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Association study of 5-HT2A receptor gene polymorphism in anorexia nervosa in Polish population

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Summary

Aim*: Anorexia nervosa (AN) is a disorder of complex etiopathogenesis including the genetic factors. The previous studies on the role of –1438A/G promoter polymorphism in 5-HT2A receptor gene brought conflicting results, and it is possible that the analysed polymorphism increases the risk of AN only in some ethnic groups. The aim of the study was to assess the frequency of –1438 A/G polymorphism in Polish patients with AN and ethnically matched healthy controls.*

Method*: The genotyping of 5-HT2A receptor polymorphism was performed in 67 AN patients and 114 healthy controls. The frequencies of alleles and genotypes were compared with Chi^2 test.*

Results*: The deviations from the Hardy-Weinberg equilibrium were not observed in any group. The frequencies of A/A, A/G and G/G genotypes in AN group were respectively: 37.3%, 50.7% and 11.9%; and in the control group: 40.4%, 47.4% and 12.3% (chi^2=0.2; df=2; p=0.91). The prevalence of A and G alleles in the AN group was respectively 62.7% and 37.3%; and in the control group 64.0% and 36.0%; and did not show any statistically significant difference (chi^2=0.67; df=1; p=0.79).*

Conclusions*: These results suggest that –1438A/G polymorphism in the promoter region of the 5-HT2A receptor gene does not increase the risk of AN in the Polish population.*

Key words: 5-HT2A receptor, anorexia nervosa

Introduction

Anorexia nervosa (AN) is a disorder of complex aetiology, in which social, cultural, psychological and biological factors play an important role. Because of familiar clustering of eating disorders and higher morbidity among monozygotic than dizygotic twins, genetic background of AN is extensively studied [1]. In these studies two main strategies are used: linkage and association analysis. In former studies, searching of

the whole genome to find chromosomal markers typical for the studied disorder is used. In association studies frequencies of several genotypes (polymorphisms) are compared in patients and in healthy controls. Differences in frequency of genetic markers may indicate the role of specific gene variants or surrounding polymorphisms in aetiology of the studied disorder. In such a type of study, choice of the studied genetic polymorphism is arbitrary, however the probability of a true positive result increases with the choice of the so called candidate gene. Such a gene is usually associated with the postulated neurobiological aberration, playing an important role in the aetiology of the disorder.

In the course of AN, changes in several systems of neurotransmitters and neuromodulators are present, but most of them are probably caused by long lasting food deprivation. Abnormalities in serotonergic systems (5-HT) like an increased level of main serotonin metabolite 5-hydroxyindole acetic acid in cerebrospinal fluid [2] or abnormal function of serotonergic receptors in the central nervous system revealed in neuroimaging studies [3] persist after normal body weight and hormonal function resumption. 5-HT system role in aetiology of AN is also supported by reports of high efficiency of selective serotonin reuptake inhibitors in the maintenance of treatment of this disease [4].

Many authors stress an important role of personality dimensions in eating disorder morbidity. Patients with AN are typically characterised by emotional rigidity, harm avoidance and obsessive and perfectionist behaviour. These personality dimensions may be also connected with the serotonergic system impairment [5, 6].

Serotonin exerts its' biologic action through seven types of receptors (5-HT1-5-HT7) which with the exception of the 5-HT3 receptor are all coupled with G protein. The 5-HT2A receptor is present in various regions of central nervous system like brain cortex, basal nuclei and hypothalamus. This receptor is also active in regulating smooth muscles activity in the digestive tract, vascular and urinary system. It also regulates the action of platelets [7]. In the animal model, hypothalamic 5-HT2A receptors modulate appetite-stimulating action of neuropeptide Y [8]. 5-HT2A receptor polymorphism may be also associated with serotonin modulated personality dimensions [9]. On the basis of these data, the 5-HT2A receptor gene located on chromosome 13q14q21 is an interesting candidate gene in eating disorders studies because of the role of the protein coded by this gene in appetite regulation and postulated impact on several personality dimensions.

First reports on the association of polymorphism (1438 A/G) in the promoter region of the 5-HT2A gene were published in 1997 [10]. Since then, this preliminary report was confirmed several times, but few other studies did not confirm the initial observation. Moreover, meta-analysis and a multicenter study, which used the family based approach (based on the triad: 2 parents and patient) did not confirm the reported association (results of previous studies are presented in Table 1).

