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An associations study of the Dopamine Transporter (DAT) gene and catechol-O-methyltransferase (COMT) gene in anorexia nervosa in the Polish population

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Summary

Background: Anorexia nervosa (AN) is a common, severe psychiatric disorder that affects 1%-2% of the young female population. AN is characterised by profound weight loss and body image disturbances. Twin and family studies have suggested the role of a genetic component in the etiology of AN, although the exact mechanism of inheritance is unknown. Recent data suggest contribution of dopaminergic neuronal pathway disturbances in the pathogenesis of AN.

Material and method: We investigated the polymorphism of two genes: substitution Val108(158)Met of catechol-O-methyltransferase (COMT) gene and DAT 3`UTR VNTR gene with 40bp fragment repeated 3-11 times. The group of 91 AN probands and 135 controls was studied.

Results: We have not found an association between AN and COMT polymorphism (genotypes: Met/Met, Met/Val, Val/Val; p=0.08) and between AN and DAT polymorphism (p=0.44).

Conclusion: The analyzed COMT and DAT polymorphisms do not seem to play a role in the pathogenesis of AN in Polish population.

Key words: DAT and COMT polymorphism, genetics, anorexia nervosa

Introduction

Anorexia Nervosa is a complex and multifactorial disease with incompletely known vulnerability factors. Epidemiological research on AN have shown that it is a familial disorder [1]. Twin studies have shown that the concordance rate for monozygotic twins is higher (on overage 44%) then for dizygotic twins (on average 12.5%) [2]. The difference in concordance rates shows that genetic component may have key role in ethiopathogenesis of AN and genetic factors are significantly involved in the individual risk for this disorder. AN as other complex traits do not show simple

Mendelian pattern of inheritance and we should not suspect simple one gene – one disorder relation. Anorexia nervosa is rather caused by interaction of many genes of small effect with the environmental factors. Allelic association studies on candidate genes can provide the statistical power needed to detect small size effects. Several genes that are responsible for monoamine metabolism and transport may contribute to AN susceptibility. Among the monoamines, dopamine and serotonin are relevant brain messengers in food-intake regulation. Dopamine is an important neurotransmitter involved in the regulation of motor, endocrine, cognitive, emotional functions and plays an essential role in feeding regulation. Dopamine and serotonin may facilitate or inhibit second-messenger intracellular activity. This conveys information concerning meal size, meals number and food-intake by activation of postsynaptic neurons of the stimulatory food-intake neuropeptides (e.g. neuropeptide Y, galanin, orexin A/B, melanin-concentrating hormone) or by the inhibitory food-intake neuropeptides (e.g. melanocyte-stimulating hormone, cocaine and amphetamine-regulated transcript, corticotropin-releasing factor), depending on the prevailing metabolic conditions, to effector organs to modulate food intake. Leptin, insulin and other peripheral peptides and steroid hormones modulate the synthesis and release of dopamine and serotonin [3, 4]. Drugs and hormones are in vivo environmental factors which interfere with dopamine transmission and may cause disinhibition of eating [5]. Based on these data, some authors proposed, that disturbances of dopaminergic neuronal pathway may contribute to the pathogenesis of AN [6-8]. One of the candidate genes is highly polymorphic catechol-O-methyltransferase (COMT) gene [9]. The COMT gene is localized in region q11.1-q11.2 of the human chromosome 22 [10]. This gene encodes two forms of the enzymes: membrane bound form (MB-COMT) and soluble form (S-COMT). The two forms differ only by a 50 amino acid fragment in the N-terminal sequence that is present in the MB form. Investigated COMT gene polymorphism was described as G/A transition at codon 158 of MB-COMT and at codon 108 of S-COMT [11]. This polymorphism has potential functional importance because it results in a valine (high-activity allele) to methionine (low-activity allel) substitution [12] and is associated with three- to four fold variation in COMT enzyme activity. Frisch et al. (2001) [13] reported an association of the high activity allele of the COMT gene with AN, suggesting that this gene may contribute to the genetic susceptibility for the disorder. Anorexia Nervosa was significantly more frequent among homozygotes for the high activity allele (51% vs 29%, $\chi^2 = 4.01$, df=1, p=0.04). In the contrast, an unpublished German study revealed an association of AN with low-activity allele of COMT gene (Klauck et al. personal communication). This difference may be caused by variations in ethnic origin of studied groups or these alleles are in linkage disequilibrium with another polymorphism which is associated with the susceptibility to the AN.

The dopamine transporter (DAT) is responsible for regulation of dopamine synaptic active reuptake [14]. The human dopamine transporter is a member of a family of Na^+/Cl^- dependent neurotransmitter transporters. The gene encoding DAT has been cloned and located on chromosome 5p15.3 [15]. The association between DAT (*SLC6A3*) gene polymorphisms and dopaminergic neurotransmission disorders, including Parkinson`s disease, attention-deficit/hyperactivity disorder (ADHD) [16-18], Tourette syndrome

[19] and major depression [20] has been reported. The 40bp DAT gene polymorphism with variable number of tandem repeats (VNTR) ranging from 3-13 copies is located in 3'-untranslated region of gene [21]. In European populations the alleles containing 9 and 10 repeats are most frequently reported (about 90% of cases) [22].

