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Genetic mapping of a locus associated with bovine chronic interstitial nephritis to chromosome 1

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Summary

Chronic interstitial nephritis with diffuse zonal fibrosis (CINF) occurs in Japanese Black cattle (Wagyu) as an autosomal recessive disorder leading to death prior to puberty, first six months or a year of life. We performed a genome-wide scan using microsatellite markers in a Wagyu pedigree segregating for CINF and mapped the *CINF* locus to bovine chromosome 1. *CINF* was closest to microsatellites *BM9019* and *INRA49* (Z score = 12·0; $P < 3{\cdot}4 \times 10^{-10}$).

Keywords: chronic interstitial nephritis, autosomal recessive disorder, linkage study, microsatellite

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Introduction

Most renal diseases are related to defects in glomerular filtration and tubular reabsorption. Glomerular basement membrane defects are hereditary in humans and include Alport syndrome and benign familial hematuria. Familial juvenile nephronophthisis and polycystic disease are hereditary and manifested as tubular or interstitial structural defects (see reviews: Hildebrandt 1995; Scheinman 1998; Zerres & Rudnik-Schoeneborn 1996).

In Japanese Black cattle (Wagyu), a juvenile renal disease has been characterized as interstitial fibrosis with inflammatory cell infiltration, clusters of atrophic and cystic tubules, and thickening of the tubular and membranes of Bowman's capsule. The disorder is thought to have genetic basis (Kuwamura *et al.* 1997). In addition, a renal disease, for which genetic information is not available, has been found in Holstein and Japanese Shorthorn breeds based on a correlation between increased urinary N-acetyl-β-d-glucosaminidase activity and renal lesions (Sato *et al.* 1999). In summary, little is known about genetically based renal diseases in cattle.

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We recently identified renal failure associated with growth retardation in a local Wagyu herd based on a preliminary diagnosis of increased levels of blood urea nitrogen (BUN) and creatinine. Although Kuwamura *et al.* (1997) observed that growth retardation was apparent in this herd after six months of age, growth retardation was prominent among affected cattle between three to five months of age. The incidence of renal failure in specific pedigrees was ≈ 10%, suggesting that growth retardation could be due to a hereditary disorder. After examining the pedigree structure, a Wagyu sire widely used by commercial farmers in Japan, appeared as a possible progenitor for the disease. Rigorous culling of affected pedigrees as a method to eliminate this genetic disorder was economically unacceptable. Therefore, we took advantage of a molecular genetic approach that enabled us to determine the heritability of the disease, identity the causative gene, and establish a DNA based test to identify non-carrier replacement stock. Here we report that the renal failure results from a chronic interstitial nephritis with diffuse zonal fibrosis (CINF) and we have mapped the *CINF* locus to BTA 1.

Materials and methods

Diagnosis and histology

Wagyu calves suspected as having CINF were measured for BUN and creatinine levels according to standard protocols. Kidneys were excised from two normal and two CINF-affected calves. Tissue samples were fixed in 10% neutral formalin, embedded in parafin, and sectioned at 5 μm. Semi-serial sections were stained by haematoxylin and eosin (HE) or Masson's trichrome stains.

Linkage analysis

Total genomic DNA was prepared using standard protocols from peripheral blood obtained from seven phenotypically normal and 17 CINF-affected cattle, their five sires and 17 dams. All

sires and dams in the study were descendants of Sire A. A battery of 230 markers (Kappes *et al.* 1997) covering all bovine autosomes at 15 cM to 20 cM and heterozygous in Sire A were used in the first screening.

The PCR reactions were performed in a volume of 15 μl reaction volume containing 6·25 pmoles each of the respective forward and reverse primers by fluorecent labelled and 0·375 unit of *Taq* DNA polymerase (TAKARA). The thermal cycling conditions were optimized for each primer set as recommended (Kappes *et al.* 1997), and the other reaction conditions were set as recommended by the manufacturer. Following PCR, alleles were resolved by electrophoresis in polyacrylamide gels using an ABI 377 sequencer and genotype data were captured by means of GENESCAN and Genotyper software (Perkin-Elmer Applied Biosystems). Correction of genotype errors and linkage analysis were performed with the GENEHUNTER package (Kruglyak *et al.* 1996).

Fluorescent in situ hybridization

Yeast artificial chromosome (YAC) clones were screened from a bovine YAC library by a PCR-based method as described (Takeda *et al.* 1998). YAC 249E9, harboring microsatellite loci *BM9019* and *INRA49*, was used for construction of a cosmid library. Briefly, DNA (100 μg) from YAC 249E9 was partially digested with *Sau*3AI; the resulting 20–30 kb fragments were collected by agarose gel electrophoresis, followed by ligation into pWe15 cosmid vector. Approximately 100 bovine DNA-derived cosmid clones were isolated by hybridization with total bovine DNA as probe. Bovine metaphase chromosome spreads were hybridized with a cosmid clone essentially as described (Wada *et al.* 1994) using reagents supplied in the Oncor Chromosome In Situ Kit.

