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RESEARCH ARTICLE

Hoxb8 Gene Polymorphism in Fur Chewing Chinchilla – A Preliminary Study

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ABSTRACT: The fur chewing in animals is being mentioned among the obsessive- compulsive disorders. It is a group of behavioral dysfunctions of unknown origin, occurring in almost all vertebrates. Hoxb8 gene is involved in embryonic development and is extensively expressed in the CNS, in the regions responsible for grooming behavior in mammals. The loss of function mutation in Hoxb8 gene induces phenotypic symptoms comparable to the human trichotillomania - obsessive – compulsive disorder, in the course of which the patients uncontrolled pluck their hair from different areas of the body. The aim of the study was to analyze Hoxb8 gene sequence in fur chewing chinchillas for the presence of single nucleotide polymorphisms (SNPs). On the basis of the analyzed sequences, the presence of two point mutations was detected: transversion A>C (g.1451C>A), resulting in the substitution of proline to glycine and the transition A>G (g.1654A>G), resulting in substitution of aspartic acid to glycine. In the analyzed group were detected both homozygous AA, GG, and heterozygous AG.

Keywords: Hoxb8 gene, fur chewing, chinchilla

INTRODUCTION

Obsessive - compulsive disorders (OCD) affect 2-3% of the human population. Their treatment is extremely difficult and is mainly based on cognitive behavioral therapy, and in some cases, pharmacotherapy. There are many scientific works trying to determine the biological basis of OCD, but the understanding of this type of diseases is based on the creation of a suitable animal model that reproduces the phenotypic effects and, if possible, the neuronal symptoms (Rauch *et al.*, 2001; Spruijt *et al.*, 1992; Welch *et al.*, 2007; Chou-Green *et al.*, 2003; Garner *et al.*, 2004). Obsessive - compulsive disorders, aside from induced phenotypic effects, are often an ailment of animals, both farmed and accompanying. Due to the vague causes and the lack of effective treatments, they are a big problem for breeders. And that, horses and pigs biting metal rods (Bachmann 2002; Dodman 1996 birds plucking their feathers (Jenkins 2001), dogs and cats licking and gnawing a hair on the legs and tail (9), or chinchillas chewing their fur coat (Ponzio *et al.*, 2012)(Figure 1), became the object of study of many scientists. Strong interest in obsessive compulsive disorders, directed the attention of researchers towards the genetics. Currently, in order to better understand the bases of OCD in humans, animal genetic models are using, among which mouse mutants are exhibiting symptoms of fur chewing, inter alia, the Hoxb8 gene mutation (Greer 2002). 1.1 Hoxb8 gene mutation Hoxb8 is part of a mammalian homeobox complex, containing 39 transcription factors. These factors are best known for their roles in the early stages of embryonic development when determining the anterior-posterior position of animals (Capecchi 1997). But this is not their only function.

Hoxb8 is extensively expressed in the CNS, in the regions responsible for the behavior of rodents in hair grooming. The loss of function mutation in Hoxb8 gene induces phenotypic symptoms comparable to the human trichotillomania - obsessive – compulsive disorder, in the course of which the patients uncontrolled pluck their hair from different areas of the body (13). Therefore researchers decided to use a line of genetically modified mice as a model of the disease (Greer 2002). Studies have shown that mice with a mutation of the gene Hoxb8 chewed their hair coat themselves and others mice in the same cage, leaving large areas of the body without the coat. In one cage, usually was "dominant" animal , which bit fur of others, and if it was left alone, bit hair of its own. All mice in this line, both homo- and heterozygotes have shown a normal reaction to heat, cold, pain and pleasure (Kurien 2005).

MATERIALS AND METHODS

The material for the study was the lower limbs muscle tissue obtained from chinchillas carcasses from fur chewing and normal ones. No animal was slaughtered directly for the needs of this research. Tissues were from carcasses, representing a by-product formed during the normal process conducted for the purpose of obtaining fur on one of the Polish chinchilla farms. Tissues, until the DNA isolation, were stored in a freezer at -20°C. DNA isolation were performed with using of the commercial kit to isolating nucleic acids. Primers for the PCR reaction, due to the high complementarity of gene sequence in different species, were designed on the base of mouse Hoxb8 gene sequence, available in the NCBI database (NCBI Ref. Seq.: NM_010461.2). Each PCR amplification was conducted

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in a 20 µL reaction mixture, which included 0.4 µM of each primer, 200 µM dNTPs, 3mM MgCl2, 0.25 U of Taq polymerase (Promega), 1× bufor Taq, and approximately 30 ng of genomic DNA as a template. The amplification went through 30 cycles of 94°C denaturation for 30 s, annealing at 61°C for 30 s, extension for 45 min, and a final extension for 8 min at 72°C in Arktik Thermal Cycler (Thermo Scientific). PCR products were sequenced using ABI Prism 377 DNA Sequencer and analyzed in bioinformatic software (FinchTV, CodoneCode Aligner).

RESULTS

PCR products resulted in two fragments containing the coding sequence: first a 208 bp (1257-1465 bp, N = 14) and the second with a length of 201 bp (1500-1701 bp, N = 16). On the basis of the analyzed sequences, the presence of two point mutations was detected: transversion A>C (g.1451C>A), resulting in the substitution of proline to glycine and the transition A>G (g.1654A>G), resulting in substitution of aspartic acid to glycine. In the analyzed group were detected both homozygous AA, GG, and heterozygous AG.

CONCLUSIONS

As a result of the attempts to understand and know the causes of obsessive – compulsive disorders carried out so far, it seems to be reasonable to conduct additional studies, which could significantly broaden the knowledge on the subject. Studying the Hoxb8 gene polymorphism in fur chewing chinchillas will enable us to verify the hypothesis of the genetic background of the disease.

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Figure 1: Fur chewing chinchillas

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CONFLICTS OF INTEREST
"The authors declare no conflict of interest".