Association between serotonin transporter gene polymorphisms and depressed mood caused by job stress in Japanese workers

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Abstract. To estimate the genetic factors influencing depressed mood caused by job stress, a total of 243 employees at a manufacturing company and a local hospital in Japan (mean age 40.8±10.3 years) were recruited with informed consent. The Brief Job Stress Questionnaire was used to assess the present status of stress. Alcohol consumption and smoking were assessed as lifestyle factors. DNA samples were prepared to detect gene polymorphisms of serotonin transporter (5HTT), aldehyde dehydrogenase 2, D2 dopamine receptor, and cytochrome p450 2A6. The relationship between job stress, lifestyle factors and these polymorphisms was assessed for each gender. The level of depressed mood for female subjects was significantly higher among the carriers of two short (s/s) alleles of the 5HTT regulatory region compared with the carriers of one (s/l) or two (l/l) long alleles (Mann-Whitney U test, $p<0.05$). The odds ratio of depressed mood also confirmed this relationship for the female subjects, whereas there was no relationship for the male subjects. When social support was taken into consideration, the depressed mood score for those who had high support was significantly lower than for those who had low support, irrespective of 5HTT polymorphisms and gender. Job stress may elicit biological responses that contribute to depressed mood in relation to 5HTT polymorphisms, and social support may reduce depressed mood irrespective of 5HTT polymorphisms.

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Introduction

Genetic factors have been implicated in many lifestyle-related diseases such as cancer, cardiovascular disease, and osteoporosis (1-3). Genetic factors such as gene polymorphisms have also been linked with major depressive disorder, alcoholism, and nicotine dependence (4-7). Certain serotonin transporter (5HTT) gene polymorphisms have been associated with several dimensions of neurosis and psychopathology, especially anxiety traits (8). The acquisition of personal habits such as smoking and drinking has also been associated with gene polymorphisms; specifically, D2 dopamine receptor (DRD2) and cytochrome p450 2A6 (CYP2A6) polymorphisms have been linked to smoking (6,7) and aldehyde dehydrogenase 2 (ALDH2) polymorphisms to drinking (9).

On the other hand, the number of workers who suffer from job stress is increasing in Japan because of a prolonged recession, the increasing number of elderly employees, and the structural reorganization of companies (10). Increasing job stress may cause stress-related diseases and disorders such as coronary heart disease, hypertension, depression, insomnia, and substance abuse (11-14).

In order to avoid an increase in the incidence of stress-related diseases, it is necessary to measure the present level of job stress. Many questionnaires have been developed to assess job stress, e.g. the Job Content Questionnaire (JCQ) (15), WHO MONICA Psychosocial Optional Study Questionnaire (16), and the Brief Job Stress Questionnaire (17). The Brief Job Stress Questionnaire is particularly useful: consisting of only 57 questions it is easy to administer in the workplace, and being based on the JCQ and the MONICA questionnaire, it is reliable and valid. However, stress-related diseases are also lifestyle-related diseases that might result from complex interactions between genetic and environmental factors. Thus, in addition to stress estimation, the assessment of personal habits such as smoking and drinking is required as well. Job stress itself may also be involved in the development of detrimental lifestyle factors.

In the present study, the relationship between lifestyle and job stress estimated using the Brief Job Stress Questionnaire was analyzed referring to polymorphisms of the 5HTT, ALDH2, DRD2, and CYP2A6 genes.

Subjects and methods

Subjects. A total of 304 staff members at a local manufacturing company and a local hospital in the western part of Japan were recruited with informed consent. Since 61 of the employees declined to participate in the study, a total of 243 subjects was selected, of which 138 were male. We obtained written informed consent from all subjects. This study was approved by the Medical Ethics Committee of Kawasaki Medical School and Kawasaki Medical School Hospital (no. 52).

Lifestyle factors and measurement of job stress. Drinking and smoking were assessed as lifestyle factors. Alcohol consumption was evaluated by a self-assessment questionnaire and was expressed as grams of ethanol consumed per week. Smoking was assessed using the Brinkman index, which is determined as the number of cigarettes per day multiplied by the number of years since starting to smoke (18).

