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A mutation of the *WFDC1* gene is responsible for multiple ocular defects in cattle

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ABSTRACT

Multiple ocular defects (MOD) in cattle is an autosomal recessive hereditary disorder characterized by dysplasia of the lens, retinal detachment, persistence of the hyaloid artery, and microphthalmia. The locus responsible for MOD has been mapped to the proximal region of bovine chromosome 18. In the present study, we refined the localization of the MOD locus to a 1.0-Mb interval by haplotype analysis using a pedigree of affected animals. Comparison of nucleotide sequence of genes in this region revealed a one-nucleotide insertion in the *WFDC1* gene, which resulted in a frame shift mutation and premature termination codon at the middle of the protein. WFDC1 is a small secretory protein containing a WAP-type four disulfide core domain. Specific expression of Wfdc1 was observed in the lens, retina, and optic nerves of embryonic and adult mouse eyes by immunohistochemical staining and *in situ* hybridization. The present finding demonstrated the essential role of *WFDC1* in mammalian eye development.

Introduction

Multiple ocular defects (MOD) in cattle is an autosomal recessive hereditary disorder characterized by dysplasia of the lens, retinal detachment, persistence of the hyaloid artery, and microphthalmia [1] (Fig. S1). In the eyes of affected animals, the formation of many components of the eyes, including the lens and retina, are severely affected. Complete absence of the anterior chamber, hypoplastic and ectopic lenticular tissues, and retinal dysplasia with total detachment were observed in the affected eyes. The most characteristic feature of the affected eyes is the column of the hyaloid artery emerging from the optic nerve head through the vitreous cavity (Fig. S1). This phenotype of multiple defects in eye formation indicates that the gene responsible for MOD plays an important role in mammalian eye development. Recently, we have mapped the locus responsible for MOD to a 6.6-cM region on the proximal part of bovine chromosome 18 [2].

There are many genes involved in mammalian eye development, and mutations in these genes cause developmental defects of the eye, including dysplasia of the lens, retinal detachment, and microphthalmia. For example, mutations in the *MAF*, *MITF*, *PROX1*, and *PAX6* genes have been reported in a wide range of eye defects in humans and mice [3]. Although there are some phenotypic similarities between MOD and these eye defects, the multiple defects in many components of the eye observed in MOD are different from the phenotypes caused by mutations in these genes. On the other hand, the persistence of the hyaloid artery and retinal detachment have been described in human familial exudative vitreoretinopathy (FEVR) [4], Coat disease [5], retinopathy of prematurity [6], and Norrie disease [7]. In particular, FEVR is a heterogeneous disease with highly variable phenotypes, showing a persistent hyaloid artery and abnormalities in other eye components, including the retina and lens, and the severe form of FEVR is accompanied by microphthalmia, retinal dysplasia and lens degeneration [4]. Mutations in the *FZD4*, *LRP5*, and *NDP* genes, involved in the Wnt signaling pathway, have been identified in patients with FEVR [7–10]. The pathological findings of MOD suggest its similarity to the severe forms of human FEVR disease, but the chromosomal localization of these genes does not correspond to that of the MOD locus on bovine chromosomes.

In the present study, we determined the precise localization of the MOD locus on bovine chromosome 18 by haplotype analysis, identified the gene responsible for MOD in this region, and found a frame shift mutation in this gene. Expression of this gene in mouse eyes was also examined by RT-PCR, immunohistochemical analysis, and *in situ* hybridization to investigate the function of the gene in eye development.

Results

Fine mapping of the MOD critical region

The MOD locus has been mapped to a 6.6-cM region (5.6 Mb) of bovine chromosome 18, flanked by two microsatellite markers,

