

## Eating disorders with binge-eating behaviour are associated with the s allele of the 3'-UTR VNTR polymorphism of the dopamine transporter gene

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**Objective:** The dopaminergic system is associated with feelings of pleasure and reward and with positive hedonic processes related to food, sexual activity and certain substances. Because it is recognized that patients who have eating disorders with binge-eating behaviour have a high comorbidity of substance dependence, we examined the association between the variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region of the dopamine transporter gene (*DAT1*) and eating disorders with binge-eating behaviour. **Methods:** The subjects were 90 female Japanese patients with eating disorders diagnosed using DSM-IV; they were compared with 115 healthy female controls. Genomic DNA was extracted from whole blood, and standard polymerase chain reaction testing was performed. We compared the frequencies of a short allele (7 or 9 repeats) and a long allele (10 or 11 repeats) in both groups. **Results:** In the group who had an eating disorder with binge-eating behaviour, the frequency of a short allele was significantly higher compared with the control group. **Conclusion:** It seems plausible that the association between the *DAT1* VNTR and binge-eating behaviour indicates that dysregulation of dopamine reuptake may act as a common pathophysiologic mechanism in eating disorders with binge-eating behaviour and in disorders related to substance use.

**Objectif :** Le système dopaminergique est associé à des sentiments de plaisir et de récompense ainsi qu'aux processus hédoniques positifs liés à l'alimentation, à l'activité sexuelle et à certaines substances. Comme il est reconnu que les patients atteints de troubles de l'alimentation avec frénésie alimentaire présentent une comorbidité élevée de dépendance à une substance, nous avons examiné l'association entre le polymorphisme de répétitions variables (VNTR) dans la région non traduite de 3' du gène transporteur de la dopamine (*DAT1*) et un trouble de l'alimentation accompagné de frénésie alimentaire. **Méthodes :** Le groupe-sujet se composait de 90 patientes japonaises ayant reçu un diagnostic de trouble de l'alimentation selon le DSM-IV; on les a comparées à 115 témoins en santé, également de sexe féminin. On a extrait l'ADN génomique du sang total puis effectué un test standard de réaction par polymérisation en chaîne. On a comparé les fréquences d'un allèle court (7 ou 9 répétitions) et d'un allèle long (10 ou 11

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répétitions) dans les deux groupes. **Résultats :** Dans le groupe qui présentait un trouble de l'alimentation avec frénésie alimentaire, la fréquence d'un allèle court a été significativement plus élevée que dans le groupe-témoin. **Conclusion :** Il semble plausible que l'association entre le *DAT1* VNTR et la frénésie alimentaire indique qu'un dysfonctionnement du recaptage de la dopamine puisse être un mécanisme pathophysiologique courant des troubles de l'alimentation avec frénésie alimentaire ainsi que des troubles liés à une toxicomanie.

## Introduction

It is widely recognized that the cause of eating disorders is likely to involve a complex mix of multiple genes and multiple specific environmental risk factors.

Various pieces of evidence have supported the contribution of genetic factors to the origin of eating disorders. Devlin et al<sup>1</sup> performed linkage analysis of anorexia nervosa incorporating behavioural covariates. They found several regions of suggestive linkage in patients with anorexia nervosa who had a drive for thinness and obsessiveness. The same group<sup>2</sup> detected significant linkage on chromosome 10 in families of patients with bulimia nervosa.

There have been extensive association studies of the serotonin-2A receptor gene,<sup>3-6</sup> and analysis of the serotonin transporter-linked polymorphism in anorexia nervosa has been reported by several groups.<sup>7-9</sup> Recently, several association studies related to leptin, neuropeptide Y receptor, estrogen receptor, catechol-o-methyltransferase and norepinephrine transporter genes have been carried out.<sup>10-14</sup> Branson et al<sup>15</sup> indicated that binge-eating behaviour is a major phenotypic characteristic of subjects with a mutation in the melanocortin 4 receptor gene, which is a candidate gene for the control of eating behaviour. In the dopaminergic system, association studies with the D<sub>3</sub> and D<sub>4</sub> dopamine receptor genes that yielded negative associations have been reported.<sup>16,17</sup> However, the dopaminergic system, besides its well-known role in the regulation of appetite, is also considered important for such behaviour as reward and reinforcement.<sup>18</sup> In accordance with neurochemical studies, the high comorbidity between substance-related disorders and eating disorders with binge-eating behaviour is well established clinically.<sup>19-21</sup> In addition, several groups have reported that shorter alleles of the variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region of the dopamine transporter (*DAT1*) gene were associated with substance dependence.<sup>22-25</sup> These data suggest that binge-eating behaviour and substance dependence may

share a genetic causative mechanism associated with the dopaminergic system.

Based upon these data, we postulated that *DAT1* might play a key role in eating behaviour and the development of eating disorders. We investigated the association between the VNTR polymorphism in the 3' untranslated region of *DAT1* and eating disorders with binge-eating behaviour.

## Methods

The subjects were 90 female Japanese patients with eating disorders, aged 15–36 (mean 22.1, standard deviation [SD] 4.0) years. The diagnoses were made according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV)<sup>26</sup> by 1 psychiatrist (H.M.) who specialized in eating disorders and had run an eating-disorder clinic for more than 3 years. In addition, symptoms were assessed by the Eating Disorder Inventory.<sup>27</sup> The details of the diagnostic assessment have been described in a previous report.<sup>28</sup> The patients were classified as having anorexia nervosa binge-eating/purging type ( $n = 35$ ) and bulimia nervosa ( $n = 55$ ). A clinical trait common to both groups was binge-eating behaviour. These diagnoses had been stable over at least a 3-year follow-up period.

