GENETICS

Predisposition to Alcoholism, Tryptophan Oxygenase Activity, and Structure of Intron 6 in the Corresponding Gene of C57BL/6J, CC57BR/Mv, and BALB/cJ Mice

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Crossbred CC57BR/Mv mice inherited tryptophan oxygenase gene and predisposition to alcohol consumption from parent BALB/c and C57BL mice, respectively. In CC57BR/Mv mice no relationships were found between alcohol consumption, tryptophan oxygenase activity, and single nucleotide substitutions in intron 6 of the *TDO2* gene associated with predisposition to alcoholism in humans.

Key Words: *tryptophan oxygenase; intron 6; genetic predisposition; alcoholism*

Tryptophan 2,3-dioxygenase (TDO2, EC 1.13.1.12) is a key enzyme catabolizing tryptophan and regulating its plasma concentration. Since tryptophan is a serotonin precursor and disturbances in serotonin metabolism accompany various mental disorders and predisposition to alcoholism [3], it was hypothesized that structural changes in the *TDO2* gene affecting its expression can modulate the development of these diseases [4]. The study of the nucleotide sequence in *TDO2* genes in healthy people and patients with behavioral disorders revealed no differences in the encoding region, but demonstrated the presence of 2 single nucleotide substitutions (single nucleotide polymorphisms, SNPs) in intron 6 associated with Tourette's syndrome, children's hyperactivity, drug addiction, and predisposition to alcoholism [4]. We showed that both substitutions destroy binding site for transcriptional factor YY1, which plays an important role in the regulation of gene expression [9].

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C57BL mice are widely used in studies of the predisposition to alcoholism [2]. In these animals basal and glucocorticoid-induced TDO2 activity markedly surpasses that in AKR, C3H, CBA, and DBA/2 mice not preferring alcohol [1,2,7]. Study of the encoding region of *TDO2* gene revealed no variants of nucleotide sequence determining differences between C57BL/6J mice and other animals (EMBL, ID MM24493, and AC U24493). Previous studies performed on humans suggest that these differences can be revealed in intron 6 of this gene. A comparative study of crossbred CC57BR/Mv mice [5] and parent animals (BALB/c and C57BL mice) will elucidate the relationships between the primary structure of this region of *TDO2* gene, enzyme activity, and predisposition to alcohol consumption.

MATERIALS AND METHODS

Male BALB/cJ (BALB/c), C57BL/6J (C57BL), CC57BR/Mv (CC57BR), CBA/Ca (CBA), and DBA/2J (DBA/2) mice aging 3-4 months were bred in a vivarium at the Institute of Cytology and Genetics and kept

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under conditions of the natural light/dark cycle. The animals received *ad libitum* mixed fodder PK-120-1 (Informkorm) and water. Before measurements of TDO2 activity the mice were kept in individual cages over 5 days before euthanasia to prevent stress associated with group housing. Dexamethasone phosphate was injected intraperitoneally in a dose of 0.4 mg/100 g for induction of the enzyme. The mice were decapitated 5 h after treatment. The liver was homogenized in 10-fold volume of 0.05 M phosphate buffer (pH 7.0). TDO2 activity was measured in homogenates by the method described elsewhere [6] with modifications. Samples were preincubated at room temperature and 37°C for 1 h and 45 min, respectively. Alcohol consumption was determined in a free choice test for alcohol preference [8]. The results were analyzed by Student's *t* test.

DNA samples for sequencing were obtained using polymerase chain reaction (PCR) with direct and reverse primers 5'-CAGAGCAGGAGCAGACGCTGT TG-3' and 5'-GGCTCTAAACCAGGTGTTCTTTCC AG-3'. Forty-five PCR cycles were performed according to the following protocol: denaturation at 94°C for 50 sec; annealing at 64°C for 30 sec; and elongation at 71°C for 60 sec. Final DNA product was purified in 1% agarose gel. Nucleotide sequences were determined by the method of Sanger on an automatic sequencer (ABI Prism 310 Genetic Analyzer) using BigDye terminator cycle sequencing ready reaction kit (Perkin Elmer).

RESULTS

The study of *TDO2* 6 gene intron 6 structure showed that C57BL mice differed from CBA and DBA/2 mice not preferring alcohol. C57BL mice had a microdeletion in position 417 of intron 6. C-T, T-A, and A-G substitutions were revealed in positions 15, 364, and 912, respectively (Table 1). The nucleotide sequence of intron 6 in the *TDO2* gene of mice was determined and registered in GenBank database (accession number AY223809). The mice of these strains were not characterized by intrastrain polymorphism in the nucleotide sequence of DNA in intron 6. The length of intron 6 was 993 (BALB/cJ, CC57BR/Mv, CBA/Ca, and DBA/2J) and 992 b.p. (C57BL/6J). C57BL mice differed from CBA and DBA/2 mice in nucleotides in positions 629 and 974, respectively (Table 1). These data suggest that predisposition to alcoholism in humans and mice is determined by SNP in intron 6 of the *TDO2* gene. However, comparative study of C57BL, BALB/c, and CC57BR mice demolished this hypothesis. The structure of intron 6 was similar in mice of these strains. However, BALB/c and CC57BR mice differed by predisposition to alcohol consumption (Table 2). Our results indicate that CC57BR/Mv mice inherited the *TDO2* gene and predisposition to alcohol consumption from parent BALB/c and C57BL mice, respectively. Therefore, these characteristics are genetically independent. High activity of TDO2 in the liver responsible for catabolic degradation of tryptophan and serotonin deficiency in the brain is considered as a marker for predisposition to alcoholism [2]. TDO2 activity is high in C57BL mice [1,2,7]. We revealed that basal TDO2 activity in the liver of alcohol-nonpreffering BALB/c mice is as high as in alcohol-preferring C57BL and CC57BR mice. Moreover, induced enzyme activity in BALB/c mice surpassed that in CC57BR mice (Table 2). No relationships were found between liver TDO2 activity, molecular structure of the corresponding gene, and genetic predisposition to alcohol consumption (at least in adult mice kept under standard conditions). It remains unclear whether these results concern the *TDO2* gene in humans that is a V.

TABLE 1. Single Nucleotide Substitutions in Intron 6 of the *TDO2* Gene in Mice of Various Strains

Position, bp (from the start of intron 6)	C57BL/6	CC57BR/Mv, BALB/cJ, DBA/2J	CBA/Ca
15	T	C	C
364	A	T	T
417	—	A	A
629	T	T	G
912	G	A	A
974	A	A	G

TABLE 2. Alcohol Consumption and TDO2 Activity in the Liver of C57BL/6J, CC57BR/Mv, and BALB/cJ Mice (*M*±*m*)

Parameter	C57BL	CC57BR	BALB/c
Ethanol consumption, % of total fluid consumption (*n*=10)	49.0±5.7*	42.0±3.2**	28.0±3.2
TDO2 activity, μmol/g liver/h			
basal (*n*=4)	4.40±0.45	5.00±0.37	4.80±0.32
glucocorticoid-induced (*n*=7)	8.70±0.64	8.40±0.32**	9.30±0.32

Note. *$p<0.01$ and **$p<0.05$ compared to BALB/cJ mice; *n*, number of animals in the group.

good candidate for the genetic determinant of behavior. A role of this gene in the development of polyetiological mental disorders requires further investigations.

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