Mutational analysis of the human *MBX* gene in four Korean families demonstrating microphthalmia with congenital cataract

Toshinobu Miyamoto[1], Young S. Yu[2], Hisashi Sato[1], Hiroaki Hayashi[1], Naoko Sakugawa[1] Mutsuo Ishikawa[1], Kazuo Sengoku[1]

[1]Department of Obstetrics and Gynecology, Asahikawa Medical College, Asahikawa, Japan, and [2]Department of Ophthalmology, Seoul National University Children's Hospital, Seoul, Korea

SUMMARY: Miyamoto T, Yu YS, Sato H, Hayashi H, Sakugawa N, Ishikawa M, Sengoku K. Mutational analysis of the human MBX gene in four Korean families demonstrating microphthalmia with congenital cataract. Turk J Pediatr 2007; 49: 334-336.

The *MBX* gene is a novel paired-type homeobox gene. It plays a number of critical roles in the development of the eyes in the zebrafish. The knockdown of the mbx expression by morpholino antisense oligonucleotides leads to a reduction in the size of eyes and tectum in the zebrafish. We investigated whether the human *MBX* gene was associated with susceptibility to microphthalmia by analyzing four Korean families demonstrating microphthalmia with congenital cataract. Mutational analysis was performed on the human MBX gene using these families. However, no mutations could be detected. Therefore, no indications were found for an association between the MBX gene and microphthalmia with congenital cataract in humans.

Key words: MBX, mutational analysis, congenital cataract, microphthalmia.

Homeobox genes play a number of critical roles in early central nervous system (CNS) patterning[1]. The *Mbx* gene, a novel paired-type homeobox gene, was identified in the zebrafish and human genomes[2]. The *MBX* homeodomain possesses some similarities to that of Pax family proteins. Since knockdown of mbx expression by morpholino antisense oligonucleotides (mbx-MO) leads to a reduction in the size of the eyes and tectum in the zebrafish[2], mbx function has been proposed to be involved in anterior brain development, including the formation of the eyes and tectum. On the other hand, the microinjection of double-stranded RNA targeted to Pax6.1 is associated with the depressed expression of Pax6.1, thus resulting in an absent or greatly reduced eye and forebrain development in the zebrafish[3]. We previously isolated the mouse Mbx cDNA and analyzed its expression pattern during early mouse embryogenesis. The Mbx homeodomain is identical among mice, zebrafish and humans. Therefore, a high homology also exists in the domain between the mouse Mbx and Pax family. The expression of the mouse Mbx is predominantly restricted to the midbrain region at E9.5[4,5]. In Drosophila and the mouse, the Pax6 gene is required for eye formation and its mutation causes both eyeless and small-sized eye phenotypes, respectively[6,7].

In humans, microphthalmia is one of the most common ocular birth defects and a significant cause of congenital blindness[8]. The etiology of microphthalmia is diverse, with several genetic mutations associated with this condition along with potential environmental causes[9]. Mutations in the CHX10 (recessive) and SHH (dominant) genes correlate with cases of human microphthalmia[10,11]. One of the most common ocular malformations associated with microphthalmia is congenital cataracts. Homozygous mutations in the PAX6 are known to cause anophthalmia and its heterozygous mutations can cause aniridia[12]. Based on the phenotypes of the knockdown of the mbx in zebrafish and the patients

with mutations on the PAX6 in humans, we analyzed the human MBX gene in four Korean families with microphthalmia accompanied by congenital cataract only.

