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Small Eye Phenotypes Observed in a Human *tau* Gene Transgenic Rat

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ABSTRACT We developed a rat line showing small eye from transgenic rats that were obtained by microinjection of a DNA segment containing the human (h)*tau* cDNA (GenBank: BC000558: 31-677,774-1180) expressed under control of CAG promoter, which is related to Alzheimer disease, into the pronuclei rat embryos. The rat line was established by selective brother-sister mating of rats showing small eyes. Of 11 offspring in the 11th generation, there were eight animals with microphthalmia and the transgene. The remaining three rats without transgene did not show the small eyes phenotype. The globes of affected rats were 1.2 mm in length compared with normal globes (3.5 mm), and all other ocular structures were normal. The expression of hTau protein was evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium. Cytogenetic analysis suggested that the chromosome location of the transgene was chromosome 1 (1p12). This region may include genes related to lens development, such as *Cat5*.

KEYWORDS *Cat5*; cataract; rat; small eye; Tau

INTRODUCTION

It is reported that Parkinson disease is inherited as an autosomal dominant gene, and the gene is linked to chromosome 17q21-22.[1] Human (h)*tau* gene was located on the locus, and mutation of the gene was found in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). The neurofibrillary tangles composed of microtubule-associated protein Tau are related to not only the FTDP-17 but also to Alzheimer disease. The gene encoding microtubule-associated protein has been reported in rats as well, and the gene (Map1a) was linked to chromosome 3q36.[2] Many transgenic mouse lines that express hTau protein have been established to investigate the relationship between the hTau protein and tauopathy.[3] Recently, we generated three transgenic rats (founder) carrying the h*tau* gene, and they showed small eyes.

Mice and rats with ocular phenotypes, such as aniridia,[4] cataract,[5] microphthalmia,[6] and small eyes,[7] were reported, and a number of genes, including *Bld*,[8] *Cat*,[9] *Maf*,[10] and *Pax*,[11] related to these abnormalities have been reported as well. In this study, we characterized the phenotype of transgenic rats with small eyes, and the candidate gene causing the phenotype was predicted.

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MATERIALS AND METHODS

Transgenic Constructs and Animals

h*tau* cDNA (GenBank: BC000558: 31-677,774-1180) and rabbit β-globin polyA provided by Dr. Oyama (Department of Neuropathology, University of Tokyo)[12] were microinjected into the pronuclei of fertilized Jcl:SD rat (CLEA Japan Inc., Tokyo, Japan) embryos, and the embryos were transferred to the oviducts of pseudopregnant SD rats. Testing for the transgene in offspring was performed by polymerase chain reaction (PCR). The primers and conditions for the PCR are described below. In histopathological, immunochemical, and fluorescent *in situ* hybridization (FISH) analysis, four out of the 8 rats, aged 8 weeks, with small eyes were used. The rats were maintained in accordance with the Animal Care Guidelines of the Central Institute for Experimental Animals (Kanagawa, Japan).

PCR Analysis

To select rats carrying the h*tau* gene, PCR analysis was performed using the oligonucleotides, t1 (5′-AAG CTC GCA TGG TCA GTA AA-3′) and t2 (5′-GAC TTG ACA TTC TTC AGG TC-3′), and *Taq* polymerase (Takara Shuzo, Co., Ltd., Shiga, Japan) according to the manufacturer's protocol.

Histopathology

The formalin-fixed materials were embedded in paraffin, and 5-μm sections were stained by a standard method with hematoxylin and eosin (HE). The sections were examined under a light microscope to evaluate morphologic characteristics and pathologic changes. For detection of hTau protein in the tissues, all sections were stained by the dextran polymer–immunoperoxidase complex method (ENVISION kit, DakoCytomation, Kyoto, Japan) using anti-bovine Tau (mouse) serum (EMB Biosciences, Inc., San Diego, CA, USA) at 1:5000 dilution as the primary antibody and then counterstained with hematoxylin.

FISH Analysis

Determination of the chromosomal location of the h*tau* gene in the transgenic rats was undertaken by FISH analysis, and closely linked genes associated with ophthalmopathy were screened using the rat genome database (http://rgd.mcw.edu/). The chromosome samples were prepared from mitogen-stimulated splenocytes of transgenic rats. The biotin-16-dUTP-labelled *tau* cDNA clone in the pCXN2 vector[13] was used for hybridization. FISH analysis was performed essentially as described by Matsuda et al.[14] Observations were carried out with a Leica Q550 system (Leica Microsystems K.K., Tokyo, Japan), and chromosomes with fluorescent signals were identified according to G-banding standards.

RESULTS

Forty-five rats in total were obtained from the founder male rat carrying the h*tau* gene. Twenty-three out of the 45 rats had the h*tau* gene, and 3 of 23 (2 males and one female) rats showed small eyes. F2 rats were obtained by mating between a rat with small eyes and a Jcl:SD rat, and the animals were maintained by selective breeding of a small-eye line and brother-sister mating. At the 11th generation, 11 offspring were obtained, and 8 of the 11 offspring showed small eyes.

