 20 years of age). These problems contribute to many diseases and social problems including drunk driving, domestic violence, and child abuse in adulthood. Therefore, preventing minor smoking and alco-

hol use is an essential public health task. It is necessary to determine the risk factors and preventive factors that contribute to minor smoking and alcohol use in order to develop appropriate public health measures. The association of parental smoking and alcohol use with their children's smoking and alcohol use has been studied in some detail<sup>1-6</sup>. However, the association between parental attitudes or norms (parental disapproval, family rules, strict monitoring of child's smoking or alcohol use) or parent-child relationship (connectedness, communication, or family bonding) and child smoking or alcohol use has led to no consistent conclusions because of various study results<sup>7-17</sup>. Moreover, some of those studies analyzed the parental behaviors based on reports by children using the questionnaire surveys<sup>2,4,7,11,16,17</sup>. Although some results on adolescents' smoking and alcohol use are reported on subjects from Asian countries, there are few papers indicating the relationship between student and parental reports<sup>18-20</sup>. Smoking and drinking behavior and its correlates are considered to differ greatly from country to country<sup>21</sup>. To establish effective measures regarding smoking or drinking control in each country, a nationwide survey was carried out focusing on the behavior and its correlates. However, there is no previous study which analyzed the association between child smoking or alcohol use and parental behavior or attitudes in Japan based on both child and parental questionnaires. This study analyzed the linkage data from students' questionnaires and the father's and mother's questionnaires to identify the parental correlates for Japanese high school students' smoking and drinking behaviors.

### Subjects and Methods

The present study design was performed as a cross-sectional study by random sampling. Schools were randomly sampled throughout Japan and the enrolled students were asked to complete an anonymous self administered questionnaire in the classrooms. Twenty-five junior high schools and 15 senior high schools were selected from Japan. Therefore, this sampling method was a one-stage cluster sampling. The parental questionnaires were taken home by students, and these were brought back to the schools after completion by their parents. This survey was conducted as a part of nationwide survey on junior and senior high school students in 1996<sup>19,20</sup>. Because the procedures of the parent-child survey were complicated, we only asked some of the sampled schools to participate in the present survey. The students completed the questionnaire, which was placed in a large envelope with the parental questionnaires. The teacher collected these data and sent them to the research institute without opening the envelope. The same identification number was assigned to each tripartite questionnaire, linking anonymous questionnaires.

The contents of the students' survey were determined taking the contents of past surveys conducted in Japan regarding the smoking behavior of junior and senior high school students into consideration. The parental questionnaire was developed according to the contents of the students' questionnaire by the present research group.

The students' questionnaire covered their smoking behavior, alcohol use, and correlates on school life and daily life in home. The parental questionnaire examined their smoking behav-

ior, alcohol use, recognition and attitude toward their children's smoking or alcohol use were examined. The questions on parental smoking or drinking behavior included "smoking status or drinking status", "experience under 18 years of age", and "wants to quit smoking or drinking". The questions on parental attitudes include "acceptance of minor smoking or drinking", "had recommended smoking or alcohol to child", "drink alcohol in front of the child", "making the child to buy cigarettes or liquor", "disapproval of smoking or drinking by the child", and "agreement with cigarette vending machine abolition". The questions on parent-child relationship include "short time spent with the child", "students do not talk about their troubles with parent", and "parent hopes the child will enter university".

The survey was conducted from December, 1996 to January, 1997.

The school response rate was 56% (14/25) from junior high schools, and 66.7% (10/15) in senior high schools. A total of 16,732 student questionnaires were collected. Of these, 1,051 of incomplete or inconsistent and were excluded from the analyses. A total of 12,744 and 14,019 father and mother questionnaires were collected, and there were ultimately 11,362 linked tripartite questionnaires data sets. The data sets were used for the multiple logistic analyses.

The statistical analyses used student current smoking or alcohol use as a dependent variable, and student factors and parental factors as the independent variables. Students who smoked or drunk at least once in the past 30 days were defined current smokers or drinkers. The student factors included student sex and school grade. The parental factors included their smoking or drinking behavior, their attitude toward minor smoking or alcohol consumption, interaction with children, their opinion on smoking and alcohol consumption in children. The statistical analyses used a multiple logistic regression model with the variable increase method by the likelihood ratio (SPSS ver18). The odds ratios of each factor were calculated by the model including all selected dependent variable, such as sex, school grade, other student factors and parental factors.

## Results

The experimental rate of smoking among the students was 43.5% for boys, and 23.7% for girls. The current student smoking rate (smoked at least one day during the preceding 30 days) was 18.5% for boys and 6.2% for girls, and the daily smoking rate was 8.1% for boys and 1.8% for girls. The experimental rate of alcohol use was 78.3% for boys and 74.0% for girls, the prevalence of current alcohol use (had consumed an alcoholic drink on at least one day of the preceding 30 days) was 33.6% for boys and 25.8% for girls, and weekly alcohol use was 7.5% for boys and 3.1% for girls. The parental daily smoking rate was 55.3% for the fathers and 10.0% for mothers, and the prevalence of daily alcohol use was 48.2% for fathers and 6.5% for mothers (Table 1).

The present survey asked the parents that "Do you think the child is smoker or drinker?" About 30-40% of parents of smoking or drinking boys reported that their children were never smokers or never drinker. Moreover, about 40-60% of parents of smoking or drinking girls reported that their children were never smokers or never drinkers (Table 2).