Aim of the study

In this study, we examined the possible association between Val108(158)Met polymorphism of COMT gene and VNTR polymorphism of DAT gene and AN in the Polish (caucasian) sample. This is the first candidate gene association study of genes of dopaminergic system in AN.

Material and Methods

Subjects

We studied 91 (84 genotyped for DAT polymorphism only) patients with AN hospitalized in Department of Child and Adolescent Psychiatry University of Medical Sciences in Poznań. The mean age of women from the studied group was 18.5 years (SD=3.2). The study group was divided into two subgroups: 57 patients with restricting type of AN and 28 patients with bulimic type of AN, six patients diagnosed as atypical forms of AN were excluded from this subgroups. Diagnosis of AN was established by at least two psychiatrists with the use of SCID (Structured Clinical Interview for DSM-IV Axis I Disorders) according to the DSM-IV and ICD-10 criteria.

The controls were 135 (119 genotyped for DAT polymorphism) female blood donors. The mean age of women from the control group was 34.9 years (SD=10.0). They were not psychiatrically screened. The study was accepted by the local ethics committee.

Method of DNA analyses

The DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method [23]. A 217-basepair fragment of the COMT gene was amplified by PCR in PTC-100 (MJ Research) thermal cycler with primers described by Li et al. (1996). A 25 µl amplification mixture contained: 150-300 ng DNA, 0.5 µM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 0.08% NP40, 0.5 U of Taq DNA polymerase (MBI Fermentas). We used following cycling conditions: denaturation in 95˚C for 3 min, 35 cycles with a profile of 94˚C for 30 s, 55˚C for 45 s, 72˚C for 45 s, final elongation at 72˚C for 5 min. Eight microliters of each PCR product were digested overnight at 37˚C with 1.75 U of Hsp92II restriction endonuclease (Promega) in a total volume of 12 µl. Due to the presence of salt in the PCR product only 50% of the 10x restriction enzyme buffer amount recommended by the supplier was added to the digestion reaction in order to improve the efficiency of digestion reaction. Digestion products were electrophoresed on 3.25% Micropor Gamma agarose gel (Prona) and visualised by ethidium bromide staining. Band sizes were compared with DNA ladder (MBI Fermentas).

For Met allele we observed fragments of 96 bp and 40 bp and for Val allele a fragment of 136 bp was present. Additionally one constant band of 81 bp which resulting from non-polymorphic restriction site was presented.

An untranslated region (3`UTR) of the DAT gene was amplified by PCR in PTC-100 (MJ Research) thermal cycler with primers described by Vandenbergh et al. (1992) (15). A 25µl of amplification mixture contained 0.25 µg DNA, 0.1 µM of each primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 75 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, 0,01 Tween 20, 0.5 U of Taq DNA polymerase (MBI Fermentas). We used the following cycling conditions: denaturation in 95˚C for 2 min, 30 cycles with a profile of 94˚C for 30 s, 56˚C for 30 s, 72˚C for 30 s, final elongation at 72˚C for 5 min. PCR products were then electrophoresed on 3% (Micropor Gamma) agarose gel (Prona, Spain) and visualised by ethidium bromide staining. Band sizes were compared with DNA ladder (MBI Fermentas). We observed two alleles: 9-repeat allele (440 bp fragment) and 10-repeat allele (480 bp fragment).

Statistical analysis

The Pearson's χ^2 Test was applied to assess the differences in the genotype, and the Fisher's Exact Test was applied to check differences in the allelic distribution between the study group and the controls. Calculations were performed with the use of the statistical package: SPSS version 10. Two-tailed type I error rate of 5% was chosen for the analyses. Power analysis was performed using on-line calculator (http:/calculators.stat.ucla.edu/) provided by the UCLA Department of Statistics.

Results

The genotype distribution for COMT gene was in Hardy-Weinberg Equilibrium (HWE) for patients (p=0.95) but not for control subjects (p=0.001). Thy genotype distribution for DAT gene was in HWE both for patients (p=0.81) and for controls (p=0.12).

The genotype distribution and allele frequencies of Val158Met polymorphisms of the COMT gene are shown in the table I. We obtained following distribution of COMT genotypes in the AN group: Val/Val=28.6%, Val/Met=49.5%, Met/Met=22.0% and in the controls: Val/Val=17.8%, Val/Met=63.7%, Met/Met=18.5%. The frequency of COMT alleles was similar in both groups, respectively Val=52.2% and Met=47.8% in the probands and Val=49.8% and Met=50.2% in the controls. No significant differences were found neither for the genotypic distributions (p=0.078) nor for the allelic distributions (p=0.368) between the study group and the controls. We have also found no significant differences neither for genotypic distribution (p=0.131) nor for allelic distribution (p=0.202) between the patients with bulimic type of AN and controls. There was an association between Val/Val and Val/Met genotypes and AN restricting type (p=0.029) but we not observed allelic association (p=0.257) between the patients with restricting type of AN and controls.