Job stress was calculated using the Brief Job Stress Questionnaire (17), which consisted of 57 items. Using this questionnaire, stressors such as work overload and personal relations, psychosomatic responses to stress, and social support can be assessed as well as depressed mood. While many parameters can be assessed, depressed mood was used as the stress reaction in the present study.

Measurement of polymorphisms. Blood samples were obtained at an annual health examination, and genomic DNA was extracted from leukocytes. To identify polymorphisms of the serotonin transporter (5HTT), aldehyde dehydrogenase 2 (ALDH2), D2 dopamine receptor (DRD2), and cytochrome P450 2A6 (CYP2A6) genes, their polymorphic loci were amplified using polymerase chain reaction (PCR).

The promoter activity of 5HTT is modified by sequence elements within the proximal 5' regulatory region. A 20-23 base pair repeat motif within this region occurs as 2 prevalent alleles, one consisting of 14 repeats (the short allele 's') and another of 16 repeats (the long allele 'l'). Fig. 1A shows the 5HTT polymorphism types. This polymorphic region has functional significance: 'l/l' homozygote lymphoblast cells produce 1.4-1.7 times the concentration of 5HTT mRNA than 's/l' and 's/s' cells, uptake of labeled serotonin in 'l/l' homozygote cells is two times greater than in 's/l' or 's/s' cells, and the protein produced from 'l/l' cells binds 30-40% more serotonin than that of cells with the short variant (19). The primers used for 5HTT were: forward, ATGCCAGCAC CTAACCCCTAATGT and reverse, GGACCGCAAGGTG GGCGGGA (19).

Individual drinking behavior is regulated not only by the social and economic environment but also by ethanol-metabolizing capacity. ALDH2 with a single-point mutation in exon 12 and atypical homozygotes (*2/*2) or heterozygotes (*2/*1) showed marked increase in blood acetaldehyde level compared with typical homozygotes (*1/*1) after alcohol consumption (5). After amplification using a set of primers (forward, CAAATTACAGGGTCAACTGCT and reverse, CCACACTCACAGTTTTCTCTT), the products were digested with MboII: *2 was digested but not *1. Fig. 1B shows the ALDH2 polymorphism types.

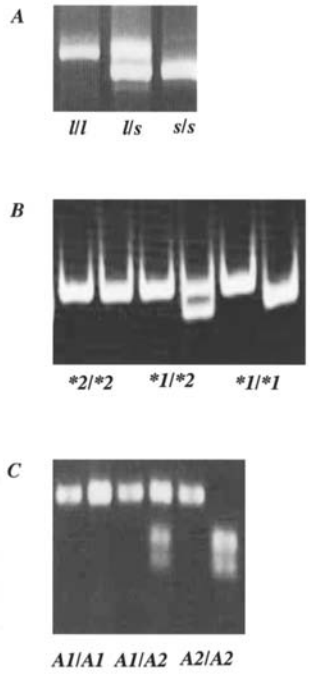

Figure 1. Representative types of polymorphisms in the 5HTT (A), ALDH2 (B) and DRD2 (C) genes.

Dopaminergic neurotransmission in the mesolimbic system is a recognized and critical target of many drugs of abuse, including alcohol, cocaine, and nicotine (20,21). Smokers carrying the A1 allele (Taq1 indigestible) in the 3' flanking region of DRD2 receptor gene quit less frequently than those with the homozygous A2 allele (Taq1 digestible) (22). Primers used for DRD2 were: forward, CCGTCGACG GCTGGCCAAGTTGTCTA and reverse, CCGTCGACCCT TCCTGAGTGTCATCA for DRD2 (23). Fig. 1C shows the DRD2 polymorphism types.

Variable CYP2A6 polymorphisms are classified into three groups by activity: normal inactivator (n), without copies of CYP2A6*2, CYP2A6*4, CYP2A6*6, and CYP2A6*12; intermediate inactivator (i), with heterozygosity for CYP2A6*9 or CYP2A6*12 and ~75% of wild-type n activity; and slow inactivator (s), with one or more copies of CYP2A6*2 or CYP2A6*4, or homozygosity for CYP2A6*9 or CYP2A6*12, and with ≤50% of n activity. All CYP2A6 polymorphisms were determined by sequence analysis according to the method of Schoedel *et al* (24).

