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The association study of a functional polymorphism of the monoamine oxidase A gene promoter in patients with affective disorders

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Recently a functional 30 bp variable number of tandem repeat polymorphism (VNTR) in the promoter region of the MAO-A gene was identified, which was shown to be associated with MAO-A transcriptional activity. Variation in the number of repeats (3-5) displayed a 2.7-4.8 fold increased transcription activity for allele containing four or five repetitive elements than allele containing three repetitive elements. In the present study we investigated this polymorphism in 78 male and 122 female patients with bipolar and unipolar affective disorders, and in 221 control subjects.

There were significant differences in the alleles and genotypes frequencies between the group of females with bipolar affective illness compared to the controls and compared to the unipolar female subjects. No differences were found in genotype distribution between unipolar female patients and control group and between bipolar and unipolar male subjects comparing to the controls. We suggest that the association may exist between uVNTR polymorphism in MAO A gene and bipolar affective disorder in women.

Key words: affective disorder, genetics, MAO A polymorphism

Introduction

Monoamine oxidase MAO is an enzyme that catalyses the oxidative deamination of biogenic amines, including brain neurotransmitters such as noradrenaline, dopamine and serotonin. All of these are supposed to play a major role in a pathophysiology of affective disorders and schizophrenia [1]. Therefore MAO has been of great interest in psychiatric research. There are two isoforms of MAO – MAO-A and MAO-B with different substrate affinity [2]. Monoamine oxidase A inhibitors are effective treatments for depression in affective disorders [3]. This makes the monoamine oxidase A gene one of the possible candidate genes in affective disorders.

The MAO-A gene was cloned and characterised in 1988. It was shown to map to region p11.23-p11.4 in chromosome X [4]. Subsequently, four gene polymorphisms were demonstrated, namely:

1. MAO-A-(CA)n – MAO-A dinucleotide repeat polymorphism [5]
2. variable number of tandem repeats in intron 1 (MAO-A-VNTR) [6]
3. MAO A restriction fragment length polymorphism in egzon 8 (RFLP) [7]
4. MAO-A u VNTR polymorphism in the promoter region [8]

Lim et al. [7] were the first to hypothesise that the MAO A gene could be implicated in the aetiology of bipolar disorder. They studied bipolar patients using MAO-A dinucleotide repeat marker and found a higher prevalence of the 3-repeat allele in the group of bipolar patients than in control subjects. Their results were confirmed by Kawada et al. [9] in a sample of bipolar patients of Japanese origin. Also Rubinsztein et al. [8] found a significant difference in the frequencies both for microsatellite and the RFLP polymorphisms between bipolar patients and controls. However subsequent studies yielded conflicting results. Nöthen et al. [10], Craddock et al. [11] and Muramatsu et al. [12] did not confirm the association between polymorphisms of MAO-A gene and bipolar disorder, using either the dinucleotide marker or VNTR and RFLP markers.

Recently, Sabol et al. [13] identified a functional 30 base pair (bp) polymorphism of a variable number of tandem repeat (VNTR) in the promoter region of the MAO-A gene, which was shown to be associated with MAO-A transcriptional activity. The Sabol group identified four alleles: allele 1 - containing three repeats, allele 2 - containing 3.5 repeats, allele 3- containing four repeats and allele 4- containing five repeats. Variation in the number of repeats (3-5) of this polymorphism displayed a 2.7-4.8 fold increased transcription activity for the allele containing four repetitive elements (allele 3) then the allele containing three repetitive elements (allele 1) [14].

Although previous studies have suggested a possible association between several polymorphisms of the MAO A gene and mood disorders, there is no evidence that these polymorphisms are related to the function of the MAO-A gene. On the other hand, the polymorphism found by Sabol et al. [13] is related to the functional activity of the MAO-A. A study performed by those authors [13] has shown that the three-repeat allele (allele 1) and five repeat allele (allele 4) were less active than allele with 3.5 repeats (allele 2) and allele with four repeats (allele 3). Deckert et al. [15] examined the frequency of the more active alleles in German and Italian patients with panic disorder. They found no differences in the male group, however, longer alleles were more common in females with panic disorder compared to the control. Samochowiec et al. [16] found that the less active (three-repeat allele) was more common in the antisocial alcoholics.