The healthy controls were 115 women aged 18–26 (mean 18.7, SD 1.1) years, whose current body weight was stable with fluctuations within 20% of the standard body weight (normal range of body mass index). Written informed consent of the patients and healthy controls was obtained after the purpose and methods of this study had been fully explained. The Ethics Committee of the University of Yamanashi approved this study.

Peripheral blood was drawn from the antecubital vein, and genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (QIAGEN Hilden, Germany).

Standard polymerase chain reaction (PCR) testing was performed in a total volume of 20  $\mu$ L containing

4 ng genomic DNA, 1.5 mmol/L MgCl<sub>2</sub>, 0.2 mmol/L of each deoxyribonucleotide, 0.5 μmol/L of each primer (sense, 5'-TGTGGTGTAGGGAACGGCCTGAG, anti-sense, 5'-CTTCCTGGAGGTCACGGCTCAAGG), 10% dimethyl sulfoxide and 2 U *Taq* DNA polymerase.<sup>29</sup> The conditions for 35 cycles consisted of denaturation at 95°C for 30 seconds, annealing at 65°C for 1 minute and extension at 72°C for 30 seconds, before a final extension step at 72°C for 4 minutes. Cycling amplification was performed by standard methods in a thermal cycler 9700 (PE Applied Biosystems, Norwalk, Conn.). The PCR products were separated on 2% agarose gel with ethidium bromide staining and visualized under ultraviolet illumination.

Identification of the bands was performed independently by 2 researchers who had not been informed of the clinical diagnosis of the patients, and their results were found to agree with each other.

The  $\chi^2$  test was used for statistical analysis to evaluate allele frequencies. The agreement of genotype frequencies with the Hardy–Weinberg equilibrium was also tested using  $\chi^2$ .

## Results

In the control group, alleles of 7 (360 base pairs [bp]), 9 (440 bp), 10 (480 bp) and 11 repeats (520 bp) were observed with frequencies of 0.9%, 3.9%, 93.9% and 1.3%, respectively. The frequency of the 10-repeat allele was overwhelmingly the highest. This frequency distribution is in accordance with that reported by Nakatome et al,<sup>30</sup> but different from data from a white population.<sup>31</sup>

Based on previous reports described earlier,<sup>22–25</sup> we compared the frequencies of a short allele (7 or 9 repeats) and a long allele (10 or 11 repeats) in both groups (Table 1). In the group with eating disorders and binge-eating behaviour, the frequency of a short allele was significantly higher as compared with the control group ( $\chi_1^2 = 4.96$ ,  $p = 0.026$ ; 2-tailed Fisher's exact test,  $p = 0.034$ ). Genotype distributions were in accordance with the Hardy–Weinberg equilibrium for patients ( $\chi_3^2 = 0.12$ ,  $p < 0.99$ ) and controls ( $\chi_3^2 = 0.05$ ,  $p > 0.99$ ).

## Discussion

In the present study, the short allele was observed at a significantly higher frequency in the patients with eating disorders and binge-eating behaviour, as compared with the control group.

Heinz et al<sup>32</sup> reported genotype influences on in-vivo dopamine transporter availability in the human striatum using [iodine 123]β-CIT single-photon emission computed tomography. Subjects who carried a genotype containing a short allele demonstrated significantly lower dopamine transporter binding in the striatum than carriers of a long allele. This finding is in accordance with the biochemical findings of Fuke et al.<sup>33</sup> They reported that a short allele had lower transcriptional activity. Furthermore, in clinical studies, associations between shorter alleles and substance dependence, such as alcohol and nicotine dependence, have been observed by several groups.<sup>22–25</sup>

The high comorbidity between eating disorders and substance-related disorders is well recognized clinically,<sup>19–21</sup> and especially that between eating disorder with binge-eating behaviour and alcohol-related disorder.<sup>34,35</sup> Dopaminergic transmission plays a key role in both appetite regulation and addiction. Because *DAT1* modulates dopaminergic transmission, it seems plausible that the association between the *DAT1* VNTR and binge-eating behaviour indicates that dysregulation of dopamine reuptake may act as a common pathophysiologic mechanism in both eating disorders with binge-eating behaviour and substance-related disorders.

A clinically important issue is the validity of diagnosis of subtypes of eating disorders, because this influences the treatment and prognosis of patients with these disorders. The *DAT1* VNTR polymorphism could be used as a biologic marker to identify patients with binge-eating behaviour among individuals with eating disorders.

Our results were obtained from a case–control association study with a relatively small sample. Although there is apparent ethnic similarity between the control and patient groups because the Japanese population is fairly homogeneous, we still cannot completely rule out genetic heterogeneity between the 2 groups. Thus, the results may not be completely free of the possibility

**Table 1: Allele frequencies for the dopamine transporter gene (*DAT1*) in patients with eating disorders and binge-eating behaviour and in controls**

Group	Type of allele; allele frequency*	
	Short	Long
Patients, $n = 90$	19 (0.106)	161 (0.894)
Controls, $n = 115$	11 (0.048)	219 (0.952)

\* $\chi_1^2 = 4.96$ ,  $p = 0.026$  (2-tailed Fisher's exact test  $p = 0.034$ ).

of stratification. Thus, this study needs to be extended to samples from different ethnic backgrounds. Confirmation of the results in independent samples or with family-based controls will be necessary to further clarify the subgroups of eating disorders.

**Competing interests:** None declared.

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