Statistical analyses. The Mann-Whitney U test and the Kruskal-Wallis test were performed as non-parametric tests to compare differences in depressed mood, alcohol consumption, and the Brinkman index, for each polymorphism. Factor analysis was performed in order to explore the relationship among job stress, polymorphisms, and lifestyle factors. Logistic regression analysis was also performed to determine the risk of increment of depressed mood between each polymorphism and lifestyle factors. Odds ratios as estimated risk with 95% confidence intervals

Table I. Characteristics of subjects.

	Total (n=243)	Male (n=138)	Female (n=105)
Mean age	40.8±10.3	41.2±9.8	40.2±10.9
Drinkers			
Number	182	109	73
Rate	74.9	79.0	69.5
Average consumption (g/week)	124.9±179.4	220.8±205.4	84.1±126.0[a]
Smokers			
Number	112	93	19[a]
Rate	46.1	67.4	18.1
Brinkman index	462.2±285.6	503.8±282.5	258.4±205.6[a]
Depressed mood score	111.1±48.5	114.7±54.7	106.4±38.6
Stressors	25.7±3.4	26.2±3.1	24.9± 3.7
Psychosomatic response to stress	18.4±3.7	18.9±3.6	17.8±3.6
Social support	11.6±2.9	12.3±3.0	10.7±2.7
Distribution of 5HTT polymorphisms			
s/s	152	88 (63.8)	64 (61.0)
s/l	77	45 (32.6)	32 (30.5)
l/l	14	5 (3.6)	9 (8.6)
s frequency		0.80	0.76
l frequency		0.20	0.24
Distribution of ALDH2 polymorphisms			
*1/*1	138	84 (60.9)	54 (51.4)
*1/*2	94	48 (34.8)	46 (43.8)
*2/*2	11	6 (4.3)	5 (8.6)
*1 frequency		0.78	0.73
*2 frequency		0.22	0.27
Distribution of DRD2 polymorphisms			
A1/A1	36	18 (13.0)	18 (17.1)
A1/A2	100	58 (42.0)	42 (40.0)
A2/A2	107	62 (44.9)	45 (42.9)
A1 frequency		0.34	0.37
A2 frequency		0.66	0.63
Distribution of CYP2A6 polymorphisms			
Normal inactivator (n)	36	22 (15.9)	14 (13.3)
Intermediate inactivator (i)	80	43 (31.2)	37 (35.2)
Slow inactivator (s)	126	73 (52.6)	53 (50.5)
n frequency		0.32	0.31
s frequency		0.68	0.69

Scores for depressed mood, stressors, psychosomatic response to stress and social support were calculated with the Brief Job Stress Questionnaire. [a]Statistical differences were observed between males and females, $p<0.001$. All allele frequencies were calculated according to the Hardy-Weinberg law.

were calculated by logistic regression using Stat View (5.0, SAS Institute Inc., Cary, NC, USA).

Results

Lifestyle factors and polymorphisms of subjects. The mean age of the male subjects was almost the same as that of the female subjects (Table I). Although the ratio of drinkers was not significantly different between males and females, the average alcohol consumption of male drinkers was more than twice that of female drinkers. The average consumption of the male drinkers was also higher than the Japanese average, which is ~200 g/week (25). The ratio of smokers was lower in females than in males, as expected, but the ratios of both were higher than the Japanese averages (43% for males and 12% for females) (26). The mean Brinkman index for male smokers was approximately twice that of female smokers. Since lifestyle factors related to drinking and smoking were

