

α -Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats

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Total gene expression analysis (TOGA) was used to identify genes that are differentially expressed in brain regions between the alcohol-naïve, inbred alcohol-preferring (iP), and -nonpreferring (iNP) rats. α -Synuclein, expressed at >2-fold higher levels in the hippocampus of the iP than the iNP rat, was prioritized for further study. *In situ* hybridization was used to determine specific brain regions and cells expressing α -synuclein in the iP and iNP rats. Similar to α -synuclein mRNA levels, protein levels in the hippocampus were higher in iP rats than iNP rats. Higher protein levels were also observed in the caudate putamen of iP rats compared with iNP rats. Sequence analysis identified two single nucleotide polymorphisms in the 3' UTR of the cDNA. The polymorphism was used to map the gene, by using recombination-based methods, to chromosome 4, within a quantitative trait locus for alcohol consumption that was identified in the iP and iNP rats. A nucleotide exchange in the iNP 3' UTR reduced expression of the luciferase reporter gene in SK-N-SH neuroblastoma cells. These results suggest that differential expression of the α -synuclein gene may contribute to alcohol preference in the iP rats.

Alcoholism is a complex disorder influenced by both environmental and genetic factors. The genetic contribution to alcoholism likely results from the action of multiple, possibly interacting, genes. Identification of the genes that influence alcohol drinking is an important area of research in the alcohol field and has involved several strategies, including the use of human studies and the development of genetic animal models of alcoholism. To date, the only consistently replicated findings from human studies are the functional polymorphisms in the alcohol metabolizing enzymes, alcohol dehydrogenase, and mitochondrial aldehyde dehydrogenase, which have protective effects in certain populations. Genome-wide analyses have, however, localized several chromosomal regions that may harbor genes that contribute to alcoholism susceptibility (1–3).

Animal models of alcohol preference have been used to identify both chromosomal loci and candidate genes that may influence alcohol-drinking behavior. The alcohol-preferring (P) and -nonpreferring (NP) rat lines were developed through bidirectional selective breeding from a randomly bred closed colony of Wistar rats [Wrm: WRC(WI)BR] on the basis of alcohol consumption and preference (4). In these lines, P rats display the phenotypic characteristics considered necessary for an animal model of alcoholism (5). Subsequently, inbred P (iP) and NP (iNP) strains were established that have maintained highly discordant alcohol consumption scores.

Quantitative trait locus (QTL) analysis and total gene expression analysis (TOGA, a registered trademark of Digital Gene Technologies) were conducted as complementary methods to identify genes that influence alcohol consumption in iP and iNP rats. A genome screen identified QTLs on chromosomes 3, 4, and 8 (6, 7). The chromosome 4 QTL produced a highly significant logarithm of odds score of 9.2 that accounts for 10% of the

phenotypic and $\approx 30\%$ of the genetic variation in alcohol consumption. TOGA identified differential expression of both known genes and 3' ESTs. Of the genes that were differentially expressed in the iP and iNP, α -synuclein was prioritized for further investigation because it was located in a region of mouse chromosome 6 syntenic to the rat chromosome 4 QTL, and it was shown to modulate dopamine transmission (8), which is likely involved with neurodegenerative and neuropsychiatric disorders such as alcoholism.

To better define the nature and effects of α -synuclein expression, (i) gene expression was localized to specific brain regions, (ii) protein expression was quantified in selected brain regions, and (iii) α -synuclein cDNA was screened for polymorphisms that might influence its expression.

Materials and Methods

TOGA Analysis. RNA was isolated from 10 alcohol-naïve male adult iP and 10 alcohol-naïve male adult iNP rats from (i) hypothalamus; (ii) hippocampus; (iii) caudate-putamen, nucleus accumbens, and olfactory tubercles; and (iv) prefrontal, frontal, and parietal cortex. TOGA analysis was performed as described (9).

Real-Time Quantitative PCR. The relative expression levels of α -synuclein in iP and iNP rats were determined by real-time quantitative RT-PCR with the ABI PRISM 7700 sequence detection system (PE Biosystems, Wellesley, MA). Each reaction contained 50 pg of cDNA template, 5 μ M forward and reverse primers, AmpliTaq Gold polymerase, and the SYBR Green PCR master mix (Applied Biosystems). The primers were selected by the integrated software package accompanying the ABI PRISM 7700 as follows: syn-1F 5'-ACAGTGAATACATGGTAGCAG-GCTC-3' and syn-1R 5'-GCCACAACAATATCCACAGCAC-3'. Each sample was amplified for 40 cycles. For each cDNA template, the cycle threshold (Ct) necessary to detect the amplified product was normalized to the Ct values of a control gene: cyclophilin (cyc-F) 5'-AGTGGAGAAACCCTTT-GCCA-3' and cyc-R 5'-GGTCCCCGAGGCTCTCTCTA-3'.