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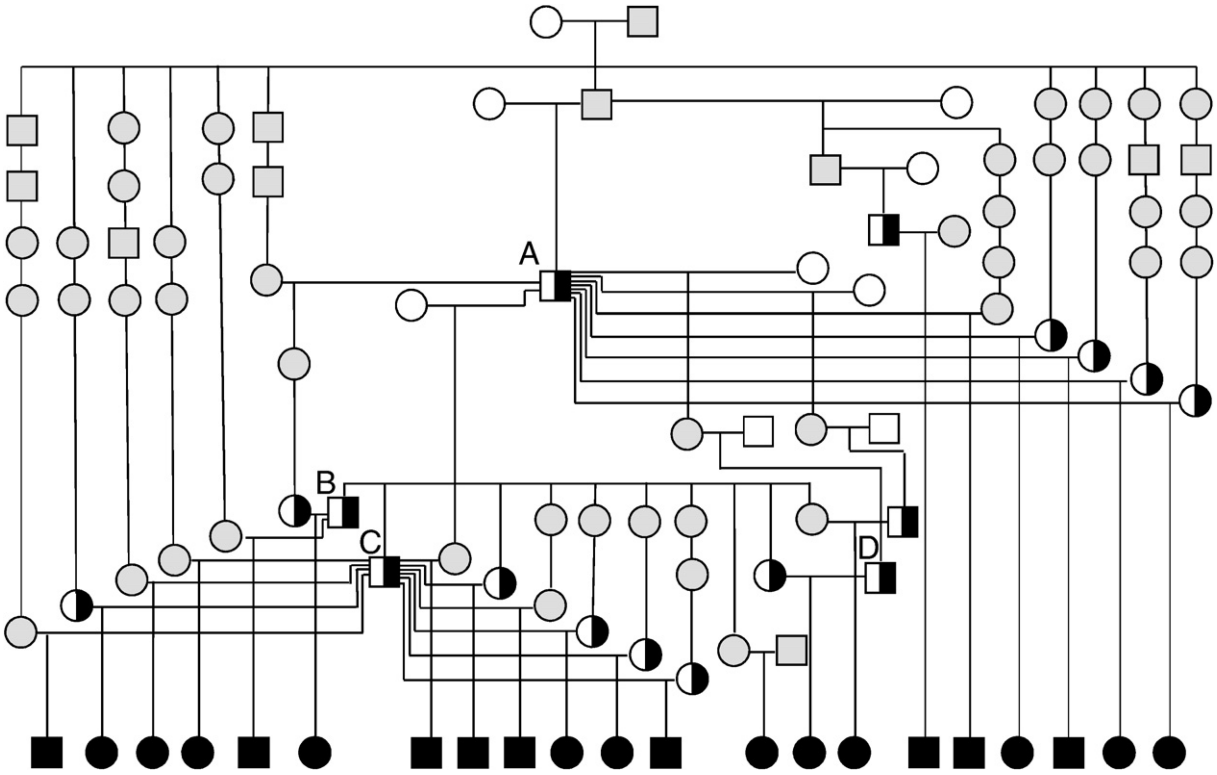

Fig. 1. Inbred pedigree of cattle with MOD. The pedigree was comprised of descendants of a single founder sire, and DNA samples of 6 sires, 12 dams, and 21 affected and 68 normal calves of the pedigree were collected. Filled and half-filled boxes or circles represent affected animals and carriers, respectively. Gray boxes or circles indicate possible carriers whose DNA samples could not be obtained but presumed to transmit the mutant allele from the founder to the affected animals.

BMS1322 and *DIK2175* [2]. We developed new microsatellite markers in this region, and seven markers (Table S1) showing heterozygosity in the carrier sires were genotyped in the animals of the inbred pedigree (Fig. 1) to refine the critical region for the MOD locus. The genotyping data (Fig. 2) indicated that all the affected animals were homozygous for *ABS13*, while one and two recombination events were observed with two adjacent markers, *MOK1801* and *MOK1805*, respectively. In addition, a single nucleotide polymorphism (SNP) in the *NECAB2* gene also indicated no recombination with the *MOD* locus. As shown in Fig. 2, the haplotypes of the markers in this region were highly conserved in all affected animals and the carrier sires, indicating that the mutant allele of the MOD locus is identical by descent and originated from the common founder in the pedigree. The haplotype data clearly indicated that the *MOD* locus lies in a region between *MOK1801*and *MOK1805* (Fig. 2). According to the cattle genome assembly BTA4.0 (B. Taurus linear scaffolds as of 2007-Sep-13 in HGSC at Baylor College of Medicine), this region spans 1.0 Mb and contains 12 known genes (Fig. 3A).

Marker	Position (Mb)	A		B		C		D		(14)		(1)		(2)		(1)		(2)		(1)	
BMS1322	8.80	3	2	3	2	3	2	3	2	2	2	2	2	2	2	2	2	2	2	3	2
MOK1801	8.96	1	5	3	5	1	5	1	5	5	5	5	5	5	5	5	5	5	5	1	5
NECAB2	9.53	a	g	a	g	g	g	g	g	g	g	g	g	g	g	g	g	g	g	g	g
ABS13	9.74	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2
WFDC1	9.76	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
MOK1805	9.97	1	3	3	3	2	3	1	3	3	3	3	3	3	3	3	3	1	3	3	3
DIK4861	10.22	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MOK1804	10.44	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
MOK1803	10.51	3	4	4	4	1	4		4	4	4	4	4	4	4	4	4	3	4	4	4
MOK1802	10.53	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2
MOK1807	12.25	3	3	1	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
MOK1806	12.54	2	2	3	2	3	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2
DIK2175	14.45	2	1	2	1	2	1	2	1	1	1	2	1	3	1	2	1	2	1	1	1

Sires | Affected offspring

Fig. 2. Haplotype of markers on MOD critical region. Genotypes of 11 microsatellite and one SNP markers in the carriers and affected animals of the pedigree are shown. Genotypes of the *WFDC1* gene, with (−) and (+) showing the mutant and wild-type alleles, respectively, are also indicated. The haplotype associated with the mutant allele is shown by gray boxes. The numbers of animals with each haplotype are shown in parentheses. A to D represent carrier sires in Fig. 1. The positions of these markers in cattle genome assembly BTA4.0 (B. Taurus linear scaffolds as of 2007-Sep-13 in HGSC at Baylor College of Medicine) are indicated.