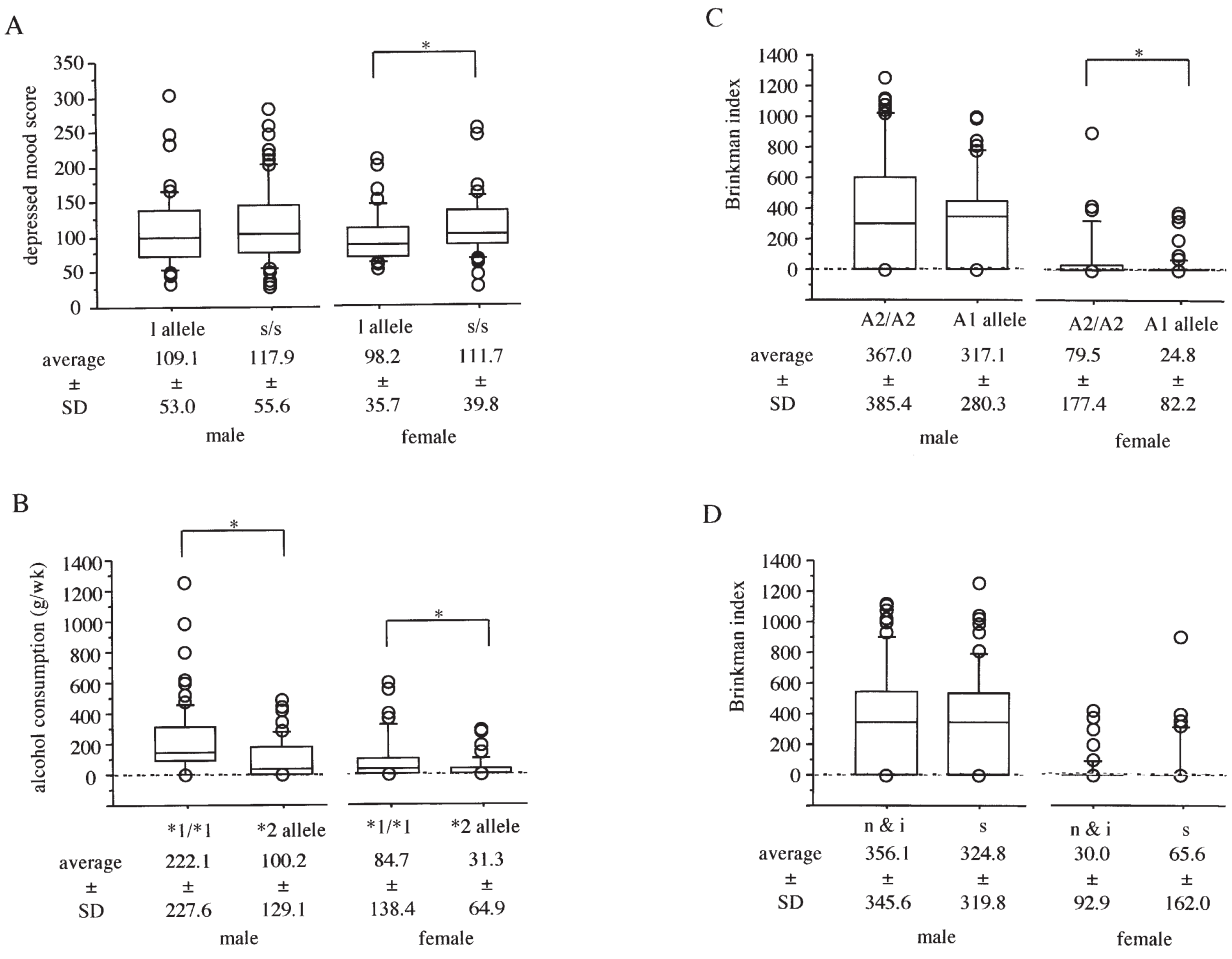

Figure 2. Relationship between lifestyle factors and each polymorphism for each gender (the Mann-Whitney U test was performed). (A) Relationship between depressed mood score and 5HTT polymorphisms. Depressed mood score in females carrying the s/s allele was significantly higher than in those with the l allele. (B) Relationship between alcohol consumption and ALDH2 polymorphisms. Carriers of the ALDH2 *2 allele showed significantly lower amounts of alcohol in both genders. (C and D) Relationships between the Brinkman index and DRD2 polymorphisms (C), and CYP2A6 polymorphisms (D). The DRD2 A1 allele group showed significantly lower Brinkman index than the A2/A2 group in females. *$p<0.05$./

Table II. Association between lifestyle factors and polymorphisms using factor analysis.