***In Situ* Hybridization.** Rat α -synuclein cDNA was cloned into the pCR4-TOPO vector (Invitrogen). From this construct, antisense and sense cRNA probes, labeled with 5'-[α -³⁵S]thio]UTP and 5'-[α -³⁵S]thio]CTP, were prepared. The tissues were hybridized according to published procedures (10). Briefly, 16- μ m brain sections were dehydrated and covered with 100 μ l of hybridization buffer (50% formamide/50 mM Tris, pH 8.0/2.5 mM

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Abbreviations: P, preferring; NP, nonpreferring; iP, inbred P; iNP, inbred NP; QTL, quantitative trait locus; TOGA, total gene expression analysis; cM, centimorgans; VTA, ventral tegmental area; SNP, single-nucleotide polymorphism; NSE, neuron-specific enolase.

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EDTA, pH 8.0/50 μ g/ml tRNA/1 $\times$ Denhardt's solution/0.2 M NaCl/10% dextran sulfate) containing 1.0×10^6 cpm of labeled probe for 16–18 h at 55°C. The sections were washed, dehydrated, and incubated in formamide buffer (0.3 M NaCl/50% formamide/20 mM Tris/1 mM EDTA, pH 7.5) for 10 min at 60°C, RNase A for 30 min at 37°C, and graded SSC washes (2 $\times$ SSC to 0.5 $\times$ SSC), followed by dehydration. The dried sections were exposed to Kodak MR film for 4–7 days, dipped in hypercoat LM-1 emulsion (Amersham Pharmacia), and exposed for 2–3 weeks at 4°C.

Sequence Analysis. Reverse transcription was used to generate cDNA (RETROscript kit; Ambion, Austin, TX) from RNA samples isolated from six iP and six iNP rats. Based on the rat α -synuclein cDNA sequence (ref. 11; GenBank accession no. AF007758), four primer pairs were designed: syn-2F 5'-GTGTGGAGCAAAGATACATC-3' and syn-2R 5'-GGCTTCAGGCTCATAGTCTTG-3'; syn-3F 5'-CAGTGAGGCT-TATGAAATG-3' and syn-3R 5'-GAGAACAGCAACC-AAAAAC-3'; syn-4F 5'-TAAGTGACTACCACTTATT-TCC-3' and syn-4R 5'-CACCATTATATACAAACACAA-3'; and syn-5F 5'-ATTTTAAATATGTGAGCATG-3' and syn-5R 5'-TTTTTTTTTTTGTGCAATGAGA-3'. By using iP and iNP cDNA, these primers were used to amplify four overlapping DNA fragments, covering the entire α -synuclein cDNA. Resulting PCR products were purified (GenElute PCR Cleanup kit, Sigma) and sequenced (Thermo Sequenase Cycle Sequencing kit, United States Biochemical).

Luciferase Reporter Constructs. Five primers were designed to amplify fragments encompassing the single-nucleotide polymorphism (SNP) at +439, the SNP at +679, or both: 5'-CTCT-AGAGAATGTCGTTGCACCTACTGT-3' (syn-6), 5'-CTCTAGAGAATGTCGTTGCACCCAC-3' (syn-7), 5'-CTCTAGACAGCACACAAGAGCCTGC-3' (syn-8), 5'-CTCTAGATGGATATTGTTGTGGCTTC-3' (syn-9), and 5'-CTCTAGAC-CACCATTTATATACAAACAGTAAAT-3' (syn-10). Using iP genomic DNA, three fragments were amplified by using primers syn-7 and syn-8, syn-7 and syn-10, and syn-9 and syn-10, respectively (Fig. 5). The same three fragments were amplified from iNP DNA substituting syn-6 for syn-7, because the +423 SNP was included in the primer. The six amplified fragments were ligated into the pCR2.1-TOPO vector and sequenced to confirm that there were no errors. The plasmid was digested with *Xba*I, and the gel-isolated fragment was ligated into the *Xba*I-digested pGL-3 promoter vector between the luciferase gene and the SV40 late poly(A) signal (Promega). The resulting luciferase constructs were then digested with *Bgl*II and *Bam*HI to determine fragment orientation. The constructs containing either the forward or reverse orientation of each fragment were isolated by using the EndoFree Plasmid Maxi kit (Qiagen, Valencia, CA).

