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Preliminary communication

Val1483Ile polymorphism in the fatty acid synthase gene was associated with depressive symptoms under the influence of psychological stress

Hirohito Tsuboi [a,*], Hiroyuki Sakakibara [b], Kimiko Yamakawa-Kobayashi [c], Asami Tatsumi [d], Tomoko Inamori [c], Reiko Hamamoto [e], Atsuko Suzuki [e], Kayoko Shimoi [b]

[a] Department of Drug Management and Policy, Institute of Medical Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan
[b] Institute for Environmental Sciences, University of Shizuoka, Shizuoka, Japan
[c] School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan
[d] Department of Community Health Nursing, Hamamatsu University School of Medicine, Hamamatsu, Japan
[e] Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka, Japan

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ABSTRACT

Background: To study the association between lipid-metabolism and depressive symptoms, genetic polymorphisms in serotonin transporter linked promoter region (*5-HTTLPR*) and fatty acid synthase gene (*FASN*) were investigated.
Method: A cross-sectional study was conducted on 177 women (n = 166) and men (n = 15) recruited from workers in a hospital and nursing homes in Japan. Depressive symptoms were assessed by the Center for Epidemiologic Studies Depression (CES-D) scale and perceived psychological stress was measured using visual analogue scale (VAS). The genotypes of *5-HTTLPR* (insertion/deletion; L/S), and *FASN* (Val1483Ile) were determined by the PCR methods. Linear regression analysis was performed, in which CES-D scores served as a dependent variable, and VAS scores, gene polymorphism, and confounders as independent variables.
Results: Under the influence of perceived stress, S/S carriers of the *5-HTTLPR* gene showed significantly higher CES-D scores in comparison with L/L + L/S carriers ($F = 8.2$, standardised beta = 0.15, $p < 0.05$). Regression analysis also confirmed that CES-D scores in participants with Val/Val + Val/Ile genotypes of the *FASN* gene were significantly higher than those with Ile/Ile genotype ($F = 8.4$, standardised beta = 0.16, $p < 0.05$). In relation to physical features, BMI among participants with S/S genotype of *5-HTTLPR* was significantly lower compared with those with L/L + L/S genotypes.
Conclusions: The Val1483Ile polymorphism in the *FASN* was associated with depressive symptoms under the influence of psychological stress. The S variant of *5-HTTLPR* was related with less obese.

1. Introduction

Literature suggested the association between depression and lipid metabolism: ex. lowered serum cholesterol levels were observed in depressive patients, particularly in those with suicidal behaviour (Fawcett et al., 1997), whereas the comorbidity between depression and hyperlipemia was also reported (Akbaraly et al., 2009). We are attempting to clarify this association from the point of view of different genotypes. Genetic variation in the 5′ flanking transcriptional region of serotonin transporter gene (*5-HTTLPR*), which originates long (L) and short (S) alleles, plays a role in predisposition to major depression in interaction with stressful life events (Caspi et al., 2003). The serotonergic system was hypothesised to regulate behavioural and metabolic responses associated with the development of obesity through feeding and satiety (Barsh and Schwartz, 2002). Fatty acid (FA) metabolism may also

* Corresponding author at: Department of Drug Management and Policy, Institute of Medical Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa-shi 920-1192, Japan. Tel./fax: +81 76 234 4403.
E-mail address: yayevgeny@p.kanazawa-u.ac.jp (H. Tsuboi).

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explain one of the mechanisms to link the psychological and somatic disorders. FA synthase (FAS), which is encoded by the FAS gene (*FASN*), is the central enzyme in de novo lipogenesis, catalysing the conversion of malonyl CoA into palmitate (Semenkovich, 1997). The Val1483Ile polymorphism in the *FASN* is linked to central obesity and insulin sensitivity, and putatively affects FAS action (Moreno-Navarrete et al., 2009). Although the evidence of the relationships between lipid-metabolism and affection remains controversial, it is relevant to reason on the biological pathway. The aim of this study was to investigate the common effects of the *5-HTTLPR* and the *FASN* genes.

2. Participants and methods

A cross-sectional study was carried out on 177 women (n = 166) and men (n = 15) with a mean age of 42.4 (SD ~~13.17~~), recruited from workers in a hospital and two nursing homes located in Shizuoka Prefecture, Japan. The Ethics Committee of University of Shizuoka approved the study protocol, and all participants gave informed consent to participate in this study.