Table 1 Smoking and drinking behavior of parents and their children

|                                                   | children |             |           |             | parent     |             |            |             |
|---------------------------------------------------|----------|-------------|-----------|-------------|------------|-------------|------------|-------------|
|                                                   | boys (%) | 95% C.I.    | girls (%) | 95% C.I.    | father (%) | 95% C.I.    | mother (%) | 95% C.I.    |
| smoking experiment                                | 44.9     | (43.7-46.2) | 23.7      | (22.5-24.9) |            |             |            |             |
| alcohol experiment                                | 78.3     | (78.5-80.5) | 74.0      | (73.6-76.0) |            |             |            |             |
| current smoking                                   | 18.5     | (17.8-19.7) | 6.2       | (5.6-6.9)   |            |             |            |             |
| current alcohol use                               | 33.6     | (33.0-35.4) | 25.8      | (24.9-27.3) |            |             |            |             |
| daily smoking                                     | 8.1      | (7.6-9.0)   | 1.8       | (1.5-2.2)   | 55.3       | (54.4-56.2) | 10.0       | (9.4-10.5)  |
| weekly alcohol use                                | 7.5      | (6.9-8.2)   | 3.1       | (2.7-3.6)   |            |             |            |             |
| daily alcohol use                                 |          |             |           |             | 48.2       | (47.3-49.2) | 6.5        | (6.1-7.0)   |
| smoking experience under 18                       |          |             |           |             | 7.8        | (7.4-8.3)   | 1.1        | (0.9-1.3)   |
| drinking experience under 18                      |          |             |           |             | 3.8        | (3.5-4.2)   | 1.0        | (0.8-1.2)   |
| parent wants to quit smoking                      |          |             |           |             | 8.6        | (8.1-9.1)   | 3.6        | (3.2-3.9)   |
| parent wants to quit drinking                     |          |             |           |             | 2.7        | (2.4-3.0)   | 1.6        | (1.4-1.9)   |
| parental acceptance of a child's smoking          |          |             |           |             | 20.2       | (19.5-21.0) | 10.3       | (9.7-10.8)  |
| parental acceptance of a child's drinking         |          |             |           |             | 55.4       | (54.4-56.3) | 43.3       | (42.4-44.2) |
| parental recommendation smoking to child          |          |             |           |             | 1.8        | (1.5-2.0)   | 0.9        | (0.7-1.1)   |
| parental recommendation alcohol to child          |          |             |           |             | 25.5       | (24.7-26.3) | 15.4       | (14.8-16.1) |
| parental disapproval of child smoking             |          |             |           |             | 80.0       | (79.3-80.7) | 85.1       | (84.5-85.8) |
| parental disapproval of child alcohol use         |          |             |           |             | 57.0       | (56.1-57.9) | 63.9       | (63.0-64.7) |
| making the child to go buy liquor                 |          |             |           |             | 26.2       | (25.4-27.0) | 22.1       | (21.3-22.8) |
| drinking in front of the child                    |          |             |           |             | 83.7       | (83.1-84.4) | 55.9       | (54.9-56.8) |
| short time spent with child                       |          |             |           |             | 12.0       | (11.4-12.5) | 2.3        | (2.0-2.6)   |
| student do not talk of their troubles with parent |          |             |           |             | 26.2       | (25.4-27.0) | 8.7        | (8.2-9.2)   |
| parent hopes the child will enter university      |          |             |           |             | 46.6       | (45.7-47.5) | 43.4       | (42.5-44.3) |

The model using student current smoking as a dependent variable revealed that the father's factors such as "smoking experience under 18 years of age" and "short time spent with child", and the mother's factors such as "mother wants to quit smoking", "acceptance of minor smoking", "mother making the child go buy cigarettes", "students do not talk of their troubles with mother", and "agreement with cigarette vending machine abolition" were statistically significant risk factors (Table 3). Mother's factors such as "mother having no job" and "maternal disapproval of smoking by child", and father's factor such as "father hopes his child will enter university" were significant preventive factors (Table 3).

The model using student current alcohol use as a dependent variable demonstrated that

Table 2 Proportion of parents who consider their children are never smoker or never drinker by children's smoking status or drinking status

| smoking or drinking status<br>of children |                 | father |                |             | mother         |             |
|-------------------------------------------|-----------------|--------|----------------|-------------|----------------|-------------|
|                                           |                 | number | proportion (%) | 95% C.I.    | proportion (%) | 95% C.I.    |
| boys                                      | current smoker  | 1176   | 39.5           | (36.7-42.3) | 36.4           | (3.6-39.1)  |
|                                           | non smoker      | 5095   | 87.3           | (86.4-88.2) | 86.8           | (86.0-87.8) |
| girls                                     | current smoker  | 318    | 63.2           | (57.9-68.5) | 54.7           | (49.3-60.2) |
|                                           | non smoker      | 4773   | 92.7           | (91.9-93.4) | 93.8           | (93.1-94.4) |
| boys                                      | current drinker | 2143   | 35.8           | (33.8-37.8) | 32.0           | (30.0-34.0) |
|                                           | non drinker     | 4226   | 66.2           | (64.8-67.6) | 63.2           | (61.8-64.7) |
| girls                                     | current drinker | 1327   | 48.8           | (46.1-51.4) | 44.8           | (42.1-47.4) |
|                                           | non drinker     | 3818   | 74.4           | (73.0-75.8) | 72.8           | (71.4-74.2) |

Table 3 Relating parental factors with smoking by students

|                                                                       | Odds ratio | 95% C.I.    | p value |
|-----------------------------------------------------------------------|------------|-------------|---------|
| sex ( boys=1, girls=2)                                                | 0.03       | (0.02-0.03) | <0.01   |
| school grade (+1)                                                     | 1.47       | (1.41-1.53) | <0.01   |
| smoking experience under 18 years of age by father                    | 1.48       | (1.22-1.80) | <0.01   |
| short spending time with child (less than 30 minutes a day) by father | 1.45       | (1.24-1.70) | <0.01   |
| interaction of gender and daily smoking by father                     | 1.12       | (1.02-1.23) | <0.05   |
| father hopes entrance of his child to university                      | 0.46       | (0.41-0.52) | <0.01   |
| mother wants to quit smoking                                          | 1.43       | (1.07-1.92) | <0.05   |
| acceptance of minor smoking by mother                                 | 1.27       | (1.07-1.52) | <0.01   |
| making the child go to buy cigarettes by mother                       | 1.25       | (1.07-1.47) | <0.01   |
| agreement of cigarette vending machine abolition by mother            | 1.24       | (1.10-1.41) | <0.01   |
| students do not talk on their troubles with mother                    | 1.24       | (1.03-1.49) | <0.05   |
| having no job of mother                                               | 0.75       | (0.64-0.88) | <0.01   |
| maternal disapproval of smoking by child                              | 0.66       | (0.57-0.77) | <0.01   |

father's factors such as "father had recommended alcohol to his child", "paternal drinking in front of the child", "paternal acceptance of a minor's alcohol use", and "father stating that liquor is not hazardous to health", and that mother's factors such as "mother recommended alcohol to her child", "mother drinking in front of the child", and "mother making the child go buy liquor" were significant risk factors. On the other hand, the mother's factors such as "mother having no job" and "maternal disapproval of alcohol use by child", and father's factors such as "father hopes his child will enter university", and "paternal disapproval of alcohol use by the child" were significant preventive factors (Table 4).



Table 4 Relating parental factors with alcohol use by students

| item                                                  | Odds ratio | 95% CI      | p value |
|-------------------------------------------------------|------------|-------------|---------|
| sex (boys=1, girls=2)                                 | 0.19       | (0.16-0.23) | <0.01   |
| school grade (+1)                                     | 1.26       | (1.22-1.29) | <0.01   |
| -----                                                 |            |             |         |
| father had recommended of alcohol use to his child    | 1.35       | (1.22-1.50) | <0.01   |
| drinking in front of the child by father              | 1.19       | (1.05-1.35) | <0.01   |
| accepting minor alcohol use by father                 | 1.16       | (1.06-1.28) | <0.01   |
| recognizing liquor not hazardous for health by father | 1.10       | (1.01-1.19) | <0.05   |
| father hopes entrance of his child to university      | 0.87       | (0.80-0.95) | <0.01   |
| paternal disapproval of alcohol use by child          | 0.86       | (0.78-0.95) | <0.01   |
| -----                                                 |            |             |         |
| mother had recommended of alcohol use to her child    | 1.45       | (1.29-1.63) | <0.01   |
| drinking in front of the child by mother              | 1.40       | (1.28-1.53) | <0.01   |
| making the child go to buy liquor by mother           | 1.18       | (1.07-1.31) | <0.01   |
| having no job of mother                               | 0.88       | (0.79-0.98) | <0.05   |
| maternal disapproval of alcohol use by child          | 0.71       | (0.65-0.78) | <0.01   |

