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Possible Association between Monoamine Oxidase A Gene and Bipolar Affective Disorder

To the Editor:

Lim et al. (1994) reported a significant overall association between bipolar affective disorder and alleles of the monoamine oxidase A (MAOA) locus of 56 patients (14 males and 42 females) compared with 58 controls (17 males and 41 females) (exact P value < .0019). They also observed a lower frequency of the a_2 allele in the patients than in controls, although the P value (P = .0339) does not reach the critical P value of multiple testing (P = .0057). They suggested that alleles at the MAOA locus contribute to susceptibility to bipolar disorder. However, this association might be a chance finding, as has frequently occurred with genetic case-control studies. Thus, replication is required to confirm their suggestion.

We have genotyped a sample of 58 patients (26 males and 32 females), diagnosed as having bipolar disorder, and 68 controls (33 males and 35 females). Consensus diagnoses were made independently by two psychiatrists, according to the *Diagnostic and Statistical Manual of Mental Disorders,* revised 3d edition (1987). Both patients and controls were unrelated Japanese and resided in the same geographical area. After informed consent was obtained, venous blood was drawn from each individual, and genomic DNA was isolated. Microsatellite repeat polymorphism (Black et al. 1991), which was used in the Lim et al. study (1994), was analyzed, using a silver-staining method (Hattori et al. 1993). We used the SAS package for computations and carried out an exact analysis.

Our results are shown in table 1. Lim et al. observed nine alleles, whereas we have seven alleles. Analysis of the data showed a statistically significant overall association between the disorder and alleles of the MAOA locus (χ^2 = 14.03; df = 6; P = .029). Then we tested each allele separately. The frequencies of the A4 allele were higher in the patients than in the controls (χ^2 = 4.14; df = 1; P = .042), and an opposite trend was observed for the A5 allele (χ^2 = 6.31; df = 1; P = .012). However, the differences do not reach the significant level after correction by multiplying the P value by the number of alleles (7) (Bonferroni's correction).

Table 1

Distribution of Alleles at MAOA Locus

Allele	Frequency: Cases (N = 58)	Frequency: Controls (N = 68)
A0	1	0
A1	7	7
A2	19	12
A3	6	12
A4	9	3[a]
A5	12	29[b]
A6	36	40

NOTE—Overall χ^2 = 14.03; df = 6; P = .029.
[a] χ^2 = 4.14; df = 1; P = .042.
[b] χ^2 = 6.31; df = 1; P = .012.
(Seven alleles, critical P value = .0071.)

Like Lim et al., we have found a weak but significant overall difference in allele distribution between patients and controls. Although the frequencies of individual alleles in patients and controls are different from those in the study by Lim et al., it is possible that the number of CA repeats is in linkage disequilibrium with a functional polymorphism and that the associated alleles have reversed in one of the populations while retaining the association. Alleles at the MAOA locus may contribute to susceptibility to bipolar disorder. Thus, further investigation is clearly needed.

YASUHARA KAWADA,[1] MINEKO HATTORI,[2] XIAO Y. DAI,[2] AND SHIN NANKO[2]
[1]*Department of Psychiatry, Juntendo University School of Medicine; and* [2]*Department of Psychiatry, Teikyo University School of Medicine, Tokyo*

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Exclusion of the Neuronal Nitric Oxide Synthase Gene and the Human *achaete-scute* Homologue 1 Gene as Candidate Loci for Spinal Cerebellar Ataxia 2

To the Editor:

The autosomal dominant cerebellar ataxias comprise a group of neurodegenerative disorders characterized by neuronal loss in the cerebellum and the inferior olivary and pontine nuclei and by degeneration of the spinal cord. We have previously mapped the spinal cerebellar ataxia 2 locus (SCA2) to chromosome 12q23-24.1 in a large Cuban founder population showing anticipation (Twells et al. 1993). As part of the strategy to isolate this gene we have investigated the possibility of pathological expansion of trinucleotide repeat motifs contained within two candidate loci recently assigned to this interval: the neuronal nitric oxide synthase (NOS) gene and the human *achaete-scute* homologue 1 (HASH1).