In this paper we report the data from a group of Polish bipolar and unipolar patients with affective disorders. We hypothesised that the MAO-A uVNTR polymorphism may be associated with affective disorders.

Materials and methods

Subjects

Subjects were 200 patients with affective disorders (78 males and 122 females), 106 with bipolar affective illness (52 males, 54 females) and 94 with unipolar illness (mean number of depression 3) (26 males, 68 females). The mean age was 49 (SD=14) years for the bipolar group and 50 years for unipolar group (SD=11).

Patients with mood disorders were recruited from inpatients, treated at the Department of Adult Psychiatry, University of Medical Sciences in Poznań (n=122) and at the Department of Psychiatry, Pomeranian University of Medical Science in Szczecin (n=78). Consensus diagnosis by at least two psychiatrists according to the ICD-10 and DSM-IV criteria, was made for each patient using SCID (Structured Clinical Interview for DSM-IV Axis I Disorders).

Two hundred and twenty one control subjects (90 females, 131 males) were recruited from the group of blood donors. They were not psychiatrically screened. Their mean age was 59 years (SD=14). The project was accepted by the local ethical committee.

DNA analyses

Genomic DNA was extracted from anticoagulated venous blood samples or lymphoblastoid cell lines using a salting out method [17]. The VNTR polymorphism in the promoter region of the MAO-A gene was amplified by polymerase chain reaction (PCR) with oligonucleotide primers: For 2 (sense): (5' - CCC AGG CTG CTC CAG AAA C 3'), Ref2 (antisense): (5'- GGA CCT GGG CAG TTG TGC-3'). PCR reactions contained 100 ng genomic DNA, 10 pmol of each primer, 50 mM KCl, 75 mM Tris-HCL (pH 8.3 at 25 C), 1.5 mM $MgCl_2$, 0,01% gelatine, 200mM of each dNTP, 20 mM $(NH4)_2SO_4$, 0.01% Tween 20 and 0.5 unit Taq polymerase (InViTek) in the total volume of 25 il. Cycling conditions were initial denaturation at 95°C for 3 min, followed by 35 cycles of (denaturation at 94°C for 40 s, annealing at 60°C for 30s elongation at 72°C for 50 s) and a final elongation at 72°C for 5 min. PCR was carried out in a Perkin-Elmer Cetus 9600 thermal cycler. Amplification products were separated by electrophoresis on 3% Methaphor agarose gels and bands were visualised by ethidium bromide staining. Fragment sizes were determined by comparison to molecular length standards. The 209 bp fragment refers to 3- repeats, 239 bp: 4-repeats, 269 bp: 5-repeats.

Genotyping was carried out without knowledge of the diagnostic status of the subjects.

Statistical analysis

The chi-square (χ^2) test was applied to test differences in the allelic distribution between groups of affective disorders patients and controls. Odds ratio together with their 95% confidence bounds for allele prevalence (frequency of individuals carrying a certain allele) were calculated using the computer program (SPSS version 7.5.2.). Two-tailed type I error rate of 5% was chosen for the analyses.

Results

Although Sabol's group identified four alleles of a 30 base pair (bp) motif of the MAO-A gene, we detected only three alleles, i.e. the allele containing five, four and three repetitive elements. In our sample, only five persons had the five repeat allele (four from control group and one from patient group) so we did not analyse this group. The genotype distribution in females were in Hardy-Weinberg equilibrium for all three groups (for the bipolar χ^2 =0,667, p=0,414, for the unipolar group χ^2=0,239, p=0,239, for controls χ^2=1,56, p=0,211).

The frequency of the alleles was analysed only in female subjects because the gene maps to chromosome X therefore the genotypes in males are also alleles. The genotype distribution was analysed separately for male and female subjects. We found a significant difference in genotype distribution between female bipolar patients and the control group (p=0,029). In male bipolar patients the distribution of genotypes did not differ significantly. For unipolar depression, genotype distribution did not differ significantly from that for the controls in males or in females. For bipolar female patients we found a significant difference (p=0,013) in allele frequencies comparing to the control group, but in the group of unipolar female patients we found no significant difference in allele frequencies. We observed statistically significant difference in both allele and genotype frequencies between bipolar and unipolar female patients only (p=0,029 for genotypes, p=0.006 for alleles).