## Discussion

The current study revealed parental attitudes toward child's smoking or drinking were statistically significant risk factors for child smoking or alcohol use, as well as the parental smoking and drinking behavior. Although the present study was a cross-sectional study, the results on parental factors are considered risk or preventive factors for adolescent smoking or drinking, because there was a reasonably causal relationship of parental factors with their children's factors. Many reports indicated during recent several decades that parental smoking or alcohol use is a significant risk factor for child's smoking or alcohol use<sup>1-9</sup>. The influence of parental smoking on child's smoking is relatively stronger in Japan than that in China<sup>20</sup>. This influence was reviewed in the present study. In addition, parental initiation of smoking or alcohol use before 20 years of age was a significant risk factor as well as parental attitudes regarding child's smoking or drinking or parent-child relationship.

The result of the present study that the parental smoking and alcohol use associated with their children's smoking and alcohol use was similar to the results from previous studies.

Multivariable analyses found that drinking in front of the child rather than daily alcohol consumption by parents was a significant risk factor. Furthermore, this analysis suggested the hazard of involving children in adults' smoking or alcohol use, such as making the child buy cigarettes or alcohol beverages, was suggested by this analysis. However, the model suggested that the parental attitude of the father or mother to warn children against smoking or drinking was significant preventive factor. Several studies have reported that parental attitudes (such as parental disapproval, strict family monitoring, and family rule) can prevent a small part of their adolescent smoking or alcohol use<sup>7,8,11,15-17</sup>, however, other studies have not observed any such effectiveness<sup>9,10,13,14</sup>. The present results suggested the importance of parental attitudes

toward child smoking or alcohol use. The difference may be caused by cultural differences such as size of houses or rooms and the interrelationship among family members between Japan and Western countries.

The importance of the attitudes of parents who do not involve their child in the adults' smoking or drinking, warning children of the dangers of smoking or alcohol use and improving parental smoking or drinking behavior were found to be important factors influencing their children's behavior in the present study. In addition, the present study revealed the importance of communication between adolescents and their parents for preventing minor smoking or alcohol use, confirming previous studies<sup>9</sup>. Several recent studies from Western countries described the strong influence of the family on adolescents' smoking or alcohol use<sup>5,9,11,16</sup>.

The present study also found that parents, especially the father, of current smokers or drinkers did not believe their children were not smokers or drinkers. Parents should be interested in children's smoking and alcohol use, and should be aware of the actual behaviors of their children. The gender difference in smoking prevalence among children is smaller than that among parents, and the gender differences in alcohol use is much smaller than those among parents. Since adolescent girls will give birth and care for children in the future, girls' smoking and alcohol use should be monitored carefully.

Measures for raising the interest of parents should therefore be developed by the smoking and alcohol control policy-makers in order to prevent children and adolescents from smoking and alcohol consumption.

Some limitations are considered to be associated with the present study. First, this study was conducted based on a self-administered questionnaire survey. Since smoking and drinking behavior are illegal behavior among minors under 20 years of age in Japan, the problems regarding the accuracy of the results obtained from children may arise. We considered that the influence of misclassification of reported smoking or drinking status was not large, because the number of questionnaires with invalid answers or contradictory answers was small. Moreover, the prevalence of smoking and alcohol use behavior in the present study was not significantly different from that reported in another nationwide survey<sup>19,20</sup>.

Second, this survey was a cross-sectional survey, and therefore it is difficult to determine the causal relationships among the surveyed factors. Since we considered that it is unusual to begin smoking or drinking for parents because of the child factors, the parental factors we found in the present study were therefore dealt with as either risk factors or preventive factors for child smoking or alcohol use.

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# 動脈硬化症予防プログラムにおける環境・遺伝要因の 介入効果およびリバウンドへの影響に関する研究

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## Influence of environmental and inherent factors upon intervention effect and rebound of an arteriosclerosis prevention program

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### ABSTRACT

Arteriosclerosis is the cause of death for many Japanese. Furthermore, arteriosclerosis may also be a cause of becoming bedridden at an advanced age. Therefore, we investigated the development of a program to prevent arteriosclerosis. This study evaluated 84 participants in an intervention program. We established an intervention term of 6 months with a follow-up term of 3 months to observe rebound. We regarded improvement of brachial-ankle pulse wave velocity (baPWV) as an endpoint of this study. To determine the effect of intervention and rebound of improvement, we analyzed the influence of environmental and hereditary factors. We studied five gene polymorphisms: endothelial NO synthase (eNOS) T786C, eNOS intron4 VNTR, eNOS Glu298Asp,  $\beta$ 2 adrenergic receptor polymorphism (ADRB2) Arg16Gly and ADRB3 Trp64Arg. Twenty-seven participants showed an effect of the intervention. On multiple logistic regression analysis using the 5 gene polymorphisms and life style (drink, tobacco and exercise), the b/a genotype and the b/a+a/a genotypes of eNOS intron4 VNTR prevented an effect of intervention from appearing (odds ratio (OR), 0.23, 0.20; 95% confidence interval (CI), 0.06-0.88, 0.05-0.74;  $p = 0.03, 0.01$ ). The Gly/Gly genotype of ADRB2 also prevented an effect of intervention from appearing (OR, 0.27; 95% CI, 0.08-0.88;  $p = 0.03$ ). In the relationship between rebound and gene polymorphism, 9 participants showed rebound of improvement. Multiple logistic regression analysis using the 5 gene polymorphisms and life style (drink, tobacco and exercise) did not show any significant results. However, the b/b genotype of eNOS intron4 VNTR tended to promote rebound (OR, 6.07; 95% CI, 0.40-92.01;  $p = 0.19$ ). The Gly/Gly genotype also tended to promote rebound (OR, 7.74; 95% CI, 0.45-131.09;  $p = 0.15$ ). These findings demonstrate that eNOS intron4 VNTR and ADRB2 Arg16Gly are related to the effects of intervention and rebound. These findings suggest that the development of effective programs to prevent arteriosclerosis should consider these inheritance factors. (Accepted on June 20, 2011)

**Key words :** arteriosclerosis, eNOS intron4 VNTR, ADRB2, rebound, polymorphism

## はじめに

日本人の死因の第2位と第3位である心疾患、脳血管疾患の多くは動脈硬化症が原因と考えられている。また死因だけではなく、厚生労働省の国民生活基礎調査によると65歳以上の要介護の原因の第1位は脳血管疾患となっており高齢化社会が進む現代の日本では大きな問題となっており動脈硬化症の予防は緊急な健康課題である。