Transient Transfection and Luciferase Assays. Human neuroblastoma SK-N-SH cells were cultured in Eagle's minimal essential medium containing 7.5% NaHCO₃, 2 mM Glut-max, 0.1 mM nonessential amino acids, 1 mM pyruvate, 10% FBS (Invitrogen), and maintained at 37°C in a humidified 5% CO₂ incubator. Twenty-four hours before transfection, 4.0×10^4 cells were plated into each well of a 24-well plate. Each pGL-3 luciferase test plasmid (0.5 μ g) was transfected per well by using Tfx 50 reagent (Promega). Cytomegalovirus (CMV; 2.5 ng) *Renilla* vector (pRL-CMV) was cotransfected with each pGL-3-luciferase test plasmid to serve as an internal control for transfection efficiency. Cells were incubated for 24 h, washed, and harvested by using passive lysis buffer. Cell extracts were assayed for firefly and *Renilla* luciferase activities in a TD-20/20 Luminometer, using the Dual-Luciferase Reporter Assay System (Promega). Assays were performed three to six times in triplicate,

using plasmids that were independently purified at least twice.

Genotype Determination and Gene Mapping. A dense map of markers in the chromosome 4 QTL region was genotyped previously. Thus, it was possible to map the α -synuclein SNP by selectively genotyping 54 animals at the extremes of the iP $\times$ iNP F2 alcohol consumption distribution (6). For genotyping, the fragment containing the +439 polymorphism was amplified from genomic DNA by using the syn-3F and syn-3R primers. Amplified DNA fragments were slot-blotted onto Zeta-probe membranes (Bio-Rad) and baked at 80°C for 2 h. Two allele-specific probes, NP (5'-TCGTTGCACCTACTGTCTTA-3') and P (5'-TCGTTGCACCCACTGTCTTA-3'), were end-labeled by using [γ -³²P]ATP. The α -synuclein alleles were distinguished by using buffers containing 3 M tetramethylammonium chloride (12). Filters were hybridized for 1 h at 55°C, and the critical washing temperature was 63°C for 20 min. Membranes were exposed to film, and the resulting genotypes were recorded. MAPMAKER/EXP (13) was used to place the α -synuclein SNP within a recombination-based map of chromosome 4 markers genotyped in the 381 iP $\times$ iNP F2 rats.

Western Blot Analysis. The hippocampus, striatum, amygdala, nucleus accumbens, and substantia nigra were dissected from another group of iP and iNP rats, protein extracts were prepared immediately (www.scbt.com; *Research Protocols*), and the analysis was performed on individual animals. Protein concentrations were determined with the Bradford assay (Bio-Rad). Neuron-specific enolase (NSE; molecular mass = 45 kDa) was used as the internal control to normalize the amount of protein loaded in each lane. Thirty micrograms of the protein supernatant was fractionated on a Novex 4–20% Tris-Glycine Gel (Invitrogen), and transferred to a poly(vinylidene difluoride) (PVDF) membrane (0.2 μ m, Bio-Rad). The membrane was blocked for 1 h in TTBS blocking buffer (5% nonfat dry milk/1 $\times$ Tris-buffered saline/0.1% Tween 20). Different methods were used to detect α -synuclein (molecular mass = 18 kDa) and NSE. The section of membrane containing α -synuclein was incubated overnight at 4°C with rat α -synuclein Ab (1:1,000; Cell Signaling Technology, Beverly, MA). After washing, the blot was developed by using the Phototope-HRP (HRP, horseradish peroxidase) Western Blot Detection System (Cell Signaling Technology). The membrane containing NSE was incubated for 1 h with rat NSE antisera (1:5,000, Polysciences). After washing, the membrane was incubated for 1 h with HRP-conjugated secondary Ab (1:15,000) and developed by using the Immuno-Star HRP Chemiluminescent kit (Bio-Rad). α -Synuclein levels were quantified by using IMAGEJ software (<http://rsb.info.nih.gov/ij/>) with each lane normalized to NSE levels.

Results

Differential Expression of α -Synuclein in iP and iNP. To identify genes that underlie alcohol-seeking behavior in iP and iNP rats, four brain regions, cortex, hippocampus, hypothalamus, and striatum, were analyzed by using TOGA (9). Expression differences were detected in 19,954 genes and ESTs. From this data set, 28 that exhibited 2-fold or greater change in expression were prioritized based on their expression pattern and function reported in the literature. α -Synuclein was selected to study first because of its chromosomal location in the mouse and its involvement with dopamine modulation. The 2-fold increase in α -synuclein expression in iP vs. iNP in the hippocampus is shown in Fig. 1A and real-time quantitative RT-PCR analysis confirmed this finding (Fig. 1B).