	Male			Female		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Age	0.710	0.384		0.384		0.431
Gender						
Alcohol consumption (g/week)	0.572	-0.486	-0.334	0.750		
Brinkman index	0.777			0.738		
Depressed mood			0.616	0.472		-0.365
5HTT polymorphisms		-0.428	0.489	0.349	-0.668	
ALDH2 polymorphisms		0.670	0.408	-0.354		-0.494
DRD2 polymorphisms	-0.351					0.673
CYP2A6 polymorphisms		-0.450	0.501		0.729	
Eigenvalue	1.639	1.299	1.175	1.829	1.185	1.152
Contribution ratio	0.205	0.162	0.147	0.229	0.148	0.144
			$p<0.001$			$p<0.01$

Absolute factor loadings >0.3. Three factors were selected for totals and for each gender. Each polymorphism was represented as follows: 5HTT: l allele, 0; s/s, 1. ALDH2: *1/*1, 0; *2 allele, 1. DRD2: A2/A2, 0; A1 allele, 1. CYP2A6: normal and intermediate inactivators, 0; slow inactivator, 1.

Table III. Risk determinants in the increase of depressed mood estimated by logistic regression analysis.

	Male				Female			
	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Age	1.01	0.97-1.06	1.01	0.96-1.06	0.97	0.93-1.02	1.00	0.94-1.06
Gender								
5HTT polymorphism								
s/s based on l allele	1.25	0.59-2.63	1.33	0.58-3.06	3.36[b]	1.15-9.76	2.26	0.58-8.82
ALDH2 polymorphism								
*2 allele based on *1/*1	0.63	0.30-1.36	0.61	0.26-1.41	0.76	0.29-1.99	0.43	0.12-1.50
DRD2 polymorphism								
A1 allele based on A2/A2	1.06	0.52-2.16	1.53	0.68-3.44	1.27	0.48-3.36	2.14	0.58-7.88
CYP2A6 polymorphism[a]	1.41	0.69-2.88	1.15	0.51-2.59	1.73	0.66-4.53	1.66	0.49-5.65
Alcohol consumption (g/week)	0.999	0.997-1.001	0.999	0.996-1.001	1.000	0.995-1.004	1.000	0.995-1.004
Brinkman index	1.000	0.99-1.002	1.000	0.99-1.002	1.005[b]	1.000-1.009	1.003	0.998-1.009
Social support			0.07[c]	0.02-0.21			0.03[c]	0.01-0.13

Odds ratios indicating that depressed mood was higher than the mean score were calculated according to potential risk factors. [a]CYP2A6 polymorphism: slow inactivator based on normal and intermediate inactivators. [b]$p<0.05$, [c]$p<0.0001$.

different between males and females, further analysis was performed on the basis of gender. There were no gender differences with regard to stressors, psychosomatic response to stress and social support estimated by the Brief Job Stress Questionnaire (17). No statistical difference was observed in depressed mood, while the depressed mood score in males was slightly higher than that in females, both of which were higher than those of the national mean (of 100) in Japan (17).

There was no gender difference in the allele frequencies of the gene polymorphisms, i.e. all the polymorphisms examined were in agreement with the Hardy-Weinberg law in both genders. In the 5HTT polymorphisms, the allele frequencies were s, 0.80 and l, 0.20 in males, and s, 0.76 and l, 0.24 in females. Since the number of l/l homozygotes was very small, the 5HTT group was subdivided into s/s and l allele groups. Similarly, in the ALDH2 polymorphisms, allele frequencies were *1, 0.78 and *2, 0.22 in males, and *1, 0.73 and *2, 0.27 in females. Consequently, the ALDH2 group was subdivided into *1/*1 and *2 allele groups. The DRD2 polymorphisms included allele frequencies A1, 0.34 and A2, 0.66, and A1, 0.37 and A2, 0.63, for males and females, respectively. The DRD2 polymorphisms were therefore analyzed for A1 and A2/A2 allele groups. The observed distributions of these genotypes were in good agreement with previous reports (5,6,23,27). The ratios of n, i, and s CYP2A6 polymorphisms were 0.32 and 0.68 in both genders. Since low cigarette consumption has been reported in carriers of the s polymorphism (24), the effect of s was assessed against n and i.

