学 位 論 文 の 要 旨

氏名　　吉田　学

学　位　論　文　名

New genetic model rat for congenital cataracts due to connexin46(Gja3) mutation

発　表　雑　誌　名　　Pathology International 55, 732-737, 2005
（巻，初頁～終頁，年）

著　　者　　名　　Manabu Yoshida, Yuji Harada, Sachiko Kaizu, Akihiro Ohira, Junichi Masuda and Toru Nabika

論 文 内 容 の 要 旨

INTRODUCTION

We found a mutant rat suffering from congenital nuclear cataract in our rat colony and established a genetic model for cataract. In this report, we genetically analyzed the new model and identified a point mutation in the connexin 46 (Cx46) gene, which is a strong candidate for the mutation responsible for the cataract in this strain.

MATERIALS AND METHODS

[Animals] In the process of establishing a congenic rat strain for a blood pressure quantitative trait locus, we identified rats with congenital cataracts in our colony. Through brother-sister matings, a cataract strain, SHRSP.WKY-(D1Mgh5-D1Rat44)^{Cat}/Izm (abbreviated to SHRSPwch1.9^{Cat}) was established. This rat strain showed bilateral congenital nuclear cataracts with 100% penetrance.

[Linkage analysis] A control strain without cataracts, WKY.SHRSP-(D1Wox18-D1Rat44)/Izm, was

mated with SHRSPwch1.9^{Cat}. The F1 generation was then backcrossed with SHRSPwch1.9^{Cat} to obtain 93 rats for linkage analysis. The initial screening was done using 46 simple sequence repeat (SSR) markers selected from throughout the genome. On a chromosome linked with cataracts, additional SSR markers were selected for further analysis.

[Expression studies] The expression of Cx46 was studied with immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR). In RT-PCR, total RNA was extracted from eyes obtained from neonatal rats. Reverse transcription was conducted using the M-MLV reverse transcriptase with oligo dT primers. PCR was performed using two sets of primers. In the immunohistochemical experiments, frozen sections of eyes either from the cataract rats or from the control rats were stained with rabbit anti-rat Cx46 IgG..

[Sequencing] The coding region of the Cx46 gene was amplified by PCR using 7 sets of primers. Direct sequencing was performed using the dideoxy sequencing method with a capillary sequencer.

RESULTS AND DISCUSSION

In the initial two-point linkage analysis using 46 SSR markers selected from the entire genome, The clusterin locus on chromosome (chr) 15 was found to be linked with the development of cataracts at a highly significant level (LOD=24.7). Then several SSR markers were selected to map the cataract gene on chr 15. The cataract gene was mapped to the D15Rat6 locus with no recombinations (LOD=27.4). A review of the Rat Genome Database revealed that Gja3, the gene for Cx46, is located only 3.4 Mbp from D15Rat6. As a homozygous deletion of Gja3 resulted in congenital cataracts in a knockout mouse, this

gene was regarded as a strong candidate for the cataract gene in SHRSPwch1.9^{Cat}. The lenses derived from both the cataract and the control rats were stained positively with anti-Cx46 antibody. RT-PCR indicated that the Cx46 gene was expressed in the eyes of the cataract rats without any significant truncation. These observations strongly suggested that the cataracts in this rat strain were not due to a lack of Cx46 expression.

We next sequenced the coding region of the Cx46 gene in SHRSPwch1.9^{Cat}. A comparison of the sequence with that of the rat Cx46 registered in GenBank revealed two single nucleotide substitutions, G to A at 330 and G to C at 1069. The former introduced an amino acid substitution (Glu to Lys) at codon 42, while the latter was synonymous. Further analysis of 6 different inbred strains (SHRSP/Izm, ACI/NKyo, BN/SsNSlc, DON, F344/NSlc, and WTC) revealed that the G330A substitution was unique to the cataract rats while G1069C was a genetic polymorphism shared by the 6 strains. No recombination was observed between the G330A mutation and the cataract phenotype in the backcrossed population. Further, comparison of amino acid residues among the connexin family indicates that Glu at codon 42 is highly conserved among the connexin family in various species. These findings support that the G330A (Glu42Lys) substitution in the Cx46 gene was a mutation responsible for cataracts in SHRSPwch1.9^{Cat}.

CONCLUSION

We established a new animal model for cataracts caused by a mutation in the Cx46 gene. This model will be a useful tool in studies both on the pathogenesis of cataracts and on the function of connexins.