Table I

Frequencies of Val158Met polymorphisms of the COMT gene in Polish (caucasian) patient with AN.

Group	N	Genotype distribution			Allele frequency	
		Val/Val	Val/Met	Met/Met	Val	Met
patients	91	26 (28.6%)	45 (49.5%)	20 (22.0 %)	71 (52.2%)	65 (47.8%)
restricting type	57	20 (35.1%)	27 (47.4%)	10 (17.5%)	47 (56.0%)	37 (44.0%)
bulimic type	28	4 (14.3%)	14 (50.0%)	10 (35.7%)	18 (42.9%)	24 (57.1%)
controls	135	24 (17.8%)	86 (63.7%)	25 (18.5%)	110 (49.8%)	111 (50.2%)

Genotype-wise (chi square Pearson test): AN vs control subject: χ^2=5.094, df=2, p=0.078;
Restricting type vs control subject: χ^2=7.078, df=2, p=0.029; bulimic type vs control subject: χ^2=4.068, df=2, p=0.131
Allele-wise (Fisher exact test): AN vs control subject: p=0.368; restricting type vs control subject: p=0.257; bulimic type control subject: p=0.202

Frequencies of VNTR polymorphisms of the DAT gene in the study group and in the controls are presented in Table II. No significant differences were found in relation to the distribution of genotypes and allele frequencies between the probands and the control group (p=0.436 for genotypes, p=0.399 for alleles). We also have not found significant differences neither for genotypic distribution (p=0.461) nor for allelic distribution (p=0.523) between the patients with restricting type of AN and controls. Similarly no difference were found between bulimic type of AN and control (p=0.553 for genotypes, p=0.347 for alleles).

Table II

Frequencies of VNTR polymorphisms in the DAT gene in Polish (caucasian) patient with AN

Group	N	Genotype distribution			Allele frequency	
		9/9	9/10	10/10	9	10
patients	84	4 (4.8%)	27 (32.1%)	53 (63.1 %)	31 (27.9%)	80 (72.1%)
restricting type	57	3 (5.9%)	17 (33.3%)	31(60.8%)	20 (29.4%)	48 (70.6%)
bulimic type	28	1 (3.6%)	8 (28.6%)	19 (67.9%)	9 (25.0%)	27 (75.0%)
controls	119	3 (2.5%)	47 (39.5%)	69 (58.0%)	50 (30.1%)	116(69.9%)

Genotype-wise (chi square Pearson test): AN vs control subject: χ^2=1.662, df=2, p=0.463;
restricting type vs control subject: χ^2=1.551, df=2, p=0.461; bulimic type vs control subject: χ^2=1.184, df=2, p=0.553
Allele-wise (Fisher exact test): AN vs control subject: p=0.399; restricting type vs control subject: p=0.523; bulimic type control subject: p=0.347

Power analysis for sample genotyped for COMT ranged between 32%-71% for a relative risk from 1.5 to 2. The DAT sample had a power 26%-64% for relative risk ranging from1.5 to 2. The results of power analysis suggest that in our group there is a relatively small chance to obtain false negative association if relative risk is smaller than 2.

Discussion

Genetic background of AN is not precisely defined. Previous studies gave conflicting results. In our study we did not find the association between the polymorphisms of COMT and DAT gene and AN. The genotype association of COMT gene and restrictive type AN may be object of discussion, however it was not confirmed in allelic association. This association may be spurious and result from deviation of HWE in control sample. The deviation from HWE may be a consequence of genotyping errors, selection, migration, allelic distortion, or population admixture. In our analysis we can exclude genotyping errors, but all other mentioned above causes may explain deviation from HWE in control subjects because the controls were female blood donors not psychiatrically screened.

Power analysis showed that size of research group would be enough to demonstrate the lack of association in case of relative risk for alleles higher then 2. In the other case replication study on larger sample size is warranted.

In the case of complex phenotypes such as AN it is likely that multiple polymorphisms of different genes may influence disease risk. Factors which may explain presence of association found in the other studies are: sampling from various ethnic groups, sizes of the groups, selection of specific subgroups of patients. Positive associations obtained by some researchers may therefore reflect an effect present only in one subgroup of patients. Despite of our negative findings, in the light of other studies a minor effect or an effect in a subset of anorectic patients regarding studied COMT and DAT gene polymorphism cannot be excluded. The findings suggest that more research on the functional consequence of COMT and DAT variants is warranted.

Conclusions

1. There is no association between RFLP polymorphism of COMT gene and VNTR polymorphism of DAT gene and AN in the Polish (caucasian) population.
2. There is no association between RFLP polymorphism of COMT gene and VNTR polymorphism of DAT gene and bulimic and restricting type of AN in the Polish (caucasian) population.

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