Discussion

The main finding of our study is an association between the functional polymorphism in the promoter region of the MAO-A gene and bipolar affective illness in female subjects. It may suggest that this functional VNTR polymorphism of the MAO-A gene is likely to have some relevance to the susceptibility to bipolar mood disorder. In our sample, the allele containing three repeats was significantly more frequent in the group of female bipolar patients compared with female control subjects and with unipolar female subjects. We also found significant difference in the genotype distribution in the female bipolar patients compared to the control subjects and unipolar female subjects. On the other hand, we did not find differences in genotype/allele distribution in male bipolar and unipolar subjects comparing to the control group.

Our results are in contrast with those of Furlong et al. [1] who found no association of promoter VNTR polymorphism and affective disorders. Also Kunugi et al. [14] found no association of this polymorphism either in males or females and affective disorders in the Japanese population. Jorm et al. [18] studied this polymorphism in the group of Caucasian patients with depressive symptoms although no significant association was observed, they found a trend towards some association in females with 3.5 repeat allele (allele 2) and anxiety traits.

Our results correspond in a way to those of Deckert et al. [15] who observed an association between this polymorphism and female gender with panic disorder. These authors found that in females with panic disorder the more active allele was significantly more frequent, whereas in our study the less active allele was significantly more

frequent in bipolar female patients. Since drugs used in the treatment of depression (such as moclobemide) inhibit the MAO-A activity, therefore our results are difficult to interpret. They may resemble findings with serotonin transporter gene in affective illness, where a less active variant of this transporter polymorphism was prevalent, and drugs inhibiting this transporter (SSRI) exert a therapeutic action (19).

In summary, we found an association between the less active form of functional polymorphism in the promoter region of the MAO-A gene and bipolar affective illness in females.

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References

1. Furlong RA, Ho L, Rubinsztein JS, Walsh C, Paykel ES, Rubinsztein DC. *The analysis of the monoamine oxidase A (MAO-A) gene in bipolar affective disorder by association studies, meta analyses, and sequencing of the promoter*. Am. J. Med. Gen. 1999; 88: 398-406.
2. Krishnan KRR. Monoamine Oxidase Inhibitors. In Schatzberg AF, Nemeroff ChB. *Textbook of psychopharmacology*. Washington: American Psychiatric Press; 1998. p. 239-249.
3. Frazer A, Hensler JG. Serotonin, In: Siegel GJ, Agranoff B, Albers RW, Molinoff RB ed. *Basic neurochemistry*. New York: Raven;1994. p. 304-306.
4. Bach AWJ, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan S-W, Seeburg PH, Shih JC. *cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties*. Proc. Natl. Acad. Sci. USA 1988; 85: 4934-4938.
5. Black GCM, Chen ZY, Craig IW, Powell JF. *Dinucleotide repeat polymorphism at the MAOA locus*. Nucleic Acids Res.1991; 91: 689.
6. Hinds HL, Hendricks RW, Craig IW, Chen ZY. *Characterization of a highly polymorphic region near the first exon of the human MAOA gene containing a GT dinucleotide and a novel VNTR motif*. Genomics 1992; 13: 896-897.
7. Lim LC, Powell J, Sham P, Castle D, Hunt N, Murray R et al. *Evidence for a genetic association between alleles of monoamine oxidase A gene and bipolar affective disorder*. Am. J. Hum. Gen. 1995; 60: 325-331.
8. Rubinsztein DC, Leggo J, Goodburn S, Walsh C, Jain S, Paykel ES. *Genetic association between monoamine oxidase A microsatellite and RFLP alleles and bipolar affective disorder: analysis and meta-analysis*. Hum. Mol. Genet. 1996; 5 (6): 779-82.
9. Kawada Y, Hattori M, Dai XY, Nanko S. *Possible association between monoamine oxidase A gene and bipolar affective disorder*. Am. J. Hum. Genet. 1995, 56: 335-336.
10. Nöthen MM, Eggermann K, Albus M, Borrmann M, Rietschel M, Korner J, Maier W, Minges J, Lichtermann D, Franzek E, Weigelt B, Knapp M, Propping P. *Association analysis of the monoamine oxidase A gene in bipolar affective disorder by using family-based controls*. Am. J. Hum.Genet. 1995; 57: 975-977.
11. Craddock N, Daniels J, Roberts E, Rees M, McGuffin P, Owen MJ. *No evidence for allelic association between bipolar disorder and monoamine oxidase A gene polymorphisms*. Am. J. of Med. Gen. 1995; 60/4: 322-324.
12. Muramatsu T, Matsushita S, Kanba S, Higuchi S, Manki H, Suzuki E, Asai M. *Monoamino oxidase genes polymorphisms and mood disorder*. Am. J.of Med. Genet. 1997, 74: 494-496.
13. Sabol SZ, Hu S, Hamer D. *A functional polymorphism in the monoamine oxidase A gene promoter*. Hum. Gen. 1998; 103 (3): 273-279.