動脈硬化の原因としては、生活習慣を主とした環境要因と遺伝要因が複雑に関連している。遺伝要因として、動脈硬化症と関連する多くの遺伝子が報告されているが、その中から今回の研究では、eNOS T786C, eNOS intron4 VNTR, eNOS Glu298Asp, ADRB2 Arg16Gly, ADRB3 Trp64Argの5つの遺伝子多型との関連性を調べた。eNOSにより合成される内皮細胞性一酸化窒素は血小板の凝集抑制、血小板の移動の活動の調整、血管の拡張作用、血管平滑筋の弛緩などの作用を持ち動脈硬化の予防に大きな働きをしていることが多くの研究で解明されている。βアドレナリン受容体はβ1, β2, β3の3つのサブタイプに分かれ、β2受容体は気管支平滑筋の弛緩や骨格筋の血管平滑筋の弛緩などの作用を持ち、β3受容体は脂肪細胞の脂肪分解の促進などの作用を持ち肥満と大きな関連を持つ。Kazuko Mらによりβ2のGly16アレル、β3のTrp64アレルが体重増加、血圧の上昇に大きく関連することが報告されるなど動脈硬化との関連性があることが疑われる。

これらの遺伝子多型と動脈硬化予防の介入効果との関連を調べるためbaPWVをエンドポイントとして研究を行った。また介入によりbaPWVが改善された場合にもリバウンドという問題がその後の健康に大きく影響を及ぼし、リバウンドの予防が重要な問題となっている。本研究の目的は動脈硬化の予防を目的としたプログラムにおける環境要因・遺伝要因が介入効果及び改善効果のリバウンドに及ぼす影響に関して検討することである。

## 研究方法

### A 研究対象者

本研究は鳥取大学医学部倫理審査委員会の倫理審査の承認を受けた後実施された。対象は某事業所の従業者で、2006年9月から始まった動脈硬化症予防を目的としたプログラムの参加者84名である。

### B 介入方法

無作為に参加者を介入群と情報提供群の2つの群に分け、1グループ3ヶ月間の介入期間と情報提供期間をそれぞれ設定し(図1)、各参加グループには情報提供期間の後に介入期間を設け、参加によって全グループが介入による利益を受けられるようにした。介入期間終了後6ヶ月間を追跡期間として設けた。情報提供期間の初日にインフォームドコンセントを取り、各個人に面接を行って研究目的や方法、参加による利益・不利益、個人情報保護など、その他参加に当たって本人に十分理解を得てもらうべき事項について説明し、参加者には署名による同意を得た。更に食事改善やメタボリックシンドロームに関するパンフレットを配布し、参加者の改善意欲を促した。介入期間においては週に1度、12週間(3ヶ月間)にわたり各項目の測定を行った。この項目は体重、体脂肪率、腹囲、左右の上腕と足首の血圧、baPWV<sup>1)</sup>である。また介入期間と情報提供期間の開始時と終了時には血液検査を行った。その他の介入の内容としては、期間中に産業医による保健指導と栄養士による食事指導を行い、生活習慣(喫煙習慣、飲酒習慣、食生活など)の改善を促した。また介入開始時に動脈硬化症に関する学習を目的とした健康教室や開始から約1ヶ月半後には中間健康教室を行い、参加者の各個人の詳細な途中経過の確認と参加者同士のグループでのディスカッションで参加者の意欲向上を図った。介入期間中には介入群の各参加者にライフコーダ万歩計(スズケン、愛知)を渡し、1日1万歩を目標とした低強度持久力トレーニング(ウォーキング)を勧め、日々の歩数・消費カロリーなどを測定した。追跡期間においては、介入終了6ヶ月後に各項目の測定を行った。

| 期間                   | 個別説明・IC<br>ベースライン調査                           | 情報提供期間                     | 追跡期間                                            |
|----------------------|-----------------------------------------------|----------------------------|-------------------------------------------------|
| 開始時                  | ①測定<br>②採血<br>健康教室                            | ③運動・生活習慣調査<br>④ライフコーダ万歩計配布 | 個別説明・IC<br>ベースライン調査<br>①測定<br>②採血<br>③運動・生活習慣調査 |
| 1ヶ月目<br>2ヶ月目<br>3ヶ月目 | ↑ ↓ [週に1回] 測定・産業医の指導<br>[月に1度] 栄養士の食事指導       |                            |                                                 |
| 終了時                  | 終了時検査<br>①測定<br>②採血<br>③運動・生活習慣調査<br>総括ミーティング |                            | 終了時検査<br>①測定<br>②採血<br>③運動・生活習慣調査               |
| 介入終了<br>6ヶ月後         |                                               |                            | 終了時検査<br>①測定<br>②採血<br>③運動・生活習慣調査<br>④最終アンケート   |

|        | 第1G      | 第2G                | 第3G      | 第4G      | 第5G      |
|--------|----------|--------------------|----------|----------|----------|
| 2006年度 |          |                    |          |          |          |
| 2007年度 | ↑ ↓ 追跡期間 | 情報提供期間<br>↑ ↓ 追跡期間 | 情報提供期間   | 情報提供期間   |          |
| 2008年度 |          |                    | ↑ ↓ 追跡期間 | ↑ ↓ 追跡期間 | 情報提供期間   |
| 2009年度 |          |                    |          |          | ↑ ↓ 追跡期間 |

図1 研究開始時から終了時までの測定・指導の流れ

- ① 測定項目：体重，体脂肪率，腹囲，右上腕SBP (systolic blood pressure)，右上腕DBP (diastolic blood pressure)，baPWV (brachial-ankle pulse wave velocity)
- ② 採血項目：総コレステロール，LDLコレステロール (low density lipoprotein)，HDLコレステロール (high density lipoprotein)，中性脂肪，インスリン，ヘモグロビンA1c

3ヶ月間の介入期間の終了時のbaPWVの値が介入開始時の値と比較し5%減少していたとき、「介入効果あり」と判断した。また、介入効果ありの対象者に関して、介入終了6ヶ月後にbaPWVの値が介入開始時のbaPWV値を超えている対象者をリバウンド群，その他を非リバウンド群と定義し2つのグループに分けて解析した。

C 測定項目

1 体格

体格の測定項目として身長，体重，体脂肪率，腹囲を計測した。また以下の式から

Body Mass Index (BMI) を算出した。BMI=体重 (kg) /身長 (m) ×身長 (m)}.