α -Synuclein mRNA Expression Pattern. To identify additional brain regions where α -synuclein was expressed, *in situ* hybridization

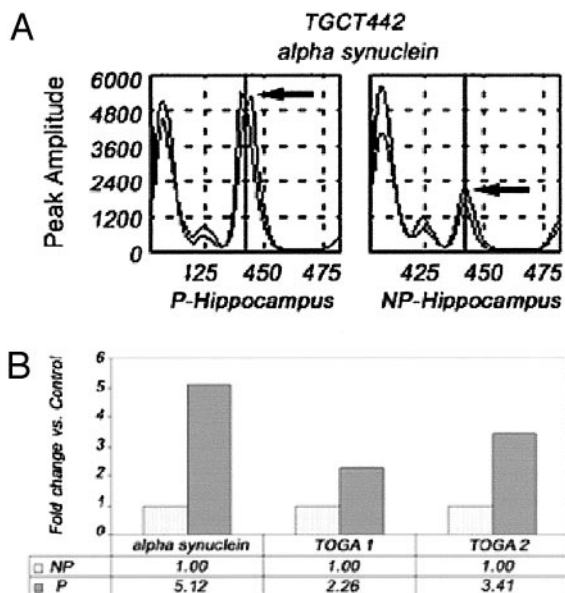


Fig. 1. α -Synuclein expression is increased in iP vs. iNP rat hippocampus. The TOGA profile for the product corresponding to rat α -synuclein is displayed in A. The displayed TOGA profile represents a small region of one of the 256 TOGA electropherograms for each of the templates prepared from the pooled samples of P-hippocampus (hippocampus from the iP rat strain) and NP-hippocampus (hippocampus from the iNP rat strain). A line is drawn through the TOGA PCR product corresponding to rat α -synuclein that has a digital address of TGCT442 (A, arrows). The two traces represent the duplicate TOGA runs for each sample. The relative expression levels of α -synuclein in the iP vs. iNP rat hippocampus was also evaluated by real-time quantitative RT-PCR (B). In B, the relative levels of α -synuclein in the iP as compared with the iNP are presented for the real-time quantitative RT-PCR analysis and for comparison to both duplicates in the TOGA experiment.

was performed in the iP and iNP rats (Fig. 2). α -Synuclein was widely expressed throughout the brain, with expression localized to specific brain regions and cells. An example of the distinctive pattern of expression of α -synuclein is shown in Fig. 2 E–G. α -Synuclein expression is evident in the medial habenular nucleus but there is no expression in the ependymal cells lining the dorsal third ventricle (D3V) (Fig. 2 F and G). Although expression in the ventral tegmental area (VTA) was not as prominent as that in the hippocampus (Fig. 2D), it is clear that expression is high in a subpopulation of cells in the VTA (Fig. 2K).

α -Synuclein Protein Levels in iP and iNP Rats. To compare α -synuclein protein expression in iP and iNP rats, brain regions were analyzed based on α -synuclein mRNA expression in regions rich in dopaminergic pathways (14): hippocampus, caudate putamen, amygdala, and nucleus accumbens. Similar to α -synuclein mRNA levels, the level of protein expression in the hippocampus was 1.7-fold higher in iP rats than iNP rats (Fig. 3). The same trend was observed in the caudate putamen, with a 1.6-fold difference between iP and iNP rats (data not shown). No difference in expression was detected in the amygdala or the nucleus accumbens between the iP and iNP rats. It is clear from this study that there are regional differences in α -synuclein expression in rat brain.

Polymorphism in the α -Synuclein cDNA. To determine whether a sequence difference might underlie the expression difference observed between the iP and iNP rats, the α -synuclein cDNA was sequenced in each strain (Fig. 4). Two SNPs were discovered in the 3' UTR. The iP sequence displayed a T to C substitution at +439 relative to the translation start site (+1), and an A to G

substitution at +679, whereas the iNP sequence was identical to the published rat α -synuclein cDNA sequence at both of these positions (ref. 11; GenBank accession no. AF007758). Six additional polymorphisms were also identified in which the cDNA sequences of iP and iNP rats were identical with each other but differed from the published rat α -synuclein cDNA sequence (Table 1). An alignment of the iP and iNP sequences with another α -synuclein cDNA sequence (www.tigr.org, TC 284516) determined that the iP and iNP sequences were more similar to this sequence than the published sequence and that the A to G substitution both at +679 and +807 are unique SNPs.

Activity of 3' UTR SNPs in Transiently Transfected SK-N-SH Cells. To determine whether the 3' UTR SNPs were functional, reporter plasmids were constructed containing either both SNPs or one of the SNPs (Fig. 5). All constructs were transiently transfected into human neuroblastoma SK-N-SH cells, which constitutively express the endogenous α -synuclein gene. Activities of the reporter constructs were expressed as fold changes compared with the pGL-3 promoter vector. The nucleotide exchange at +679 resulted in a 2-fold greater expression in the iP reporter construct P₂ compared with the iNP construct NP₂ ($P < 0.05$). The SNP at +439 produced no significant difference in expression between constructs P₁ and NP₁. There was a trend for the expression of the NP₁₊₂ construct to be lower than the P₁₊₂ construct. Reporter constructs with α -synuclein 3' UTR in the reverse orientation gave similar results; the iNP constructs with the +679 SNP repressed expression (data not shown). These results suggest that the SNP at +679 may be responsible for the differential expression observed between the iP and iNP strains.