14. Kunugi H, Ishida S, Kato T, Tatsumi M, Sakai T, Hattori M, Hirose T, Nanko S. *A functional polymorphism in the promoter region of monoamine oxidase A gene and mood disorders*. Mol. Psych. 1999; 4: 393-395.
15. Deckert J, Catalano M, Syagailo YV, Bosi M, Oklandnova O, Di Bella D et al. *Exess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder*. Hum. Mol. Gen.1999; 8: 621-624.
16. Samochowiec J, Lesch KP, Rottmann M, Smolka M, Syagailo YV, Oklandova O et al. *Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial alcoholism*. Psych. Res. 1999; 86: 67-72.
17. Oruć L, Verheyen GR, Furać I, Jakovljević M, Ivezić S, Raeymaekers P, Van Broeckhoven C. *Association analysis of the 5HT2C receptor and 5HT transporter genes in bipolar disorder*. Am. J. of Med. Genet. 1997; 74: 504-506.
18. Rubinsztein DC, Leggo J, Goodburn S, Walsh C, Jain S, Paykel ES. *Genetic association between monoamine oxidase A microsatellite and RFLP alleles and bipolar affective disorder: analysis and meta-analysis*. Hum. Mol. Genet. 1996; 5 (6): 779-82.
19. Oruć L, Verheyen GR, Furać I, Jakovljević M, Ivezić S, Raeymaekers P, Van Broeckhoven C. *Association analysis of the 5HT2C receptor and 5HT transporter genes in bipolar disorder*. Am. J. of Med. Genet. 1997; 74: 504-506.

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Table 1

Genotype distribution and allele frequencies of MAO-A gene polymorphism in female subjects of bipolar, unipolar and control group

	Genotype 3&3 repeats n (%)	Genotype 3&4 repeats n (%)	Genotype 4&4 repeats n (%)	TOTAL Genotypes n (%)	Allele 3 n (%)	Allele 4 n (%)	TOTAL Alleles n (%)
bipolar	15 (27.8%)	24 (44.4%)	15 (27.8%)	54 (100%)	54 (50%)	54 (50%)	108 (100%)
unipolar	8 (11.8%)	28 (41.2%)	32 (47.1%)	68 (100%)	44 (32.4%)	92 (67.6%)	136 (100%)
control	18 (20%)	26 (28.9%)	46 (51.1%)	90 (100%)	62 (34.4%)	118 (65.6%)	180 (100%)

Difference bipolar versus control – χ^2 =7.581 df=2 p=0.023 for genotypes, χ^2=6.790 df=1 p=0.013 for alleles
Difference unipolar versus control – χ^2=3.436 df=2 p=0.179 for genotypes, χ^2=0.152 df=1 p=0.719 for alleles
Difference bipolar verus unipolar – χ^2=7.074 df=2 p=0.029 for genotypes, χ^2=7.800 df=1 p=0.006 for alleles

Table2

Genotype (alleles) distribution of MAO-A gene in male subjects of bipolar, unipolar and control group

	Allele 3	Allele 4	TOTAL
BIPOLAR	22 (42.3%)	30 (57.7%)	52 (100%)
UNIPOLAR	6 (23.1%)	20 (76.9%)	26 (100%)
CONTROL	47 (35.9%)	84 (64.1%)	131 (100%)

Differences bipolar versus control – χ^2= 0.655 df=1 p=0.499
Difference unipolar versus control – χ^2= 1.590 df=1 p=0.260
Differences bipolar versus unipolar – χ^2= 2.786 df=1 p=0.134