Table 1

Previous studies of (-1438 A/G) promoter region polymorphism in 5-HT2A receptor gene in patients with AN

Authors	Type of the study	Patient number/ control number	Population studied	p value
Collier et al. 1997 [10]	CCS	81/226	British	p=0.02
Hinney et al. 1997 [13]	CCS; FS	100/355; 57 trios	German	NS
Campbell et al. 1998 [14]	CCS	152/150	British	NS
Sorbi et al. 1998 [16]	CCS	77/107	Italian	p=0.005
Enoch et al. 1998 [15]	CCS	68/169	American	p=0.0001
Ziegler et al. 1999 [17]	CCS; MA	78/170	German	NS
Nacmias et al. 1999 [18]	CCS	109/107	Italian	p=0.0001
Ando et al. 2001 [19]	CCS	75/127	Japanese	NS
Nishiguchi et al. 2001 [20]	CCS	62/374	Japanese	NS
Gorwood et al. 2002 [21]	FS	316 trios	European (Western Europe)	NS

Abbreviations: CCS – case-control studies, FS – family studies (parents + patient), MA – meta-analysis

The aim of this study was assessment of –1438 A/G region of 5-HT2A gene polymorphism in Polish patients with AN. The choice of this polymorphism was based on the results of previous studies.

Material and method

Subjects

We studied 67 non-related women of Polish origin diagnosed with AN. The patients were diagnosed according to DSM-IV and ICD-10 criteria during hospitalisation in the Department of Child and Adolescent Psychiatry University of Medical Sciences of Poznań, Poland. In the study group, 10 patients fulfilled the criteria of bulimic type of AN, whereas 57 patients had a restrictive type of AN. The mean age of women from the study group was 18.7 years (Standard Deviation, SD+/-3.6 years), range 13-25 years. The control group was made up of 114 healthy women of the same ethnic origin (mean age 35.8 years; SD+/-12.3; range 22-40 years), who were recruited from blood donors and students. Age difference between the study group and controls is allowed in such a study because it decreases the risk of inclusion of the potential cases to the control group). 10 ml of venous blood was taken from all women. All of them signed the informed consent for the study. The study was approved by the Ethical Committee of our university.

Genotyping of the promoter region of the 5-HT2A receptor gene

DNA was isolated from the peripheral blood leucocytes with the salting out procedure [11]. Promoter region of 5-HT2A gene polymorphism was studied with the use of a method reported by Collier et al [10]. This method consists of using appropriate starters and polymerase chain reaction (PCR) in relation to an analysed DNA fragment. The reaction product was then digested by the Hpa II restrictive enzyme. The difference in length of the digested DNA fragments (effect of studied polymorphism presence) causes various velocities of DNA fragments in electrophoresis on 2% agar gel. Analysis of polymorphism was performed without the knowledge of the diagnostic status of the studied women.

Statistical analysis

Frequency of alleles and genotypes was calculated on the base of genotype analysis results assessment. Because of the small number of patients, the study group was not divided into the bulimic and restrictive subgroups. In the study and control groups, no deviation from the Hardy-Weinberg equilibrium was found. Allele and genotype frequencies were compared with the use of Chi^2 test, and p value less than 0.05 was considered statistically significant. Statistical analysis was performed with the SPSS package [12].

Results

In the study and control groups we did not observe any deviation from the Hardy-Weinberg rule. We did not obtain any difference in alleles distribution between the study group and controls; the frequency of allele G (guanine) polymorphism –1438 (A/G) in the 5-HT2A genotype was 36% among AN patients, and 37.3% in the control group. Allele A (adenine) was present respectively in 64% and 62.7% women (Chi^2=0.06; df=1; p=0.79). We did not observe differences in the frequency of genotypes between the study and control groups. In the AN group frequency of A/A genotype was 37%; A/G – 50.7% and G/G – 11.9%. In the control group frequencies of genotypes were respectively: 40.4%; 47.4% and 12.3% (Chi^2=0,20; df=2; p=0,91), results were presented in Table 2.

Table 2

Frequencies of alleles and genotypes of (-1438 A/G) promoter region polymorphism in 5-HT2A receptor gene in patients with AN and in the control group

Group	No	Number of women and frequency of genotypes		
		G/G	G/A	A/A
Anorexia nervosa	67	8 (11.9%)	34 (50.7%)	25 37.3%)
Controls	114	14 (12.3%)	54 (47.4%)	46 (40.4%)
p value		p=0.91		

Discussion

In this study we did not find any association between alleles and genotypes in the promoter region of the 5-HT2A receptor gene and AN. These results suggest a low probability of the studied polymorphism role in the aetiology of AN in Polish population.