α -Synuclein Is Mapped to Chromosome 4 in the Rat. α -Synuclein has been mapped to chromosome 4q21.3-q22 in humans (15) and chromosome 6 in mice at 29 centimorgans (cM; ref. 16). It has not been mapped in the rat. Using the SNP that was discovered at +439, rat α -synuclein was mapped by using recombination-based methods to chromosome 4, 1.3 cM distal to *D4Rat34* and 0.3 cM proximal to *D4Rat35* (Fig. 6). According to this mapped position, α -synuclein is located at the peak (57.3 cM) of the identified QTL on chromosome 4 that is associated with alcohol consumption in the iP $\times$ iNP F2 rats (6) (Fig. 7).

Discussion

In this study, a difference in α -synuclein mRNA expression in the hippocampus between iP and iNP rats was discovered and confirmed. α -Synuclein protein expression levels were different between the iP and iNP lines in the hippocampus and the caudate-putamen. A polymorphism in the 3' UTR of α -synuclein cDNA altered expression of the luciferase reporter gene in transient transfection assays. The polymorphisms were used as markers to map α -synuclein to a locus on chromosome 4 at the peak of a previously identified QTL for alcohol preference in the iP and iNP rats.

The α -synuclein gene codes for a 140-aa protein, is expressed throughout the central nervous system, and is particularly abundant in presynaptic nerve terminals (11, 17, 18). Recent data suggest that α -synuclein regulates dopamine synthesis by interacting with tyrosine hydroxylase, inhibiting its activity and leading to a reduction in dopamine synthesis (8). Overexpression of human α -synuclein has been shown to cause death of dopaminergic neurons in human primary cultures (19). In addition, transgenic mice exhibited degeneration of dopaminergic terminals and α -synuclein-immunoreactive inclusions in neurons in the hippocampus, neocortex, and substantia nigra (20). To date, however, the functional role of α -synuclein regarding dopaminergic dysfunction remains unclear.

Expression efficiency of the rat α -synuclein gene in the luciferase assay was significantly altered because of a polymor-