Univariate comparison between lifestyle factors and polymorphisms. The differences of depressed mood score and lifestyle factors in each polymorphism group were examined. Significantly higher depressed mood score was observed only in females carrying the s/s allele of 5HTT rather than the l/l and l/s alleles (Fig. 2A). The ALDH2, DRD2, and CYP2A6 groups had no significant differences in depressed mood score regardless of gender (data not shown). Alcohol consumption of the ALDH2 *1/*1 allele group was higher than that of the ALDH2 *2 allele group irrespective of gender (Fig. 2B), because ALDH2*2 lacked activity for acetaldehyde metabolism. However, the alcohol consumption of the male ALDH2 *2 allele group was almost the same as that of the female ALDH2 *1/*1 allele group. No statistically significant differences were observed between drinking habits and 5HTT, DRD2, or CYP2A6 polymorphisms irrespective of gender (data not shown). Although DRD2 and CYP2A6 polymorphisms have been associated with smoking (7), these polymorphisms did not affect males (Fig. 2C and D). In females, however, the DRD2 A1 allele group showed a significantly lower Brinkman index than the A2/A2 group. Multivariate analyses such as factor analysis and logistic regression analysis were further applied in order to remove possible confounding factors and to clarify the risks of depressed mood score.

Multivariate analysis to determine the risk of depressed mood score. Age, gender, alcohol consumption, the Brinkman index, depressed mood score, and polymorphisms of the 5HTT, ALDH2, DRD2, and CYP2A6 genes were selected as a determinant for factor analysis (Table II). For both genders, this analysis allowed us to select three factors with statistically significant differences. The contribution ratio of each factor was almost the same between males and females, but the determinants constituting each factor differed. In males, factor 1 was defined as the lifestyle factor because age, alcohol consumption and the Brinkman index showed strong correlations, whereas DRD2 polymorphism was also included. Factor 2 was defined as the alcohol factor because age, alcohol consumption, and 5HTT, ALDH2 and CYP2A6 polymorphisms were correlated. Factor 3 was defined as the

stress factor because depressed mood was strongly correlated with 5HTT polymorphisms. In addition, ALDH2 polymorphisms and alcohol consumption showed positive and negative correlations, respectively. The 5HTT s/s allele was associated with a high depressed mood score. In females, factor 1 was defined as the stress factor because of a positive correlation among age, alcohol consumption, Brinkman index, depressed mood, and 5HTT polymorphisms, and a negative correlation of ALDH2 polymorphisms. Since depressed mood score and 5HTT polymorphisms appeared as factor 3 in males, their contribution ratio in female was higher than in males.

As the average score for depressed mood was ~110 in all subjects, a logistic regression analysis was performed to determine possible risk factors which increased depressed mood score to >110. Logistic regression analysis indicated that, only in females, did the s/s polymorphism of 5HTT increase the risk of a higher depressed mood score compared with the l allele, of which the odds ratio was 3.36 (Table III). The odds ratio was low but a high Brinkman index was also recognized as a risk in females. Polymorphisms for ALDH2, DRD2, and CYP2A6 were not risks for the increase in depressed mood score for either gender. When social support was included as a determinant, it was found to reduce depressed mood score in both genders. Moreover, in females, this protective effect was independent of 5HTT polymorphisms.

Discussion

Job stress is one of the major risk factors for depression. The Brief Job Stress Questionnaire used in the present study characterizes many aspects of stress. Firstly, not only the stress reactions of workers but also stressors in the workplace can be estimated. Secondly, not only negative but also positive psychological reactions can be estimated. Thirdly, it uses multi-axis methods to assess somatic reactions and modifying factors as well as these psychological reactions (17). This questionnaire clarifies the three major factors of job stress, i.e. stressors, psychosomatic responses to stress, and social support. Stressors include age, gender, quantitative, qualitative and somatic workload, job control, interpersonal relations, work environment and job fitness. Psychosomatic responses to job stress consist of lack of vigor, irritability, fatigue, anxiety, depressed mood, and somatic symptoms. Social support consists of supervisor, coworker and family support. Excessive stressors can cause work-related diseases, and social support can ameliorate these conditions. This questionnaire does not consider factors of family life or personality. In the present study, we focused on depressed mood among psychosomatic responses, because depressed mood is considered to be the highest correlation coefficient to work-related stressful conditions using covariance structure analysis (17). The mean level of depressed mood of our subjects was higher than that of a previous study in Japan (17), which may indicate that the subjects in the present study work under high job stress conditions.