2 血圧測定・baPWV

収縮期血圧 (systolic blood pressure: SBP) および拡張期血圧 (diastolic blood pressure: DBP) およびbaPWVをform PWV/ABI (オムロンコーリン株式会社，東京) を使用し測定した。

3 血液生化学

採取した血液から血清総コレステロール(TC)，high density lipoprotein (HDL) コレステロール，low density lipoprotein (LDL) コレステロール，中性脂肪 (TG)，空腹時血糖，インスリン，ヘモグロビンA1c (HbA1c)，インスリン抵抗性 (homeostasis model assessment for insulin resistance; HOMA-R) の測定を行った。TC，HDL，LDL，TG，HbA1cは自動分析装置オリンパス5430 (オリンパス，東京) と自動分析装置

用試薬デタミナー (132614-147439 協和メデックス, 東京) を使用し, 空腹時血糖は自動分析装置 BM9030 (日本電子, 東京) と自動分析装置用試薬クイックオート II GLU-HK (シノテスト, 神奈川) を使用し, インスリンは自動分析装置アーテクト (アボットジャパン, 千葉) と自動分析装置用試薬アーテクト・インスリン (アボットジャパン, 千葉) を使用しそれぞれ測定を行った。

#### 4 5種の遺伝子多型

**eNOS T-786Cの同定;** QIAamp DNA Blood Mini Kit (QIAGEN, 東京) を用い, プロトコルにしたがってDNA抽出を行なった。その後ABI PRISM 7900HT-SDS v2.1 [Applied Biosystems (ABI), Foster City, CA, USA] を用いて TaqMan PCR法による遺伝子多型の同定を行なった。使用したプライマーおよびプローブは以下の通りである; Forward プライマー: 5'-CACCAGGGCATCAAGCTCTT-3', Reverse プライマー: 5'-GCAGGTCAGCAGAGAGACTAG-3', プローブ (T) VIC: 5'-CCCTGGCTGA-3', プローブ (C) FAM: 5'-CCTGGCCGGCTGA-3' (すべてABI)。

**eNOS intron4 VNTRの同定;** QIAamp DNA Blood Mini Kitを用い, プロトコルにしたがってDNA抽出を行った。その後, Polymerase chain reaction (PCR) 法により多型部位のDNA増幅を行い遺伝子多型の同定を行った。使用したプライマーは以下の通りである。Forwardプライマー: 5'-AGGCCCTATGGTAGTCCCTT-3, Reverseプライマー: 5'-TCTCTTAGTGCTGTGGTC AC-3'。4 repeats allele (a allele) 存在下では393-bp, 5 repeats allele (b allele) 存在下では420-bpのバンドを形成する。

**eNOS Glu298Aspの同定;** 自動核酸抽出システム [MFX-2000 (TOYOBO Co., Ltd., Osaka)] を用い, プロトコルにしたがってDNA抽出を行なった。その後, Polymerase chain reaction with confronting two-pair primers (PCR-CTPP) 法<sup>2)</sup> によって遺伝子多型の同定を行なった。使用した4種類のプライマーは以下の通りである; プライマー1: 5'-CATGAGGCTCAGCCCCAGAAC-3', プライマ

ー2: 5'-AGTCAATCCCTTTGGTGCTCAC-3', プライマー2: 5'-GAAGGAAGAGTTCTGGGGGA-3', プライマー4: 5'-GCTGCAGGCCCCAGATGA-3'。プライマー1と3は298Aspアレルの存在下で141-bpのバンドを形成し, プライマー2と4は298Aspアレル存在下で103bpのバンドを形成する。

**ADRB2 Arg16Glyの同定;** QIAamp DNA Blood Mini Kit を用い, プロトコルにしたがってDNA抽出を行なった。その後ABI PRISM 7900HT-SDS v2.1 を用いてTaqMan PCR法による遺伝子多型の同定を行なった。使用したプライマーおよびプローブは以下の通りである; Forward プライマー: 5'-GAAGCCATGCGCCGGACCACGACGT-3', Reverse プライマー: 5'-CAGCGCCTTCTTGCTG GCACCCAAT-3'。

**ADRB3 Trp64Argの同定;** QIAamp DNA Blood Mini Kit を用い, プロトコルにしたがってDNA抽出を行なった。その後ABI PRISM 7900HT-SDS v2.1 を用いてTaqMan PCR法による遺伝子多型の同定を行なった。使用したプライマーおよびプローブは以下の通りである; Forward プライマー: 5'-GTCATGGTCTGGAGTCTGGGAGTCC-3', Reverseプライマー: 5'-GGCGATGGCCACGAT GACCAGCAGG-3'。

#### D 統計解析方法

統計解析には, 統計ソフトSPSS 17.0を使用した。効果ありとなし, リバウンド群と非リバウンド群の年齢, 収縮期血圧, 拡張期血圧などの連続変数 (定量的変数) の差の検定にはt検定を, 遺伝子多型, 性別, 喫煙者, 飲酒者の割合の検定には $\chi^2$ 検定を行った。介入効果の発現と遺伝子多型, リバウンドと遺伝子多型との関連性を見るために, ロジスティック回帰分析 (性, 年齢, 喫煙習慣, 運動習慣, 飲酒習慣で調整) を用いオッズ比を算出して解析した。運動習慣に関して, 改善効果の発現オッズ比を求める多変量ロジスティック回帰分析では介入期間中の平均歩数を1万歩未満と以上で調整し, リバウンド出現のオッズ比を求める多変量ロジスティック回帰分析では追跡期間中の平均歩数を1万歩未満と以上で調整している。遺伝子多型については3つのgenetic modelに



表1 介入による各指標の全体の変化

|                          | 介入開始時         |  | 介入終了時         |  | p値    |
|--------------------------|---------------|--|---------------|--|-------|
| 対象者数 (人)                 | 84            |  | 84            |  | —     |
| 性別 (M/F)                 | 75 / 9        |  | 75 / 9        |  | —     |
| 体重 (kg)                  | 70.4 ± 1.3    |  | 68.6 ± 1.3    |  | 0.000 |
| BMI (kg/m <sup>2</sup> ) | 24.9 ± 0.3    |  | 24.3 ± 0.3    |  | 0.000 |
| 腹囲 (cm)                  | 88.5 ± 1.0    |  | 85.9 ± 1.0    |  | 0.000 |
| 体脂肪率 (%)                 | 23.4 ± 0.5    |  | 21.9 ± 0.5    |  | NS    |
| 右上腕SBP (mmHg)            | 137 ± 1.5     |  | 134.8 ± 1.6   |  | 0.035 |
| 右上腕DBP (mmHg)            | 87.1 ± 1.2    |  | 85.2 ± 1.2    |  | NS    |
| baPWV (cm/s)             | 1515.6 ± 22.2 |  | 1498.7 ± 21.0 |  | 0.025 |
| 総コレステロール (mg/dL)         | 215.9 ± 3.6   |  | 207.6 ± 3.2   |  | 0.002 |
| LDLコレステロール (mg/dL)       | 131.2 ± 3.3   |  | 126.1 ± 2.9   |  | 0.041 |
| HDLコレステロール (mg/dL)       | 58.2 ± 1.7    |  | 60.8 ± 1.8    |  | 0.022 |
| 中性脂肪 (mg/dL)             | 163.3 ± 12.7  |  | 145.0 ± 10.2  |  | 0.000 |
| 空腹時血糖 (mg/dL)            | 104.2 ± 2.9   |  | 96.4 ± 1.6    |  | 0.000 |
| インスリン (μU/mL)            | 6.7 ± 0.6     |  | 5.0 ± 0.5     |  | 0.020 |
| HOMA-R                   | 1.8 ± 0.2     |  | 1.2 ± 0.1     |  | 0.013 |