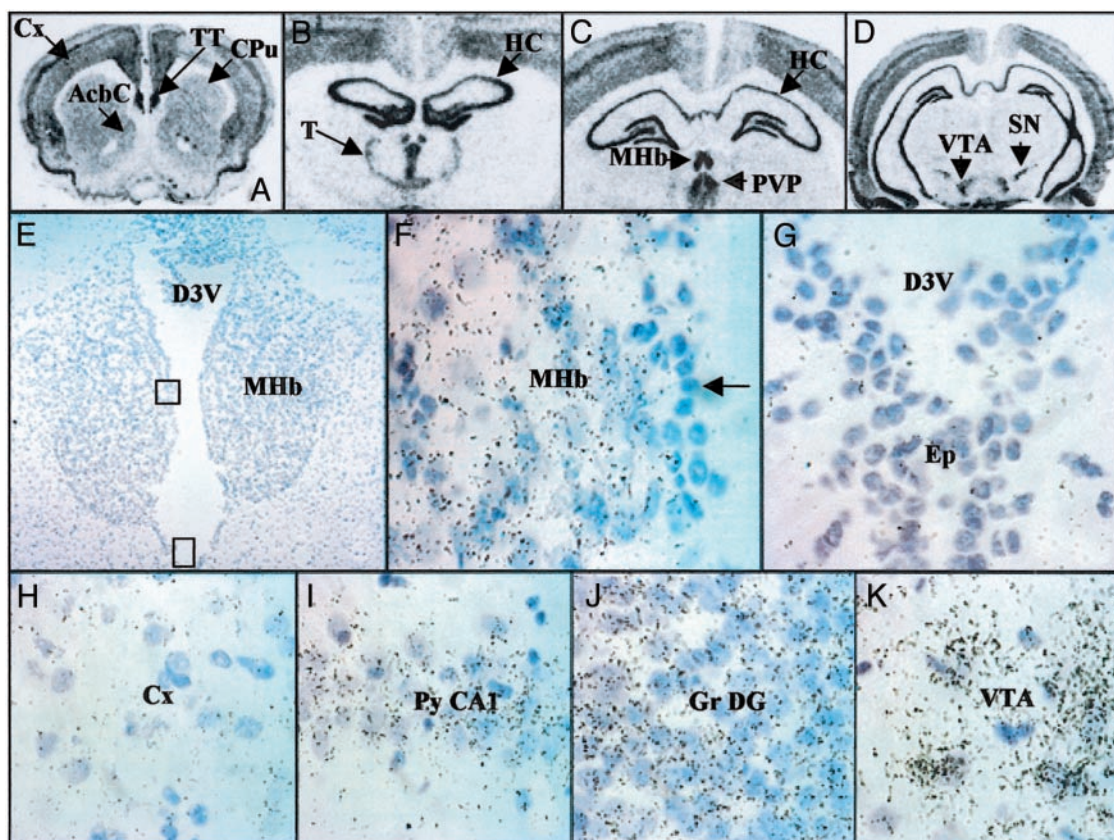


Fig. 2. Cellular localization of α -synuclein expression in iNP rat brain (A–D). After dipping the ^{35}S -radiolabeled sections in emulsion and counterstaining with eosin Y and Mayer's hematoxylin, silver granula were observed (E–K). (A–F) Expression in: (A) cortex (Cx), tenia tecta (TT), caudate putamen (CPu), nucleus accumbens core (AcbC), $\times 2.5$; (B) hippocampus (HC), thalamic nucleus (T), $\times 4$; (C) hippocampus, medial habenular nucleus (MHb), posterior paraventricular thalamus nucleus (PVP), $\times 3$; (D) substantia nigra (SN), VTA, $\times 3$; (E) MHb, dorsal third ventricle (D3V), $\times 40$; (F) Magnification of the square in E. α -Synuclein is expressed only in the cells of the MHb; the arrow indicates ependymal cells around D3V that do not express α -synuclein, $\times 800$. (G) Magnification of the rectangle in E to show lack of α -synuclein expression in ependymal cells (Ep), $\times 800$. (H–K) Expression levels in cells of cortex, pyramidal cells of CA1 (Py CA1), granular cells of dentate gyrus (Gr DG), and VTA, $\times 800$.

phism in the 3' UTR region. Although neuroblastoma cells may not fully reflect regulatory mechanisms underlying α -synuclein expression in brain cells, they show that this SNP is functional in a cell that expresses α -synuclein. The reduced expression exhibited by the +679 polymorphism is consistent with the lower α -synuclein mRNA and protein levels detected in the hippocampus of the iNP strain compared with the iP strain. Numerous reports have identified the eukaryotic mRNA 3' UTR as a primary site for the regulation of mRNA stability (21–23). In addition, regulation by the 3' UTR can also occur at the level

of mRNA subcellular localization and translation initiation (24, 25).

α -Synuclein has been implicated in the etiology of several neurodegenerative disorders, including dementia with Lewy

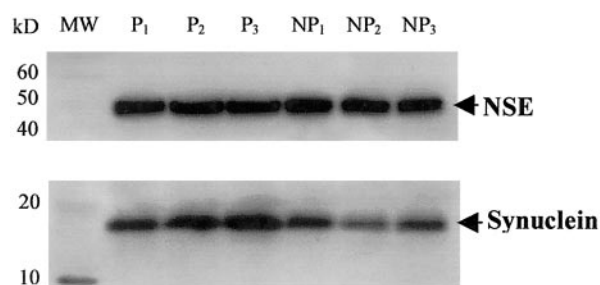


Fig. 3. In the hippocampus of iP and iNP rats, α -synuclein protein expression was analyzed by using quantitative Western blot analysis. The blots were probed with both α -synuclein Ab and NSE antisera. The NSE signal was used to normalize the amount of neuronal protein loaded per lane.

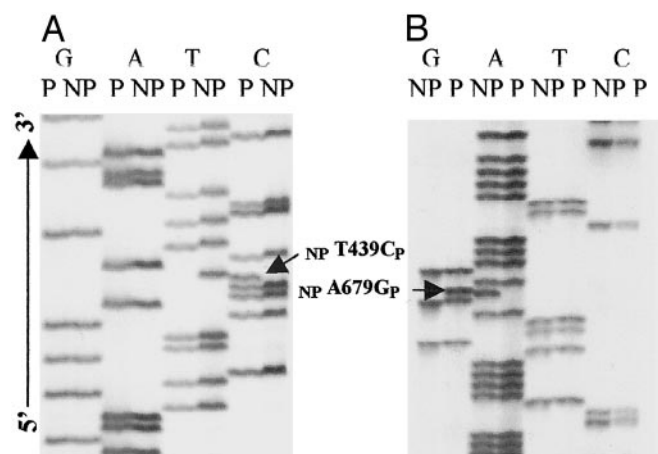


Fig. 4. Two SNPs in the 3' UTR of α -synuclein when comparing the cDNA sequences of iP and iNP rats. (A) An arrow denotes an SNP at +439 in which a C was observed in the iP strain, whereas a T was observed in the iNP strain. (B) The iNP sequence displayed an A at +679, whereas the iP displayed a G.