Univariate analysis showed the relationships between depressed mood score and 5HTT polymorphisms. The short (s) allele in the 5HTT gene is associated with lower serotonin re-uptake compared with the long (l) allele. This polymorphism is associated with many diseases or dependencies such as anxiety (27), suicide (28), smoking (29) and alcohol dependence (30), and attention deficit hyperactivity disorder (ADHD) (31). Distribution of the l/l homozygote in Japanese is ~4% (27), which is lower than in Caucasians (~30%) (19). Moreover, distribution of the s/s homozygote in Japanese is ~60%, which is higher than in Caucasians (~20%). The distribution of 5HTT polymorphisms was almost the same as this previously reported distribution (27). An epidemiological study indicated that individuals with the s/s homozygote of 5HTT exhibit depressive symptoms, diagnosable depression, or suicidality associated with very stressful life events including employment problems (long-term unemployment, employer bankruptcy, lay-off, termination), financial problems (debt, inadequate funds for living expenses), housing problems (homelessness, multiple residence changes), health problems (disabling physical illness lasting a month or more, disabling injury), or relationship problems (death of a family member, being in a physically violent relationship, break-up of a cohabitation relationship) (19). According to the Mann-Whitney U test, a high-depressed mood score in carriers of the s/s allele of 5HTT was only found in female subjects; this was also confirmed by factor and logistic regression analyses. These results indicate that the 5HTT s/s allele in female subjects was recognized to be a potential risk factor for depressed mood caused by job stress, which was commonly observed in the workplace. This might indicate that females were more sensitive to stress.

Although the 5HTT s/s allele was a risk factor for depressed mood, social support reduced this risk. A previous study (32) showed that individuals who had the 5HTT s/s allele had greater depressive symptomatology if they had experienced early or recent adversity but significantly less depressive symptomatology if they reported a supportive early environment or recent positive experiences, compared with individuals with the s/l or l/l genotype. In the present study, a depressed mood score was reduced by social support irrespective of 5HTT polymorphisms and gender. This result indicated that the 5HTT genotype did not necessarily predetermine the incidence of depressed mood caused by job stress. Although gene-by-environment interaction might affect the incidence of depressed mood, environmental factors such as adequate support might be more important to prevent the incidence of depressed mood.

Alcohol consumption by subjects with the ALDH2 *2 allele was significantly lower than in those with the ALDH2 *1/*1 allele, since the ALDH2 polymorphism is associated with drinking behavior (5). Individuals with the ALDH2 *2/*2 homozygous allele are not able to metabolize alcohol, and individuals with the ALDH2 *1/*2 heterozygous allele experience facial flushing in response to alcohol (33). While ~65% of subjects had the ALDH2 *1/*1 homozygous allele, a distribution that is typical of Japanese, most of them were able to drink alcohol. Logistic regression analysis showed no relationship between depressed mood and alcohol consumption. On the other hand, male subjects consumed more alcohol than females. Even when carrying the ALDH2 *2 allele, their alcohol consumption was comparable to that of the female ALDH2 *1/*1 allele group. It is likely that the

relaxation effect of alcohol might conceal the effect of the 5HTT s/s allele in the case of the male subjects. Since dopamine D2 receptor and CYP2A6 polymorphisms were associated with smoking status (6,22), non-parametric tests showed that the Brinkman index of the female DRD2 A1 allele group was lower than that of the A2/A2 group. Moreover, logistic regression analysis showed the Brinkman index corresponded with the risk of increasing depressed mood, especially in female subjects. Although Cinciripini *et al* (22) demonstrated that smokers carrying the A1 allele showed no reduction in negative mood during antidepressant therapy, our results indicated no relationship between depressed mood and DRD2 polymorphisms. Further investigation is needed in order to clarify the effects of the A1 allele.

Diagnosable depression is associated not only with environmental factors but also genetic factors. Depressed mood shown in the present study did not present as a disease, but as a response to job stress. Although the present study involved only 241 subjects, an association of depressed mood with a polymorphism of 5HTT was observed. Since Japanese have higher rates of the s/s allele compared to Caucasians, the reduction of job stress by social support might be effective in reducing depressed mood. Further research is needed to corroborate a relationship between mental disorders and environmental and genetic factors.

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