BMI: Body Mass Index, SBP: systolic blood pressure, DBP: diastolic blood pressure, baPWV: brachial-ankle pulse wave velocity, LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-R: homeostasis model assessment for insulin resistance, NS: Not Significant

関して解析した。eNOS T786CにはTとCの2つのalleleがありC alleleが動脈硬化症と関連があると仮定した。TT, TC, CCそれぞれの遺伝型の影響を評価するための Codominant genetic model, TTとTC + CCを比較するための Dominant genetic model, TT + TCとCCを比較するための Recessive genetic modelの3つの genetic model が得られる。eNOS intron4 VNTR ではa alleleが動脈硬化症と関連があると仮定し、Codominant genetic model (b/b, b/a, a/a), Dominant genetic model (b/bとb/a + a/a), Recessive genetic model (b/b + b/aとa/a) が得られる。eNOS Glu298AspではAsp alleleが動脈硬化症と関連があると仮定し、Codominant genetic model (Glu/Glu, Glu/Asp, Asp/Asp), Dominant genetic model (Glu/GluとGlu/Asp + Asp/Asp), Recessive genetic model (Glu/Glu + Glu/AspとAsp/Asp) が得られる。ADRB2 Arg16GlyではGly alleleが動脈硬化症と関連があると仮定し、Codominant genetic model (Arg/Arg, Arg/Gly, Gly/Gly), Dominant genetic model (Arg/ArgとArg/Gly + Gly/Gly), Recessive genetic model (Arg/Arg + Arg/GlyとGly/Gly) が得られる。ADRB3 Trp64ArgではArg alleleが動脈硬

化症と関連があると仮定しCodominant genetic model (Trp/Trp, Trp/Arg, Arg/Arg), Dominant genetic model (Trp/TrpとTrp/Arg + Arg/Arg), Recessive genetic model (Trp/Trp + Trp/ArgとArg/Arg) が得られる。有意水準は0.05とし、p値はすべて両側検定の有意確率を表す。

## 結 果

介入による全体での各指標の変化に関して、体脂肪率と拡張期血圧を除くすべての項目で有意な改善効果が見られた (表1)。baPWV 5%減少の効果あり群と効果なし群のそれぞれの介入による前後比較の結果を表2, 3に示した。baPWV 5%減少の効果あり群ではTC, 中性脂肪, インスリンを除く全ての項目で有意な差が見られた。一方、効果なし群では体重, BMI, 腹囲, 体脂肪率, 中性脂肪, 空腹時血糖で有意な差が見られた。エンドポイントのbaPWVに関しては悪化するという結果になった。表4はbaPWV5%減少の効果あり群と効果なし群の遺伝子多型同定結果を示している。intron4 VNTRのDominant genetic modelとADRB2のRecessive genetic modelで有意差が見られた。

表2 baPWV5%減少効果が表れた対象者の介入による各指標の変化

|                          | 介入開始時         | 介入終了時         | p値    |
|--------------------------|---------------|---------------|-------|
| 対象者数 (人)                 | 27            | 27            | —     |
| 性別 (M/F)                 | 24 / 3        | 24 / 3        | —     |
| 体重 (kg)                  | 68.0 ± 2.1    | 66.1 ± 2.0    | 0.000 |
| BMI (kg/m <sup>2</sup> ) | 24.3 ± 0.6    | 23.7 ± 0.6    | 0.000 |
| 腹囲 (cm)                  | 85.9 ± 1.9    | 83.8 ± 1.8    | 0.002 |
| 体脂肪率 (%)                 | 23.0 ± 0.9    | 20.5 ± 0.8    | 0.000 |
| 右上腕SBP (mmHg)            | 141.1 ± 2.4   | 131.1 ± 2.1   | 0.000 |
| 右上腕DBP (mmHg)            | 88.8 ± 1.8    | 82.5 ± 1.8    | 0.000 |
| baPWV (cm/s)             | 1645.8 ± 39.8 | 1474.8 ± 30.7 | 0.000 |
| 総コレステロール (mg/dL)         | 210.0 ± 5.7   | 196.6 ± 5.8   | NS    |
| LDLコレステロール (mg/dL)       | 121.1 ± 5.0   | 114 ± 4.4     | 0.001 |
| HDLコレステロール (mg/dL)       | 61.0 ± 3.1    | 62.2 ± 2.9    | 0.022 |
| 中性脂肪 (mg/dL)             | 181.0 ± 29.3  | 136.3 ± 24.0  | NS    |
| 空腹時血糖 (mg/dL)            | 111.8 ± 8.0   | 97.7 ± 3.7    | 0.011 |
| インスリン (μU/mL)            | 5.7 ± 1.0     | 3.7 ± 0.4     | NS    |
| HOMA-R                   | 1.6 ± 0.3     | 0.9 ± 0.2     | 0.011 |

BMI: Body Mass Index, SBP: systolic blood pressure, DBP: diastolic blood pressure, baPWV: brachial-ankle pulse wave velocity, LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-R: homeostasis model assessment for insulin resistance, NS: Not Significant

表3 baPWV5%減少効果が表れなかった対象者の介入による各指標の変化

|                          | 介入開始時         | 介入終了時        | p値    |
|--------------------------|---------------|--------------|-------|
| 対象者数 (人)                 | 57            | 57           | —     |
| 性別 (M/F)                 | 51 / 6        | 51 / 6       | —     |
| 体重 (kg)                  | 71.5 ± 1.6    | 69.8 ± 1.6   | 0.000 |
| BMI (kg/m <sup>2</sup> ) | 25.2 ± 0.4    | 24.6 ± 0.4   | 0.000 |
| 腹囲 (cm)                  | 89.7 ± 1.1    | 86.9 ± 1.2   | 0.000 |
| 体脂肪率 (%)                 | 23.6 ± 0.6    | 22.6 ± 0.7   | 0.012 |
| 右上腕SBP (mmHg)            | 135.0 ± 1.9   | 136.5 ± 2.1  | NS    |
| 右上腕DBP (mmHg)            | 86.3 ± 1.6    | 86.5 ± 1.6   | NS    |
| baPWV (cm/s)             | 1453.9 ± 22.7 | 1510 ± 27.4  | 0.000 |
| 総コレステロール (mg/dL)         | 218.8 ± 4.6   | 212.9 ± 3.7  | NS    |
| LDLコレステロール (mg/dL)       | 136.0 ± 4.1   | 132 ± 3.5    | NS    |
| HDLコレステロール (mg/dL)       | 56.8 ± 2.1    | 60.1 ± 2.3   | NS    |
| 中性脂肪 (mg/dL)             | 154.8 ± 12.6  | 149.3 ± 10.0 | 0.033 |
| 空腹時血糖 (mg/dL)            | 100.5 ± 1.9   | 95.8 ± 1.7   | 0.011 |
| インスリン (μU/mL)            | 7.3 ± 0.8     | 5.7 ± 0.7    | NS    |
| HOMA-R                   | 1.9 ± 0.2     | 1.4 ± 0.2    | NS    |

