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Short communication

Five mutations in the $GABA_A$ α6 gene 5′ flanking region are associated with a reduced basal and ethanol-induced α6 upregulation in mutated Sardinian alcohol non-preferring rats ☆

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Abstract

The presence of four nucleotide changes and a three base-pair deletion in the $GABA_A$ α6-subunit promoter is described in Sardinian alcohol non-preferring rats, selectively bred for their ethanol aversion. These mutations are associated with the R100Q α6 intragenic mutation that was previously characterized in the same animals. The possibility that these mutated nucleotides alter the ethanol-induced upregulation of the α6 gene was investigated by measuring cerebellar α6 mRNA levels after a chronic ethanol liquid diet in sNP rat. Real-time quantitative PCR showed an increased α6 gene expression after ethanol ingestion in normal and mutated rats. However, lower amounts of α6 mRNA levels were detected both in control and in ethanol-treated sNP rats carrying the five promoter and the intragenic mutations in a homozygous state. Using the electromobility shift assay, specific DNA binding sites were found in cerebellar extracts of the α6 regions comprising the five mutations. These results suggest that one or more of the mutated binding sites that were found in the 5′ flanking α6 region may be a consensus sequence for regulatory factors which are responsible for both basal and ethanol-induced α6 gene expression.

Theme: Cellular and molecular biology
Topic: Gene structure and function: general

Keywords: Alcohol; Animal models; GABA A; Gene regulation; Transcription

The functional and pharmacological properties of $GABA_A$ receptors mostly depend on their isoform composition, which is extremely variable in different brain areas. The $GABA_A$ α6 subunit is uniquely expressed in the cerebellar granule cells and vestibular cochlear cells and confers insensitivity to benzodiazepine agonists [12]. Cerebellar expression of the α6 gene depends on its 5′ flanking sequence in which there is a minimal promoter [1,6,13] containing several consensus sequences for regulatory factors which might be critical in enhancing promoter activity in the cerebellum.

Ethanol, a positive $GABA_A$ receptor modulator, produces changes in the mRNA expression of the α subunits in different brain regions [5], including the cerebellum where an upregulation of the $GABA_A$ α6 subunit is observed following a chronic ethanol treatment [14].

In a previous study [18], we have characterized an intragenic point mutation (R100Q) in the $GABA_A$ α_6 gene of a rat line selectively bred for its alcohol non-preferring

☆ This work is dedicated to the memory of Dr. Anna Porcella.

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phenotype (sNP) [3], a mutation which was also found in ANT [7] and ANA [2] rats. We have also described a positive association between the α6 mutation and some polymorphisms of the β2, α1, γ2 $GABA_A$ gene cluster [4] in sNP rats.

We describe here the presence of four nucleotide changes and a three base-pair deletion in the 5′ flanking sequence of the α6 gene of sNP rats carrying the intragenic R100Q mutation and the $GABA_A$ genes cluster polymorphism. Real-time PCR was used to assess whether these mutated nucleotides might influence the basal and the ethanol-modulated expression of the α6 gene in sNP rats. Finally, the presence of specific DNA–protein interactions in normal and in mutated sites was investigated using cerebellar extracts and an electromobility shift assay (EMSA) to check whether these mutations could generate differential DNA–protein patterns in wild type and mutated rats.

Genomic DNA was prepared from tail biopsies according to a simplified procedure (as previously described; [9]). The five polymorphisms were found by sequencing the 5′ α6 gene 1172 base-pair region amplified using primers 1 and 5 (Table 1). The PCR reactions were sequenced with internal primers (Table 1) using the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Kit and loaded in the ABI PRISM™ 310 Genetic Analyzer.

Animals carrying the intragenic mutation were found to have four nucleotide changes and a three base-pair deletion at the following positions from the start site: −195(T→A), +70(C→T), +163(T→G), +281(C→T), and +105/108(−GAA) (Fig. 1). This result supports the hypothesis that an ancestral mutated $GABA_A$ α6 gene, present in the founder Wistar strain, carrying the six mutated nucleotide positions, has been selected for more than 40 generations, exclusively in the sNP rat line.