Human MBX cDNA was isolated in 2002[2], and the nucleotide sequence is now available from GenBank under accession numbers AF398527 and AF398528. Human MBX cDNA consists of two distinct splice variants producing long and short forms (MBX-L and MBX-S) that differ by only five amino acids. This MBX-L cDNA consists of 1,155 nucleotides and spans an open reading frame from nucleotide 7 to 1155, encoding 363–amino acid proteins. We herein report the results of mutation analysis of the MBX gene in four Korean families with microphthalmia and congenital cataract only. The full-length cDNA sequences were compared with the human genomic sequences and all exon-intron borders were determined. The coding region is composed of exon 1 to exon 4. The mutation screen of MBX in the patients from the four families was performed in all coding regions. The analyzed patients were the following: MC31, MC47, MC45, MC44, MC41, MC42 (family 1), MC30b, MC28, MC29, MC30 (family 2), MC13b, MC13, MC14 (family 3), and MC17b, MC17 and MC18 (family 4) (Fig. 1). All patients participating in this study gave their informed consent for a molecular analysis to be performed on their blood samples, and the study protocol was approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis, Seoul National University. Genomic DNA was obtained from peripheral lymphocytes using QIAGEN Blood and Cell Culture DNA Midi Kit (QIAGEN, Hilden, Germany). Nested polymerase chain reactions (PCRs) were carried out with primers designed to be homologous to the intronic regions adjacent to each exon, from exon 1 to exon 4, and the samples of human DNAs. All oligonucleotide primers are listed in Table I. The resultant PCR products were used for a direct sequence analysis. However, no mutation or polymorphism was detected in any patient (data not shown).

Knockdown of the mbx expression leads to a reduction in the size of the eyes and tectum in the zebrafish[2]. It was suggested that mbx may play a critical role in the formation of the eyes and tectum in the zebrafish. The expression of the mouse Mbx is almost completely restricted to the midbrain region at E9.5[4,5]. The findings of these studies were very interesting, however, there have to date been no reports on the functions of the mouse *MBX* gene. Creating knockout mice is no longer such a difficult task.

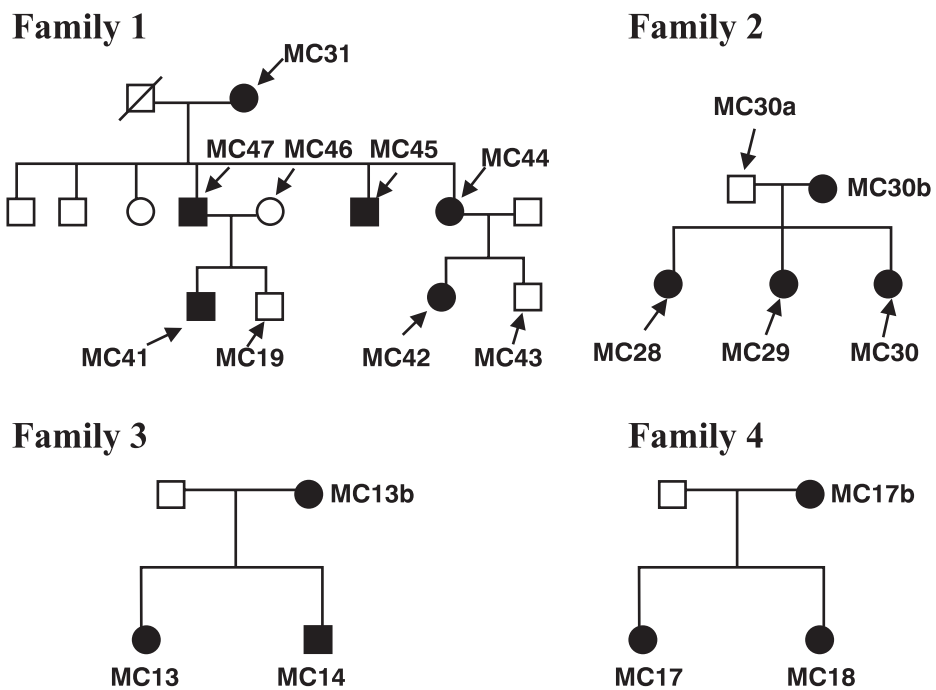

Fig. 1. Pedigree of families 1, 2, 3 and 4.