BMI: Body Mass Index, SBP: systolic blood pressure, DBP: diastolic blood pressure, baPWV: brachial-ankle pulse wave velocity, LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-R: homeostasis model assessment for insulin resistance, NS: Not Significant

表4 効果あり群と効果なし群の遺伝子多型同定結果

| 遺伝子多型          | Codominant genetic model |           |           | Dominant genetic model |           |            | Recessive genetic model |           |             |
|----------------|--------------------------|-----------|-----------|------------------------|-----------|------------|-------------------------|-----------|-------------|
|                | 遺伝子型                     | 効果あり群     | 効果なし群     | 遺伝子型                   | 効果あり群     | 効果なし群      | 遺伝子型                    | 効果あり群     | 効果なし群       |
| eNOS T786C     | TT                       | 21(77.8%) | 36(64.3%) | TT                     | 21(77.8%) | 36(64.3%)  | TT+TC                   | 25(92.6%) | 54(96.4%)   |
|                | TC                       | 4(14.8%)  | 18(32.1%) | TC+CC                  | 6(22.2%)  | 20(35.7%)  |                         |           |             |
|                | CC                       | 2(7.4%)   | 2(3.6%)   |                        |           |            | CC                      | 2(7.4%)   | 2(3.6%)     |
| eNOS intron4   | b/b                      | 23(85.2%) | 35(62.5%) | b/b                    | 23(85.2%) | 35(62.5%)  | b/b+b/a                 | 27(100%)  | 53(96.4%)   |
|                | b/a                      | 4(14.8%)  | 18(32.1%) | b/a+a/a                | 4(14.8%)  | 21(37.5%)* |                         |           |             |
|                | a/a                      | 0         | 3(5.4%)   |                        |           |            | a/a                     | 0         | 3(5.4%)     |
| eNOS Glu298Asp | Glu/Glu                  | 22(81.5%) | 48(85.7%) | Glu/Glu                | 22(81.5%) | 48(85.7%)  | Glu/Glu+Glu/Asp         | 27(100%)  | 56(100%)    |
|                | Glu/Asp                  | 5(18.5%)  | 8(14.3%)  | Glu/Asp+Asp/Asp        | 5(18.5%)  | 8(14.3%)   |                         |           |             |
|                | Asp/Asp                  | 0         | 0         |                        |           |            | Asp/Asp                 | 0         | 0           |
| ADRB2 Arg16Gly | Arg/Arg                  | 4(14.8%)  | 8(14.3%)  | Arg/Arg                | 4(14.8%)  | 8(14.3%)   | Arg/Arg+Arg/Gly         | 22(81.5%) | 33(58.9%)   |
|                | Arg/Gly                  | 18(66.7%) | 25(44.6%) | Arg/Gly+Gly/Gly        | 23(85.2%) | 48(85.7%)  |                         |           |             |
|                | Gly/Gly                  | 5(18.5%)  | 23(41.1%) |                        |           |            | Gly/Gly                 | 5(18.5%)  | 23(41.1%)** |
| ADRB3 Trp64Arg | Trp/Trp                  | 15(55.6%) | 37(66.1%) | Trp/Trp                | 15(55.6%) | 37(66.1%)  | Trp/Trp+Trp/Arg         | 26(96.3%) | 54(96.4%)   |
|                | Trp/Arg                  | 11(40.7%) | 17(30.4%) | Trp/Arg+Arg/Arg        | 12(44.4%) | 19(33.9%)  |                         |           |             |
|                | Arg/Arg                  | 1(3.7%)   | 2(3.6%)   |                        |           |            | Arg/Arg                 | 1(3.7%)   | 2(3.6%)     |

eNOS: Endothelial NO synthase, Glu: Glutamate, Asp: Aspartic acid, ADRB2: Adrenergic receptor beta-2, ADRB3: Adrenergic receptor beta-3, Trp: Tryptophan

\*  $\chi^2$ 検定:  $p$ 値 = 0.043

\*\*  $\chi^2$ 検定:  $p$ 値 = 0.050

表5 性・年齢・生活習慣\*で調整した遺伝子多型と介入効果の多変量ロジスティック回帰分析結果

| 遺伝子多型          | Codominant genetic model |       |              |       | Dominant genetic model |       |             |       | Recessive genetic model |       |              |       |
|----------------|--------------------------|-------|--------------|-------|------------------------|-------|-------------|-------|-------------------------|-------|--------------|-------|
|                | 遺伝子型                     | オッズ比  | 95%信頼区間      | $p$ 値 | 遺伝子型                   | オッズ比  | 95%信頼区間     | $p$ 値 | 遺伝子型                    | オッズ比  | 95%信頼区間      | $p$ 値 |
| eNOS T786C     | TT                       | 1.000 |              |       | TT                     | 1.000 |             |       | TT+TC                   | 1.000 |              |       |
|                | TC                       | 0.280 | 0.075-1.050  | 0.059 | TC+CC                  | 0.385 | 0.120-1.234 | 0.108 |                         |       |              |       |
|                | CC                       | 1.460 | 0.154-13.864 | 0.742 |                        |       |             |       | CC                      | 2.102 | 0.232-19.024 | 0.509 |
| eNOS intron4   | b/b                      | 1.000 |              |       | b/b                    | 1.000 |             |       | b/b+b/a                 | 1.000 |              |       |
|                | b/a                      | 0.235 | 0.063-0.882  | 0.032 | b/a+a/a                | 0.201 | 0.054-0.744 | 0.016 |                         |       |              |       |
|                | a/a                      | 0.000 | 0.000-       | 0.999 |                        |       |             |       | a/a                     | 0.000 | 0.000-       | 0.999 |
| eNOS Glu298Asp | Glu/Glu                  | 1.000 |              |       | Glu/Glu                | 1.000 |             |       | Glu/Glu+Glu/Asp         | 1.000 |              |       |
|                | Glu/Asp                  | 1.208 | 0.319-4.569  | 0.781 | Glu/Asp+Asp/Asp        | 1.208 | 0.319-4.569 | 0.781 |                         |       |              |       |
|                | Asp/Asp                  |       |              |       |                        |       |             |       | Asp/Asp                 |       |              |       |
| ADRB2 Arg16Gly | Arg/Arg                  | 1.000 |              |       | Arg/Arg                | 1.000 |             |       | Arg/Arg+Arg/Gly         | 1.000 |              |       |
|                | Arg/Gly                  | 1.444 | 0.336-6.216  | 0.621 | Arg/Gly+Gly/Gly        | 0.867 | 0.215-3.487 | 0.840 |                         |       |              |       |
|                | Gly/Gly                  | 0.361 | 0.070-1.862  | 0.223 |                        |       |             |       | Gly/Gly                 | 0.271 | 0.083-0.888  | 0.031 |
| ADRB3 Trp64Arg | Trp/Trp                  | 1.000 |              |       | Trp/Trp                | 1.000 |             |       | Trp/Trp+Trp/Arg         | 1.000 |              |       |
|                | Trp/Arg                  | 1.904 | 0.661-5.481  | 0.233 | Trp/Arg+Arg/Arg        | 1.796 | 0.653-4.941 | 0.257 |                         |       |              |       |
|                | Arg/Arg                  | 1.149 | 0.086-15.352 | 0.916 |                        |       |             |       | Arg/Arg                 | 0.969 | 0.074-12.668 | 0.981 |