Table 1. α -Synuclein sequence differences

Name	Position							
	439	679	717	735	807	867	961	966
iP rat	C	G	T	T	G	T	Deletion	T
iNP rat	T	A	T	T	G	T	Deletion	T
TC 284516	C	A	T	T	A	T	Deletion	T
AF 007758	T	A	C	G	A	C	A	C

bodies, multiple system atrophy, and Parkinson's disease (26–28). In Parkinson's disease, there is selective loss of dopaminergic neurons and the presence of Lewy bodies, which are largely composed of α -synuclein. Dopamine functions as an important neurotransmitter in many neurochemical pathways and has been implicated in the etiology of several neuropsychiatric disorders, including schizophrenia, major depression, and drug and alcohol dependence. In fact, both schizophrenia and major depression have been associated with alcoholism and alcohol dependence (29–31), and major depression has also been documented at a higher frequency in patients with Parkinson's disease (32).

Dopaminergic neurons projecting from the VTA to the nucleus accumbens play a major role in mediating the rewarding properties of drugs of abuse, including alcohol (33). Ethanol administration increases dopamine release in the nucleus accumbens of rats, and dopamine antagonists reduce self-administration of ethanol (34, 35). P rats seem to have an innate deficiency in this dopaminergic pathway. Dopamine levels have been shown to be 25–30% lower in key limbic structures (e.g., nucleus accumbens) of P rats (13) and in high-drinking iP $\times$ iNP F2 rats (36). Furthermore, there is a selective reduction in the number of projections from dopamine neurons projecting from the VTA to the nucleus accumbens in P rats relative to NP rats (37). Consequently, a protein such as α -synuclein that may play a role in the modulation of dopaminergic neurotransmission (18, 38) becomes a candidate gene of considerable interest for alcohol-seeking behavior.

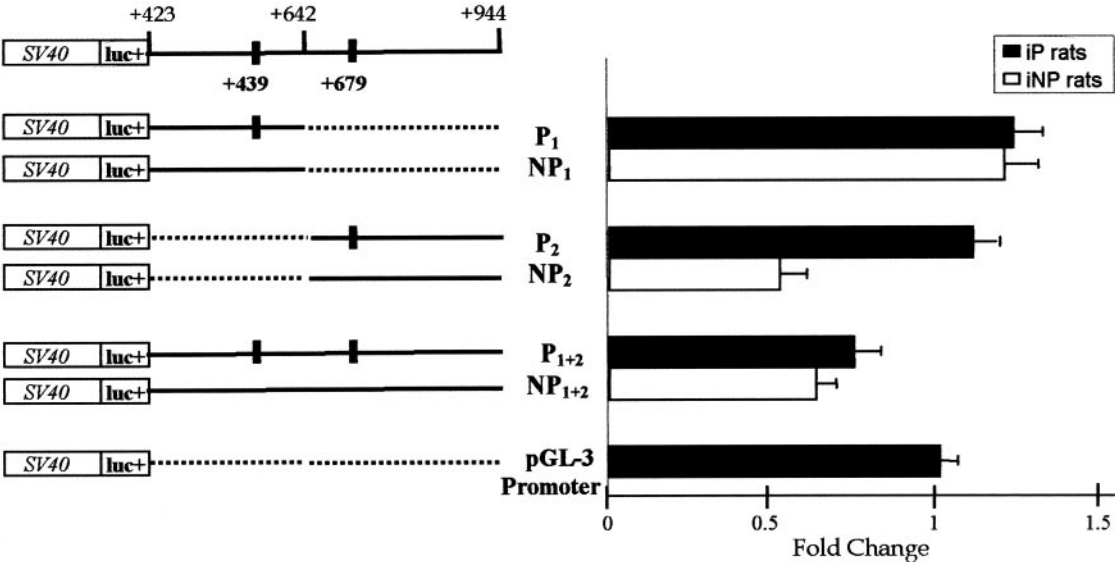


Fig. 5. Functional importance of the two 3' UTR SNPs. Plasmids pGL-3 promoter, P₁, P₂, P₁₊₂, NP₁, NP₂, and NP₁₊₂ were transfected into SK-N-SH cells. SV40 and luc+ denote the SV40 promoter and luciferase gene, respectively. The polymorphisms, located at +439 and +679, in the 3' UTR are noted. The activity of each construct was normalized by cotransfection of the internal control plasmid, pRL-CMV, and was expressed as fold change compared with the activity of the pGL-3 promoter vector, which was designated as 1. The bars and fold change show the mean $\pm$ SEM of the results from eight (forward orientation) independent transfection experiments performed in triplicate, using two different plasmid preparations. Significance of difference in mean values within and between multiple constructs was analyzed by using ANOVA.