The neuronal NOS gene has been assigned to chromosome 12q24.2-24.3 by FISH (Xu et al. 1993). This gene is expressed in all regions of the brain, with the highest levels detected in the basket and granule cells of the cerebellum (Fostermann et al. 1990). Neuronal NOS is responsible for the production of nitric oxide (NO), a neurotransmitter, which also plays a role in nervous system morphogenesis and synaptic plasticity. NO may also act as a cytotoxin in the brain; in stroke, NOS-producing cells generate high levels of NO, which kill neighboring cells, but remain intact themselves (Nowicki et al. 1991). This indicates that increased levels of NO in neuronal cells could be a mechanism causing neurodegeneration.

Cosmids containing genomic sequence corresponding to the neuronal NOS gene (WX) were systematically screened with a collection of tri- and tetranucleotide repeat oligonucleotides, resulting in the detection of an $(AAT)_n$ motif within an intronic region of the gene. The 3.8-kb *Bam*HI fragment containing the repeat was subcloned, and the sequence of the flanking regions was determined. Primers selected for PCR amplification are 5′–3′ NOS F: CTG GGG GCA ATG GTG TGT and NOS R: GAG TAA AAT TAA GGG TCA GC.

The polymorphic characteristics of the NOS repeat motif were investigated in 116 chromosomes from the CEPH reference panel of unrelated individuals. Six alleles were observed with sizes of 1 = 425, 2 = 422, 3 = 419, 4 = 416, 5 = 410, and 6 = 407 bp. The corresponding allele frequencies are 1 = .017, 2 = .155, 3 = .267, 4 = .017, 5 = .526, and 6 = .017. The observed heterozygote frequency is .63 in CEPH families.

Pairwise lod scores between NOS and the SCA2 locus as analyzed in 89 affected and 19 normal individuals of the Cuban kindred are (i) including asymptomatic individuals with correction for age-dependent penetrance, $Z_{max} = 6.93$; $\theta_{max} = .06$ and (ii) affected individuals only, $Z_{max} = 4.34$; $\theta_{max} = .07$. Three recombination events were detected in affected members of the kindred, one directly and two as a result of ancestral recombination. No evidence for expansion of the $(AAT)_n$ was detected in affected individuals, many of whom are heterozygous at this locus with alleles within the normal size range.

The HASH1 gene is expressed in human fetal brain and neuroendocrine tumors and has recently been mapped to chromosome 12q24.1 (Ball et al. 1993; H. Donis-Keller, unpublished data). In *Drosophila,* the *achaete-scute* complex genes have been implicated in neurosensory development in the neural crest and CNS. The HASH1 gene contains a polymorphic $(CAG)_n$ repeat motif located in the proximal coding region, absent from the mouse and *Drosophila* homologues (Ball et al. 1993).

Analysis of the repeat motif in the Cuban pedigrees (primer sequences, are 5′–3′, F—AGC CCT TCC TGC CGC CCG CA; and R—GGC GCT GAC TTG TGA CCG CC) resulted in the detection of six alleles representing 19, 17, 15, 13, 12, and 10 repeats. Four of these alleles were observed in affected members of the pedigrees: allele 1 (.007), allele 3 (.007), allele 4 (.268), and allele 5 (.717), resulting in comparatively poor informativity in this kindred. No evidence for expansion in affected individuals and no significant difference between the distribution of alleles in affected and normal chromosomes were detected.

Pairwise analysis carried out between SCA2 and the HASH1 gene generated a maximum lod score of 4.12 at $\theta_{max} = .06$. Affected-only analysis, carried out to minimize the effect of presymptomatic individuals on the analysis, confirmed the exclusion of this candidate gene by the detection of a single recombination event in an affected individual ($Z_{max} = 3.72$; $\theta_{max} = .06$).

The majority of affected individuals are homozygous at this locus. We interpret the most likely cause of this to be the inbred nature of the population, but we are aware that previous experience with the analysis of other disease loci where the mutation is an expansion of a $(CAG)_n$ motif has shown that amplification of an expanded allele may prove suboptimal, giving an erroneous impression of homozygosity. Considered in association with the detection of only a single recombination event between SCA2 and HASH1, direct sequence analysis was undertaken in two affected individuals. No expansion was observed; the individual homozygous for the locus exhibited only a $(CAG)_{12}$ motif, and a heterozygote exhibited 12 and 13 repeats.

We have confirmed the "founder" status of this popula-