Table I. Oligonucleotide Primers Used in Mutation Analysis

Exon	Primer sequence
1	HMBXE1F1=5'-ATCAGAAGGTGGGCATCCAG-3' HMBXE1F3=5'-TCCCTTTTCCGTCTGTAGGC-3' HMBXE1R1=5'-ACAAGCTTGGCCAGTGCTCC-3' HMBXE1R3=5'-ACAGTCCTGTCCCCATGGTC-3'
2	HMBXE2F1=5'-ATATGGAGAGGGGATGGCTG-3' HMBXE2F2=5'-CTAAGGGTAAGGGACCAGGG-3' HMBXE2R1=5'-CATGCTGGCTCCTGGACAAG-3' HMBXE2R2=5'-ATGGGCTGGTGTTTGTCTGC-3'
3	HMBXE3F1=5'-CGTACATCCTCTCCCAGAGC-3' HMBXE3F2=5'-CAACCCCACTTCTTTCTTGC-3' HMBXE3R1=5'-GAGGGGCAGTTGATTACTCG-3' HMBXE3R2=5'-GACTTGCTTCACACGCTCAG-3'
4	HMBXE4F1=5'-GTGAGAAGGCTGAGTGCACC-3' HMBXE4F3=5'-GTTCTTGGGTTCTCTGCTTG-3' HMBXE4R1=5'-CCACCTAGGGATAACCACAG-3' HMBXE4R3=5'-CTGGGTTGGAAGCCAGACAG-3'

In addition, many of the homo-mutant mice have been reported after the interesting genes were isolated in mice. The mouse Mbx was isolated in 2002, over four years ago. We could not detect any mutation or polymorphism in the human MBX from the four families that were analyzed herein. Therefore, the MBX (Mbx) genes may have some other functions in mammals.

In conclusion, our investigation did not detect any association between the human MBX gene and microphthalmia accompanied by congenital cataract only.

Acknowledgements

We thank Mr. S. Bayley for checking our manuscript. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 16390471, 15591725 and 16790934) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

1. Beddington RS, Robertson EJ. Anterior patterning in mouse. Trends Genet 1998; 14: 277-284.
2. Kawahara A, Chien CB, Dawid IB. The homeobox gene mbx is involved in eye and tectum development. Dev Biol 2002; 248: 107-117.
3. Li YX, Farrell MJ, Liu R, Mohanty N, Kirby ML. Double-stranded RNA injection produces null phenotypes in zebrafish. Dev Biol 2000; 217: 394-405. Erratum in: Dev Biol 2000; 220: 432.
4. Miyamoto T, Kawahara A, Teufel A, et al. Mbx, a novel mouse homeobox gene. Dev Genes Evol 2002; 212: 104-106.
5. Ohtoshi A, Nishijima I, Justice MJ, Behringer RR. Dmbx1, a novel evolutionarily conserved paired-like homeobox gene expressed in the brain of mouse embryos. Mech Dev 2002; 110: 241-244.
6. Quiring R, Walldorf U, Kloter U, Gehring WJ. Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. Science 1994; 265: 785-789.
7. Hill RE, Favor J, Hogan BL, et al. Mouse small eye results from mutations in a paired-like homeobox-containing gene. Nature 1991; 354: 522-525. Erratum in: Nature 1992; 355: 750.
8. Levin AV. Congenital eye anomalies. Pediatr Clin North Am 2003; 50: 55-76.
9. Voronina VA, Kozhemyakina EA, O'Kernick CM, et al. Mutations in the human RAX homeobox gene in a patient with anophthalmia and sclerocornea. Hum Mol Genet 2003; 13: 315-322.
10. Ferda Percin E, Ploder LA, Yu JJ, et al. Human microphthalmia associated with mutations in the retinal homeobox gene CHX10. Nat Genet 2000; 25: 397-401.
11. Scimmenti LA, De La Cruz J, Lewis RA, et al. Novel mutation in sonic hedgehog in non-syndromic colobomatous microphthalmia. Am J Med Genet 2003; 116: 215-221.
12. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet 1994; 7: 463-471. Erratum in: Nat Genet 1994; 8: 203.