eNOS: Endothelial NO synthase, Glu: glutamate, Asp: aspartic acid, ADRB2: Adrenergic receptor beta-2, Arg: Arginine, Gly: Glycine, ADRB3: Adrenergic receptor beta-3, Trp: Tryptophan. \* 喫煙, 飲酒, 運動習慣

表6 リバウンド群と非リバウンド群における介入開始時の特性

|                          | リバウンド群        | 非リバウンド群       | p値 |
|--------------------------|---------------|---------------|----|
| 対象者数 (人)                 | 9             | 18            | —  |
| 性別 (M/F)                 | 7 / 2         | 17 / 1        | —  |
| 年齢                       | 48.6 ± 2.2    | 48.3 ± 1.6    | NS |
| 体重 (kg)                  | 69.9 ± 4.5    | 67.0 ± 2.4    | NS |
| BMI (kg/m <sup>2</sup> ) | 25.0 ± 1.3    | 24.0 ± 0.6    | NS |
| 腹囲 (cm)                  | 87.1 ± 4.0    | 85.3 ± 2.1    | NS |
| 体脂肪率 (%)                 | 22.8 ± 1.2    | 23.1 ± 1.2    | NS |
| 右上腕SBP (mmHg)            | 139.0 ± 3.6   | 142.1 ± 3.1   | NS |
| 右上腕DBP (mmHg)            | 85.0 ± 2.8    | 90.8 ± 2.2    | NS |
| baPWV (cm/s)             | 1613.1 ± 53.9 | 1662.2 ± 53.9 | NS |
| 総コレステロール (mg/dL)         | 213.2 ± 11.5  | 208.5 ± 6.5   | NS |
| LDLコレステロール (mg/dL)       | 127.4 ± 9.2   | 117.9 ± 6.4   | NS |
| HDLコレステロール (mg/dL)       | 59.1 ± 5.5    | 62.0 ± 3.9    | NS |
| 中性脂肪 (mg/dL)             | 155.8 ± 34.6  | 193.5 ± 40.8  | NS |
| 空腹時血糖 (mg/dL)            | 112.2 ± 15.6  | 111.6 ± 9.4   | NS |
| インスリン (μU/mL)            | 4.4 ± 0.7     | 6.3 ± 1.5     | NS |
| HOMA-R                   | 1.2 ± 0.2     | 1.9 ± 0.5     | NS |

BMI: Body Mass Index, SBP: systolic blood pressure, DBP: diastolic blood pressure, baPWV: brachial-ankle pulse wave velocity, LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-R: homeostasis model resistance assessment for insulin, NS: Not Significant

表7 リバウンド群と非リバウンド群の遺伝子多型同定結果

| 遺伝子多型          | Codominant genetic model |          | Dominant genetic model |                 |          | Recessive genetic model |                 |          |           |
|----------------|--------------------------|----------|------------------------|-----------------|----------|-------------------------|-----------------|----------|-----------|
|                | 遺伝子型                     | リバウンド群   | 非リバウンド群                | 遺伝子型            | リバウンド群   | 非リバウンド群                 | 遺伝子型            | リバウンド群   | 非リバウンド群   |
| eNOS T786C     | TT                       | 6(66.7%) | 15(83.3%)              | TT              | 6(66.7%) | 15(83.3%)               | TT+TC           | 8(88.9%) | 17(94.4%) |
|                | TC                       | 2(22.2%) | 2(11.1%)               | TC+CC           | 3(33.3%) | 3(16.7%)                |                 |          |           |
|                | CC                       | 1(11.1%) | 1(5.6%)                |                 |          |                         | CC              | 1(11.1%) | 1(5.6%)   |
| eNOS intron4   | b/b                      | 6(66.7%) | 17(94.4%)              | b/b             | 6(66.7%) | 17(94.4%)               | b/b+b/a         | 9(100%)  | 18(100%)  |
|                | b/a                      | 3(33.3%) | 1(5.6%)                | b/a+a/a         | 3(33.3%) | 1(5.6%)                 |                 |          |           |
|                | a/a                      | 0        | 0                      |                 |          |                         | a/a             | 0        | 0         |
| eNOS Glu298Asp | Glu/Glu                  | 7(77.8%) | 15(83.3%)              | Glu/Glu         | 7(77.8%) | 15(83.3%)               | Glu/Glu+Glu/Asp | 9(100%)  | 18(100%)  |
|                | Glu/Asp                  | 2(22.2%) | 3(16.7%)               | Glu/Asp+Asp/Asp | 2(22.2%) | 3(16.7%)                |                 |          |           |
|                | Asp/Asp                  | 0        | 0                      |                 |          |                         | Asp/Asp         | 0        | 0         |
| ADRB2 Arg16Gly | Arg/Arg                  | 2(22.2%) | 2(11.1%)               | Arg/Arg         | 2(22.2%) | 2(11.1%)                | Arg/Arg+Arg/Gly | 6(66.7%) | 16(88.9%) |
|                | Arg/Gly                  | 4(44.4%) | 14(77.8%)              | Arg/Gly+Gly/Gly | 7(77.8%) | 16(88.9%)               |                 |          |           |
|                | Gly/Gly                  | 3(33.3%) | 2(11.1%)               |                 |          |                         | Gly/Gly         | 3(33.3%) | 2(11.1%)  |
| ADRB3 Trp64Arg | Trp/Trp                  | 6(66.7%) | 9(50.0%)               | Trp/Trp         | 6(66.7%) | 9(50.0%)                | Trp/Trp+Trp/Arg | 9(100%)  | 17(94.4%) |
|                | Trp/Arg                  | 3(33.3%) | 8(44.4%)               | Trp/Arg+Arg/Arg | 3(33.3%) | 9(50.0%)                |                 |          |           |
|                | Arg/Arg                  | 0        | 1(5.6%)                |                 |          |                         | Arg/Arg         | 0        | 1(5.6%)   |

eNOS: Endothelial NO synthase, Glu: Glutamate, Asp: Aspartic acid, ADRB2: Adrenergic receptor beta-2, ADRB3: Adrenergic receptor beta-3, Trp: Tryptophan