Self-administrated questionnaire was distributed to the participants beforehand and answered one day before the examination day (working days), The questionnaire contains the demographic measures (age, gender, medication, etc.), lifestyle characteristics (smoking status: non-smoker or former~~/current~~), current alcohol consumption: ~~0~~ vs ≥once per week, and leisure-time physical activity: <once per month~~/~~≥once per week, and psychological measures. Perceived stress was given using visual analog~~ue~~ scales (10 cm) anchored with "not at all" and "quite strong"~~; depressive~~ symptoms were assessed with the ~~Center~~ for Epidemiologic Studies Depression Scale (CES-D) (Matthews et al., 1985; Shima et al., 1985).

Fasting blood was sampled between 0830 and 1030 h from the forearm vein of each participant with a heparinized and serum-separator vacutainer tubes from which sera were obtained by centrifugation. The sera samples were delivered to a laboratory (FALCO Inc., Hamamatsu), and the heparinized blood tubes were shipped to University of Shizuoka. Serum triglycerides (TG) and total cholesterol (TC) were measured enzymatically. High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined by the precipitation method using heparin-calcium. To assess insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) was used: fasting serum insulin (μU/ml) × glucose (mg/dl) / 405 (Matthews et al., 1985). The homeostasis model assessment of ~~beta-cell~~ function (HOMA-ß) was calculated as fasting serum insulin (μU/ml) × 360 / (glucose (mg/dl) − 63) (Matthews et al., 1985). Leukocytes were isolated from the heparinized blood by density centrifugation by the method of English and Andersen (1974), as described by Albrechtsen et al. (1988). Genomic DNA was then extracted from the leukocytes using the phenol-chloroform extraction method (Sambrook et al., ~~2006~~). The genotype of *5-HTTLPR* (insertion/deletion; L/S) was determined by amplified the fragments including the polymorphisms by PCR. The *FASN* genotype (Val1483Ile, rs2228305) was determined by PCR-restriction fragment length polymorphism analysis.

Data were analysed by the Japanese versions of SPSS (ver. 12.0.1) for Windows OS. For comparison of differences of each genotype, analysis of covariance was utilised. Multiple regression analysis was conducted to evaluate depressive symptoms under the influence of perceived psychological stress and covariates. A probability *p* value less than 0.05 was considered significant.

3. Results

Prior to data analysis, one person taking steroid contained medicine, one participant ingesting Graves disease remedy,

Table 1
Characteristics of participants.

	Female		Male	
	n	Mean (SD)	*n*	Mean (SD)
Age	162	43.2 (~~13.00~~)	15	33.8 (~~12.95~~)
BMI (kg/m^2)	162	22.4 (~~4.00~~)	15	23.8 (4.95)
Subjective stress (%)	162	57.9 (~~25.36~~)	15	62.4 (~~29.97~~)
~~Satisfacton of life (%)~~	~~162~~	~~57.1 (23.21)~~	~~15~~	~~53.0 (26.21)~~
~~Subjective sleep quality (%)~~	~~162~~	~~65.8 (28.86)~~	~~15~~	~~73.6 (23.55)~~
CES-D scores	162	14.9 (6.95)	15	16.6 (9.32)
Smoking status				
No or ex-smoker	120		6	
Present smoker	42		9	
Current alcohol consumption				
(almost) No	140		9	
≥Once per week	21		6	
Exercise				
<Once per month	102		6	
≥Once per week	60		9	
5-HTTLPR gene polymorphisms				
L/L	8 (4.5%)		1 (6.7%)	
L/S	53 (29.9%)		5 (33.3%)	
S/S	114 (64.4%)		9 (60.0%)	
FASN gene polymorphisms				
Val/Val	159 (89.8%)		12 (80.0%)	
Val/Ile	14 (7.9%)		3 (20.0%)	
Ile/Ile	3 (1.7%)		0 (~~0%~~)	

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Table 2
Differences of psychological factors and indices related with metabolic syndrome between overall genotypes.