First reports of the potential role of studied genetic variant were published in 1997 [10]. Collier et al. reported that allele A was present in 51% of AN patients and in 42% of controls. They calculated a relative risk value of AN in allele A carriers as 1.5. Two later studies did not confirm the previous results [11, 12], in the former, apart from the case-control approach, analysis of 57 triads consisting of parents and patients was used. Two subsequently performed studies revealed that a variant of the receptor 5-HT2A gene may play an important role in the aetiology of restrictive type of AN and may be connected with such personality dimensions like obsessive and perfectionist behaviour [13, 14]. In a meta-analysis performed by Ziegler et al [15] the role of the discussed gene in the aetiology of AN was not confirmed, although one year earlier an Italian group [16] reported results similar to those of Collier et al. Two studies performed in a Japanese population did not confirm the relationship between the studied polymorphism [-1438 A/G) and AN risk [17, 18] although in one of them an association between G allele and the risk of bulimia was reported. Discrepant opinions on the role of 5-HT2A gene in the aetiology of AN prompted the performance of a multi-centre study on a large group of patients and the use of a family-based model (patients plus parents). This was intended to eliminate a possibility of false positive results caused by ethnic stratification of the studied population [19]. Results of this study suggest that receptor 5-HT2A gene is not an important factor in the aetiology of AN. Still there was a possibility that the 5-HT2A receptor gene may play an important role only in several ethnic groups. Results of our study do not indicate that it is an important factor in the Polish population.

Several factors limit the value of our study. The small number of studied women is similar to numbers studied in previously presented studies. Enlargement of the study group would not change our results because the frequencies of genotypes and alleles in both groups are almost identical and Chi^2 p values were very distant from values considered statistically significant. The study group was not divided into the bulimic and restrictive subgroups because of a small number of patients. Such division would decrease the numbers of patients in the studied groups to respectively 57 and 10 women. Previous reports suggested the role of 5-HT2A in aetiology of restrictive type of AN [13]. Our study group consisted mostly of patients with such a type of AN and such a relationship was not observed. We should also take into the consideration that in the case of some of patients (particularly the young and with short history of disease) after a few years of observation a change in the type of disorder from restrictive to bulimic is also possible and this would alter the proportion of both types of AN in the study group.

Conclusions

Despite its limitations, the results of our study indicate that polymorphism in the

promoter region of the 5-HT2A gene (-1438 A/G) is not an important factor in the aetiology of AN in patients from the Polish population. It does not mean that the 5-HT2A receptor is not important in the aetiology of AN, but that the analyzed polymorphism of this receptor gene does not increase risk of AN.