In light of this evidence, it was important to investigate whether $GABA_A$ α6 mRNA expression was differentially regulated by chronic ethanol in wild type and mutated sNP rats. Long-term ethanol administration using a liquid diet was carried out as previously described [15] on 8 wild type (RR) and 8 mutated (QQ) rats. Control animals (8 per group) were pair-fed with dextrose substituted isocalorically

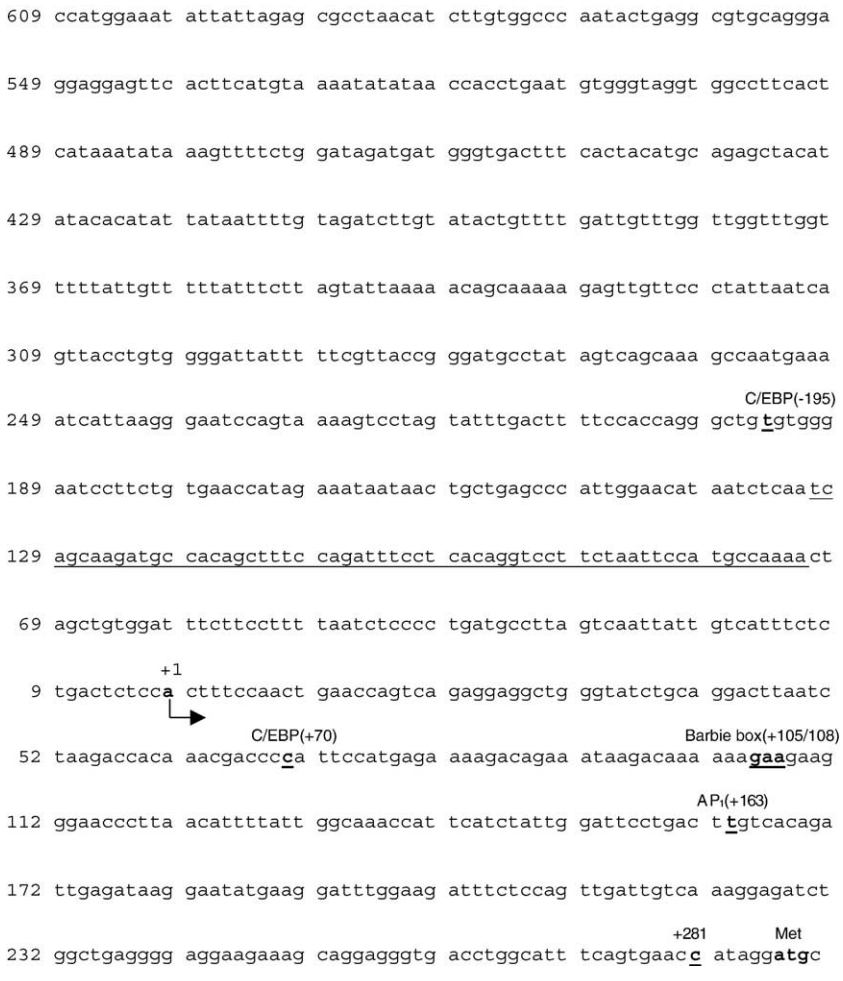

Fig. 1. Nucleotide sequence of the rat $GABA_A$ α6 subunit promoter (GenBank X97476). Nucleotide relative positions are numbered with respect to the position of a putative major transcriptional start site (+1), indicated with an arrow [4]. Nucleotides located upstream of the start site are negatively numbered, and those belonging to the 5′ untranslated region are positively numbered. The five mutations are indicated in bold underlined characters. The underlined region, spanning from the −131 to the −71 position, is critical for the enhanced promoter activity in cerebellar granule cells. The first codon (MET) is also shown.