	5-HTTLPR		*FASN*	
Genotype	S/S	L/L + L/S	Val/Val	Val/Ile + Ile/Ile
n	114	61	159	17
BMI (kg/m^2)	22.0 (0.36)	23.6 (0.56)*	22.5 (0.32)	22.8 (1.20)
Subjective stress (%)	58.2 (2.42)	58.9 (3.14)	57.7 (1.98)	61.7 (7.57)
CES-D scores	15.8 (0.71)	13.5 (0.81)**	14.6 (0.52)	19.1 (2.60)*
TG (mg/dl)	80.8 (4.36)	99.8 (6.15)**	87.0 (3.62)	89.5 (15.99)
TC (mg/dl)	211.2 (3.67)	221.1 (5.82)	215.7 (3.36)	205.8 (7.84)
HDL-C (mg/dl)	74.0 (1.67)	70.7 (2.38)	73.2 (1.44)	70.0 (4.19)
LDL-C (mg/dl)	120.4 (2.99)	130.1 (4.83)	124.5 (2.79)	117.7 (5.59)
Blood glucose (mg/dl)	85.7 (1.17)	91.3 (3.75)	87.6 (1.65)	87.8 (2.56)
HbA1c (%)	5.1 (0.04)	5.4 (0.14)	5.2 (0.06)	5.0 (0.09)
Insulin (mg/ml)	6.03 (0.352)	7.35 (0.952)	6.55 (0.439)	5.71 (0.762)
HOMA-IR	1.30 (0.088)	1.70 (0.253)	1.45 (0.115)	1.28 (0.196)
HOMA-ß	115.5 (8.73)	93.6 (18.50)	109.4 (9.40)	89.1 (10.75)

Results are expressed as mean (SE). Comparisons are controlling for age, gender, (BMI), smoking habit, alcohol consumption, and physical activities. BMI: body mass index. Subjective stress: perceived stress assessed using visual analogue scales anchored with "not at all" (0%) and "quite strong" (100%). 5-HTTLPR: Serotonin transporter gene linked polymorphism (L: long. S: short). FASN: Val(G)1483Ile(A) polymorphism in the fatty acid synthase gene. TG: Triglyceride. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. HOMA-IR: homeostasis model assessment of insulin resistance. HOMA-ß: homeostasis model assessment of beta-cell function. Note that the subject number included in this analysis is slightly lower, owing 0074o the lack of information for a few subjects.

* $p<0.05$.

** $p<0.1$.

and one who recently had a surgical operation were excluded; consequently, 162 female and 15 male participants were analysed. The characteristics of the sample are summarised in Table 1, together with the number of participants with data for each variable.

Table 2 exhibits the differences of variables concerning metabolic syndrome and psychological factors after controlling for gender, age and lifestyle factors. Since there were only nine subjects with L/L genotype of *5-HTTLPR*, the L/L and L/S genotypes were pooled and compared with the S/S genotype in further analysis. In the same manner, *FASN* polymorphism was divided into Val/Val and Val/Ile + Ile/Ile groups, and the dichotomized data were utilised for further analysis. As shown in Table 2, the subjects with S/S genotype of *5-HTTLPR* exhibited significantly lower BMI in comparison with the L/L + L/S genotypes ($p<0.05$). In *FASN*, participants with Val/Ile + Ile/Ile genotypes presented significantly higher CES-D scores ($p<0.05$) in comparison with those with Val/Val genotypes.

Depressive symptoms under the influence of psychological stress were analysed by the linear regression analysis: the CES-D scores as dependent variable, and subjective stress measured by VAS, gene polymorphism and covariates as independent variables (Table 3); consequently, the S/S genotype of *5-HTTLPR* gene significantly contributed to depressive symptoms in comparison with S/S + L/L genotypes under the influence of psychological stress (Model 1: Standardised $\beta=0.15$, $p<0.05$). Participants with the Val/Ile + Ile/Ile genotype in the *FASN* revealed a significantly higher depressive symptoms in comparison with those with Val/Val under the psychological stress (Model 2: Standardised $\beta=0.16$, $p<0.05$). Even though the *5-HTTLPR* and *FASN* genotypes were put into the analysis model together, each of them showed significance (Model 3: Standardised $\beta=0.15$, $p<0.05$; Standardised $\beta=0.15$, $p<0.05$); i.e., the *5-HTTLPR* and the *FASN* were independently related with depressive symptoms.