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References

1. Rybakowski F, Slopien A, Czerski P, Rajewski A, Hauser J. *Genetic factors in the etiology of anorexia nervosa*. Psychiatr. Pol. 2001; 35: 71–80.
2. Kaye WH, Gwirtsman HE, George DT, Ebert MH. Altered serotonin activity in anorexia nervosa after long-term weight restoration. *Does elevated cerebrospinal fluid 5-hydroxyindoleacetic acid level correlate with rigid and obsessive behavior*? Arch. Gen. Psychiatry 1991; 48: 556–562.
3. Frank GK, Kaye WH, Meltzer CC, Price JC, Greer PJ, McConaha CW, Skovira K. *Reduced 5-HT2A receptor binding after recovery from anorexia nervosa.* Biol. Psychiatry 2002; 52: 896–906.
4. Kaye WH, Nagata T, Weltzin TE, Hsu LK, Sokol MS, McConaha C, Plotnicov KH, Weise J, Deep D. *Double-blind placebo-controlled administration of fluoxetine in restricting- and restricting-purging type anorexia nervosa*. Biol. Psychiatry 2001; 49: 644–652.
5. Klump KL, Bulik CM, Pollice C, Halmi KA, Fichter MM, Berrettini WH, Devlin B, Strober M, Kaplan A, Woodside DB, Treasure J, Shabbout M, Lilenfeld LR, Plotnicov KH, Kaye WH. *Temperament and character in women with anorexia nervosa*. J. Nerv. Ment. Dis. 2000; 188: 559–567.
6. Kaye WH. *Anorexia nervosa, obsessional behavior, and serotonin*. Psychopharmacol. Bull. 1997; 33: 335–344.
7. Kroeze WK, Kristiansen K, Roth BL. *Molecular biology of serotonin receptors structure and function at the molecular level.* Curr. Top. Med. Chem. 2002; 2: 507–528.
8. Currie PJ, Coiro CD, Niyomchai T, Lira A, Farahmand F. *Hypothalamic paraventricular 5-hydroxytryptamine: Receptor-specific inhibition of NPY-stimulated eating and energy metabolism.* Pharmacol. Biochem. Behav. 2002; 71: 709–716.
9. Jonsson EG, Nothen MM, Gustavsson JP, Berggard C, Bunzel R, Forslund K, Rylander G, Mattila-Evenden M, Propping P, Asberg M, Sedvall G. *No association between serotonin 2A receptor gene variants and personality traits*. Psychiatr. Genet. 2001; 11: 11–17.
10. Collier DA, Arranz MJ, Li T, Mupita D, Brown N, Treasure J. *Association between 5-HT2A gene promoter polymorphism and anorexia nervosa.* Lancet 1997; 350: 412.
11. Miller SA, Dykes D, Plesky HF. *A simple salting out procedure for extracting DNA from human nucleated cells*. Nucleic Acids Res. 1988; 16: 1215.
12. SPSS for Windows, version 10.0.7, SPSS inc. Chicago 2000
13. Hinney A, Ziegler A, Nothen MM, Remschmidt H,Hebebrand J. *5-HT2A receptor gene polymorphisms, anorexia nervosa, and obesity*. Lancet 1997; 350: 1324–1325.
14. Campbell DA, Sundaramurthy D, Markham AF, Pieri LF. *Lack of association between 5-HT2A gene promoter polymorphism and susceptibility to anorexia nervosa*. Lancet 1998; 351: 499.
15. Enoch MA, Kaye WH, Rotondo A, Greenberg BD, Murphy DL,Goldman D. *5-HT2A promoter polymorphism -1438G/A, anorexia nervosa, and obsessive- compulsive disorder*. Lancet 1998; 351: 1785–1786.
16. Sorbi S, Nacmias B, Tedde A, Ricca V, Mezzani B, Rotella CM. 5-*HT2A promoter polymorphism in anorexia nervosa.* Lancet 1998; 351: 1785.

17. Ziegler A, Hebebrand J, Gorg T, Rosenkranz K, Fichter M, Herpertz-Dahlmann B, Remschmidt H, Hinney A. *Further lack of association between the 5-HT2A gene promoter polymorphism and susceptibility to eating disorders and a meta-analysis pertaining to anorexia nervosa*. Mol. Psychiatry 1999; 4: 410–412.
18. Nacmias B, Ricca V, Tedde A, Mezzani B, Rotella C M, Sorbi S. *5-HT2A receptor gene polymorphisms in anorexia nervosa and bulimia nervosa.* Neurosci. Lett. 1999; 277: 134–136.
19. Ando T, Komaki G, Karibe M, Kawamura N, Hara S, Takii M, Naruo T, Kurokawa N, Takei M, Tatsuta N, Ohba M, Nozoe S, Kubo C, Ishikawa T. *5-HT2A promoter polymorphism is not associated with anorexia nervosa in Japanese patients*. Psychiatr. Genet. 2001; 11: 157–160.
20. Nishiguchi N, Matsushita S, Suzuki K, Murayama M, Shirakawa O, Higuchi S. *Association between 5HT2A receptor gene promoter region polymorphism and eating disorders in Japanese patients*. Biol. Psychiatry 2001; 50: 123–128.
21. Gorwood P, Ades J, Bellodi L, Cellini E, Collier DA, Di Bella D, Di Bernardo M, Estivill X, Fernandez-Aranda F, Gratacos M, Hebebrand J, Hinney A, Hu X, Karwautz A, Kipman A, Mouren-Simeoni MC, Nacmias B, Ribases M, Remschmidt H, Ricca V, Rotella CM, Sorbi S, Treasure J. *The 5-HT(2A) -1438G/A polymorphism in anorexia nervosa: a combined analysis of 316 trios from six European centers*. Mol. Psychiatry 2002; 7: 90–94.

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