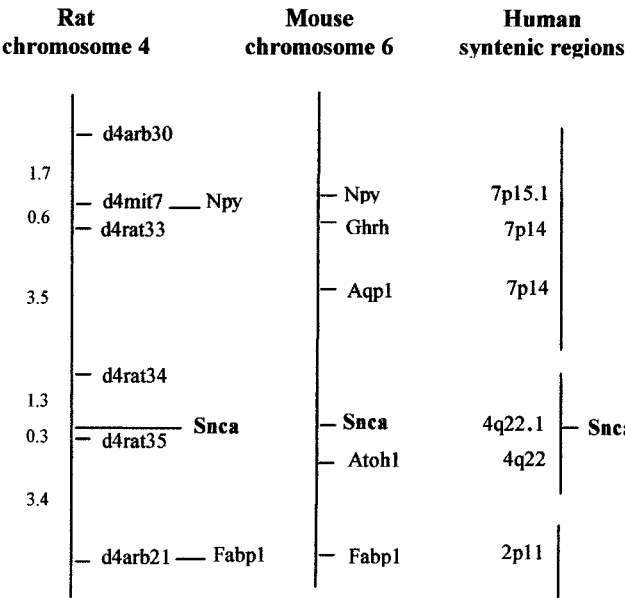


Fig. 6. The genetic map demonstrates the synteny among human, mouse, and rat with respect to the QTL region discovered on rat chromosome 4 (6). By using iP and iNP rats, Snca (α -synuclein) was mapped in cM to the chromosome 4 QTL region. Human and mouse syntentic data were obtained from www.rgd.mcu.edu/and www.informatics.jax.org.

By using a polymorphism that was identified in the α -synuclein cDNA, the gene was mapped 5.4 cM from the neuropeptide Y (*Npy*) gene, a candidate gene being studied for alcohol-seeking behavior. This region is located at the peak of the alcohol consumption QTL identified on rat chromosome 4 with a logarithm of odds score of 9.2 (6, 7). It is intriguing that the rat *Npy* and α -synuclein genes are linked, located within close physical proximity on the same chromosome, and both potentially capable of influencing alcohol consumption. With high-

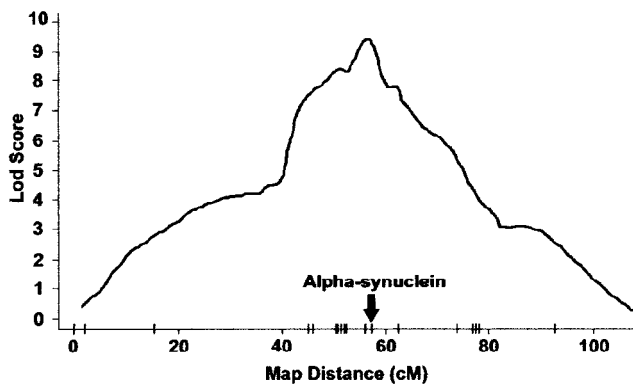


Fig. 7. α -Synuclein is located at the peak of the QTL region with a logarithm of odds score of 9.2 for alcohol consumption. The arrow marks the position, 57.3 cM, of α -synuclein on the map.

resolution mapping, it has been found that there is often more than one gene influencing a trait within a QTL region (39, 40). We speculate that the large QTL effect for alcohol consumption in the iP and iNP rats may be caused by the combined effects of multiple genes within the QTL region.

Additional studies will provide insight into the relationship between α -synuclein and alcohol consumption. Immunocytochemistry is currently underway to detect differential expression of α -synuclein in regions that may influence dopamine function,

but not previously tested by TOGA. Studies to examine alcohol consumption using α -synuclein transgenic and knockout mice and to determine how ethanol administration affects α -synuclein expression in iP and iNP rats are planned. Finally, it will be necessary to determine the mechanism by which the +679 polymorphism alters mRNA expression.

The rat chromosome 4 region containing α -synuclein is syntenic with human 4q22, a region that harbors a cluster of alcohol dehydrogenase genes. Polymorphisms in several of the genes in this gene cluster are clearly associated with a decreased risk of alcoholism (41, 42). This region also displays linkage to an alcohol-related phenotype, the maximum number of drinks consumed in a 24-h period (43). It will be interesting to determine whether variation in the human α -synuclein gene is associated with an alcohol-related phenotype. Interestingly, microarray analyses of mRNA expression in frontal and motor cortices of human alcoholic and control cases showed that α -synuclein expression was altered in the alcoholic group (44). Taken together, it is tempting to speculate that α -synuclein, a gene differentially expressed in alcohol-preferring rats and humans with alcoholism and located in chromosomal regions known to be associated with alcohol-drinking behavior, may have effects on the risk of developing alcoholism in some human populations.

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