4. Discussion

It was found that participants with Val/Ile + Ile/Ile genotypes in the *FASN* showed higher depressive symptoms in comparison with those with Val/Val genotype under the influence of subjective psychological stress, and that participants with *5-*

Table 3
Multivariate linear regression analyses showing the association of depressive symptoms with subjective stress and genotype ($n=174$).

Gene polymorphism	*5-HTTLPR* (S/S: L/S + L/L)	*FASN* (Val/Val: Ile/Ile + Val/Ile)	*F* value and adjusted Δ^2 value of each regression model
	Standardised ß	Standardised ß	
Model 1	0.15*		$F=8.2$ (8, 169) $\Delta r^2=0.25$
Model 2		0.16*	$F=8.4$ (8, 167) $\Delta r^2=0.25$
Model 3 (Model 1 + Model 2)	0.15*	0.15*	$F=8.0$ (9, 165) $\Delta r^2=0.27$

Each model was adjusted by gender, age, BMI, leisure time physical activities, smoking habit and alcohol consumption. All models of overall genotypes are significant ($p<0.0005$).

* $p<0.05$.

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HTTLPR S/S genotype exhibited higher depressive symptoms compared with those with L/S + L/L genotypes. In addition, in S/S participants, BMI was lower and serum TG levels appeared to be lower in comparison with L/S + L/L participants.

5-HTTLPR S variant has been thought to be associated with susceptibility for depression (Canli and Lesch, 2007) since a longitudinal study revealed the vulnerability of S variant to stressful life events (Caspi et al., 2003). The present study supports the result. In addition, our results ~~indicated~~ that *5-HTTLPR* S variant ~~could~~ work to reduce obesity risks, though it is unsolved whether the *5-HTTLPR* directly affect obesity-related index, or there were confounders between them. Discrepant results concerning the *5-HTTLPR* variants and obesity were obtained in previous studies. Sookoian et al. (2008) showed that S/S carriers had higher body weights in comparison with L/L carriers in obese (BMI $\geq$ 27 kg/m^2) ~~group~~ of healthy male population. Lan et al. (2009) reported that S/S genotype was a determinant of increased BMI level in non-elderly stroke patients. On the other hand, Bah et al. (2010) presented that S allele tended to be more frequent in underweight persons among normal population. Discrepant results concerning *5-HTTLPR* and ~~BMI~~ in previous studies might depend on participants' characteristics ~~such as healthy, having metabolic syndrome risks, etc~~. Since participants analysed in the current study were healthy volunteers, studies on normal population can support our results. Thus, it seems meaningless to discuss the relationships L allele and binge eating (Monteleone et al., 2006), or to think of the associations between S allele and anorexia nervosa (Hoffman et al., 2007) in relation to our results.

FASN gene encodes FAS, which is an enzyme in *de novo* lipogenesis (Semenkovich, 1997). Moreno-Navarrete, et al. (2009) recently showed that the adipose tissue FAS activity was significantly higher in subjects with the Val variant in comparison with carriers of the Ile variant. In addition, Val allele in the *FASN* is linked with impaired glucose tolerance, visceral obesity etc. (Kovacs et al., 2004; Moreno-Navarrete et al., 2009). However, no differences were found between Val and Ile alleles in lipid and glucose-metabolism indices in this study. An interesting finding in the present study was that the *FASN* was related with depressive symptoms in the same degrees as the *5-HTTLPR* under the influence of perceived stress, suggesting that Val1483Ile polymorphism in the *FASN* gene can affect the susceptibility to depression, and that the Ile variant may contribute to vulnerability to psychological stress. The pathway of the *FASN* effects on depressive symptoms is thought to differ from ~~the one~~ of *5-HTTLPR* because each genotype was independently related with depressive symptoms as shown in model 3 of Table 3.

5. Limitations and conclusion

There are limitations in the ~~current~~ study. Serotonin levels in the central nervous system were unknown. The number of male participants was small, which unavoidably put the gender factor as an independent variable of linear statistical models. Sample size could not be enough large to compare *FASN* alleles. Our results may be considered as preliminary, and further research is needed to confirm these findings. However, we presented possible relationships between depressive symptoms and *FASN* gene, and between BMI and *5-HTTLPR*.

~~6. Uncited reference~~

~~Sambrook et al., 1989~~

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Conflicts of interest

None of the authors have any conflicts of interest.

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