Results and discussion

Clinical characterization of CINF

Chronic interstitial nephritis in Wagyu calves was initially identified because of growth deficiency. The affected cattle at 180 days old (six cattle) showed 0·45 kg daily gain, whereas normal ones at 180 days old (44 cattle) gained 0·71 kg daily. Also the affected cattle had elongated hooves. Because rumen acidosis

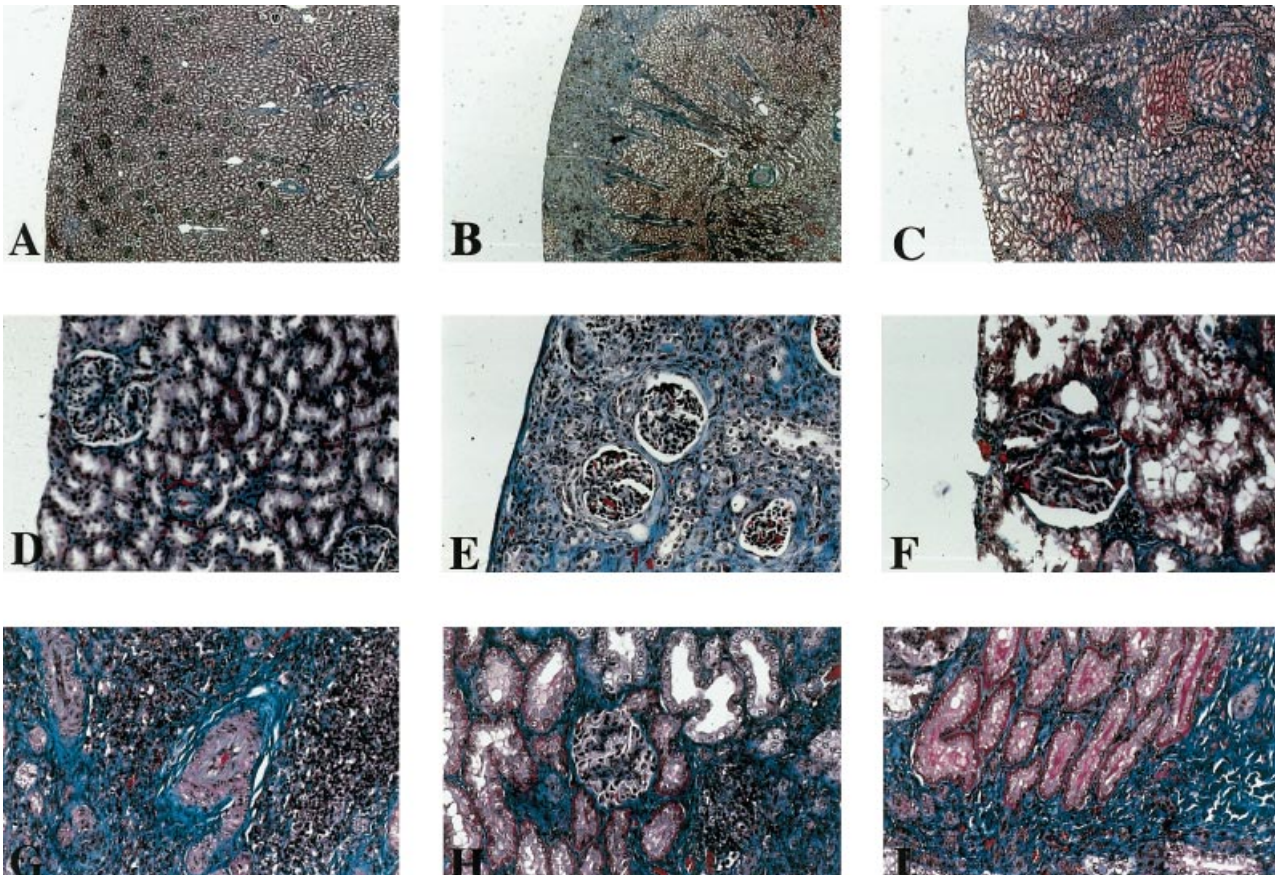

Fig. 1. Histology of kidneys from CINF-affected cattle. Slices were stained with Masson's trichrome stains. A and D, normal; B and E, affected (52 days of age); C and F–I, affected (221 days of age). A–C, × 10; D–I, × 66.

could also result in dietary laminitis, the rumen from the affected cattle was examined and due to normal contents, this possibility was excluded. Calves were diagnosed as CINF-affected when BUN values were over 0·5 mg/ml, while those below 0·3 mg/ml were considered normal. Cattle with BUN values between 0·3 and 0·5 mg/ml were monitored continuously and confirmed as CINF-affected when creatinine values exceeded 20 μg/ml. Urine tests were also carried out in three affected cattle. Urinary proteins over 0·3 mg/ml measured by the standard protocol were detected from all affected cattle, supporting the renal dysfunction in CINF.