Histopathological Analysis

The globes of affected rats were 1.2 mm in length compared with normal globes (3.5 mm), and all other ocular structures were normal (Figs. 1 and 2A). Vacuolation was observed in lens of the rats, but no lesions were observed in other tissues such as the cornea and iris. These abnormalities were observed only in rats of this line bearing the h*tau* gene. On the other hand,

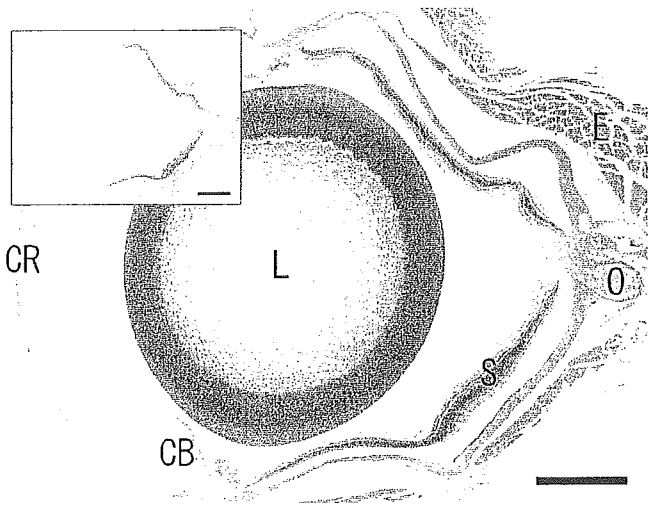
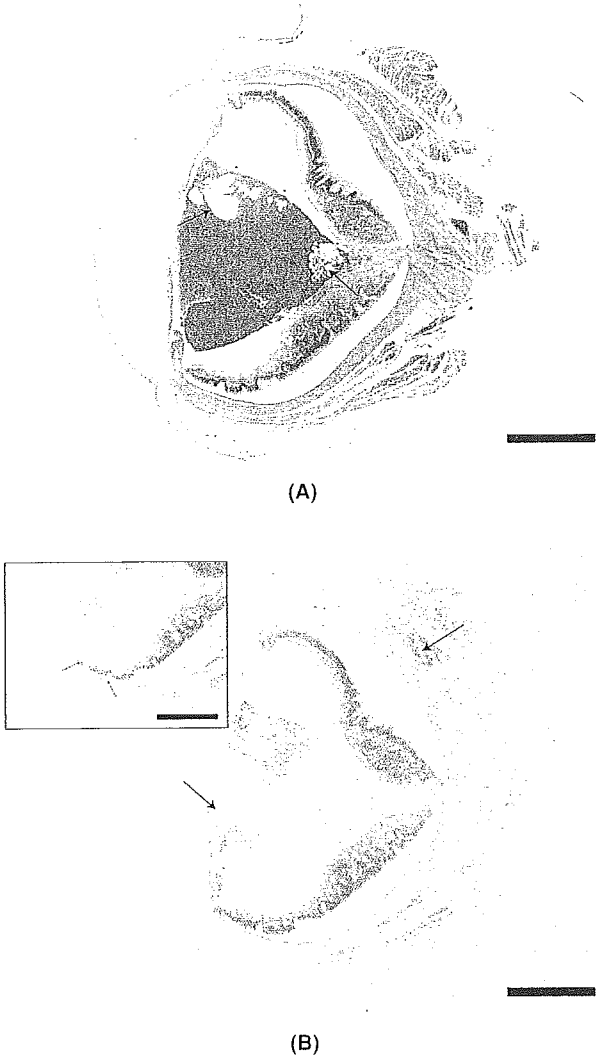


FIGURE 1 Ciliary body (CB), cornea (CR), extraocular muscle (E), lens (L), optic nerve (O), and sclera (S) from a rat not bearing the *tau* gene (H&E). Inset shows immunohistochemical stain, hematoxylin counterstain. Expression of *Tau* protein in the normal eye from a rat not bearing the *tau* gene. Bar = 1 mm.



(A)

(B)

FIGURE 2 (A) Small lens observed in human *tau* gene transgenic rats. Vacuolation is present in the lens (arrows) (H&E). Bar = 1 mm (B). Immunohistochemical stain, hematoxylin counterstain. Tau protein expression evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium (arrow). Bar = 1 mm. Inset shows detail of ciliary body and pigment epithelium. Bar = 1 mm.

ophthalmic lesions were not observed in wild-type rats and rats from other rat lines carrying the gene (data not shown). Tau protein expression was evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium (Fig. 2B).

Location of the h*tau* gene was analyzed by the FISH method, and the gene was found to be located on chromosome 1p12 (Figs. 3 and 4).

DISCUSSION

Tau protein is a microtubule-associated protein. In Alzheimer disease, Pick disease, and corticobasal degeneration, typical mutations were found in the gene.

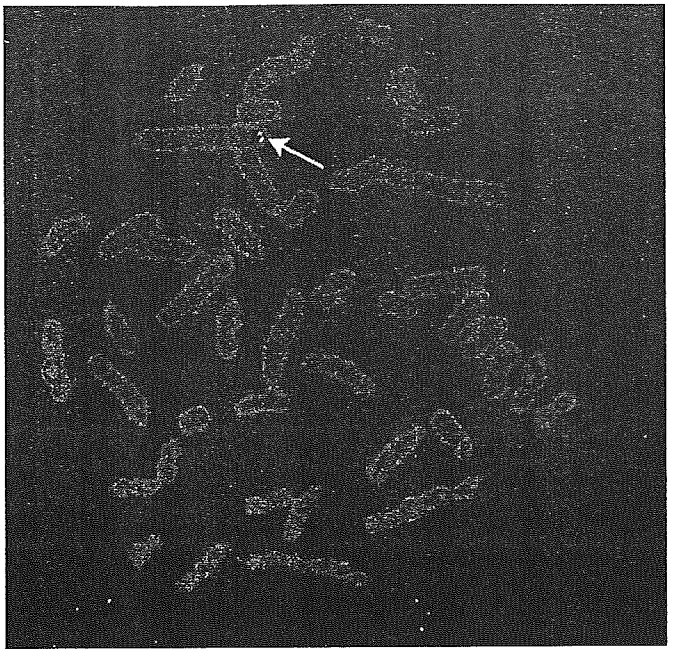


FIGURE 3 Chromosomal location of the human *tau* gene in a transgenic rat. The signal was visualized indirectly with FITC (arrow).

Mutations that affect exon 10 splicing cause frontotemporal dementia with parkinsonism.

In this study, we obtained 23 transgenic rats carrying the h*tau* gene from one founder transgenic rat, and in three out of the 23 rats small eyes appeared. Histopathologically, the lens of the rats was small in size with vacuolation. Lens development is regulated by a variety of genes, such as L-*Maf*, *Pax6*, and *Sox2*. Microphthalmic rats and mice caused by mutation of these genes were reported previously,[15,16] but all of them were not caused by the *tau* gene. Lewis et al. reported eye irritations in mice expressing mutant Tau protein but microphthalmia was not observed.[17] Only three (2 males and one female) of these 23 transgenic rats showed small eyes, suggesting that small eyes observed in this study were not caused by h*tau* gene. The human *Pax6* gene was first reported as a candidate gene for evolution of morphogenesis of the eye.[18,19] In rats, the *Pax6* gene is located on chromosome 3q32-3q36. Because the transgene in the rats with small eyes was mapped to chromosomal 1p12, it was suggested that the *Pax6* gene was not related to abnormalities in this study. Based on the database analysis of the transgene locus (1p12) in rats, several genes have been mapped in the locus. In the locus, *Cat5* was mapped as a cataract-related gene.

In conclusion, we established a rat line, that shows small eyes from transgenic rats carrying h*tau* gene.

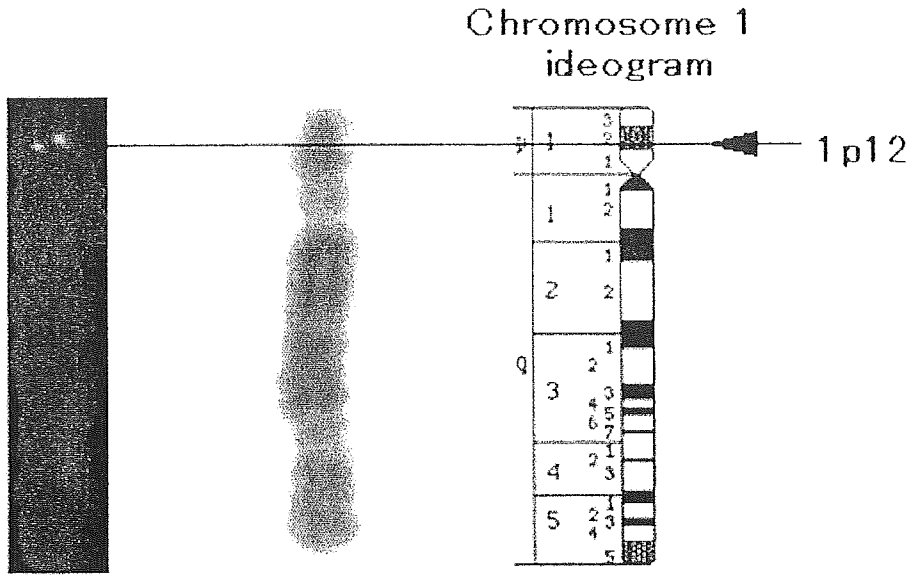


FIGURE 4 **Ideogram showing the cytogenetic location of *tau* in 1p12.**

Typical phenotypes were characterized by a small lens with vacuolations observed in the lens. The map location of the transgene suggested that the candidate gene causing small eye is located in 1p12.

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