Kidneys from two normal (10 days old) and two CINF-affected cattle (52 and 221 days old) were collected for histopathological examination (Fig. 1). The kidneys from the affected cattle were faint-coloured and had uneven surfaces with atrophy and sclerosis. Figures 1A and D show nephrons from a normal kidney, consisting of the renal corpuscles and the proximal and distal convoluted tubules. In contrast, extensive interstitial fibrosis with inflammatory cell infiltration was observed in kidneys from the affected cattle (Figs 1B &C). A high degree of inflammatory cell infiltration was observed in the affected kidney collected at 221 days old than in the affected kidney collected at 52 days old. Areas of fibrosis were observed in the outer region of the cortex in affected cattle at 52 days old (Figs 1B &E). At 221 days fibrotic areas were seen in the medullar region instead of the cortex (Figs 1C &F). They appeared as if the bands migrated from the cortex to the medullar region. In addition, there were signs of cellular and tissue regeneration in the original fibrotic areas at 52 days old (Figs 1F &I). Cystically dilated renal tubules with hyaline casts and atrophic renal tubules lined by cuboidal epithelial cells were also noted (Figs 1B,C & E–I). Kidney from a 52 d old affected calf did not have significant changes in glomeruli, while at 221 days old hypercellular glomeruli due to mesangial proliferation were present. These features were somewhat similar to those seen in human familial juvenile nephronophthisis (Antignac *et al.* 1993).

Genetic analysis of CINF

Pedigree records were used to trace filial relationships among sires to a single founder Sire A, suggesting that Sire A was the most likely source of the *CINF* locus mutation in this breeding herd. Among 217 newborn calves born to a son of Sire A and daughters of Sire A, 24 CINF-affected cattle were born. This incidence of 11·6% was close to the expected value of 12·5%, if the disease resulted from a simple recessive allele. From 1993 to 1997, a total of 441 CINF-affected cattle (245 males and 196 females) were found in this region, suggesting

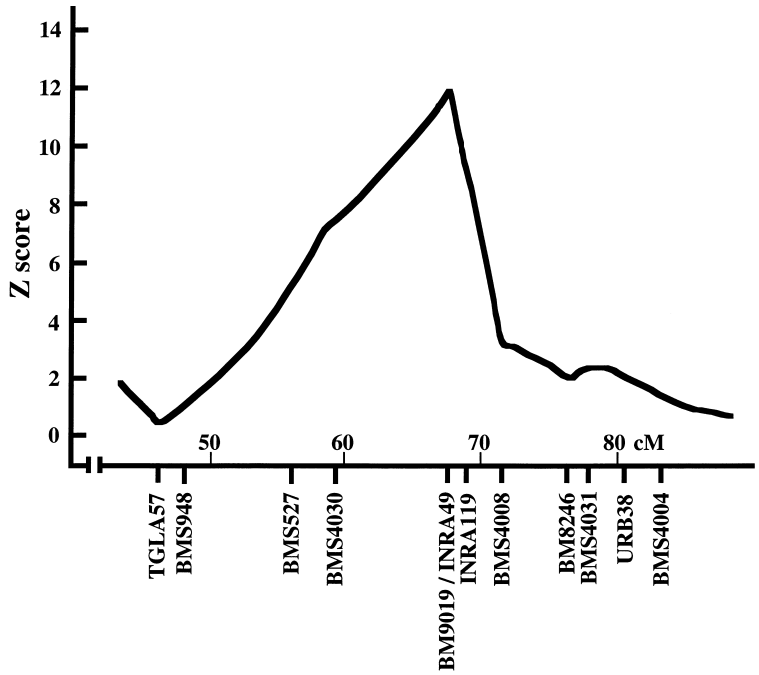

Fig. 2. Genetic mapping of *CINF* on bovine chromosome 1. Families collected were divided into six pedigrees for the determination of Z scores using GENEHUNTER. The indicated microsatellite loci were heterozygous in Sire A. Positions of the loci were from the USDA-MARC bovine linkage map (Kappes *et al.* 1997).

an autosomal genetic inheritance. Therefore, we suggest that CINF is a simple autosomal recessive disorder.

Linkage analysis of CINF locus

We collected 17 families of five sires and 17 dams whose sire is identified as founder Sire A, and seven phenotypically normal and 17 CINF-affected offspring. Genotyping of 230 microsatellites identified three possible chromosomal regions with a Z score greater than 2. An additional 110 microsatellite markers were genotyped in a second screening. As shown in Fig. 2, a maximum Z score of 12·0 was obtained in the central region of BTA 1 at 67·5 cM ($P < 3{\cdot}4 \times 10^{-10}$). The closest microsatellite loci to the *CINF* locus were *BM9019* and *INRA49*. A FISH experiment by using a bovine cosmid clone cos23 harbouring *BMS4009* as probe physically confirmed the position of *BMS4009* at the central region of BTA 1q31–33 (Fig. 3). *BMS4009* has been mapped near *BM9019* and *INRA49* loci with a recombination fraction of 0 (Kappes *et al*. 1997).

This region corresponds to human chromosome (HSA) 3 (Solinas-Toldo *et al*. 1995; Hayes 1995). Although human familial juvenile nephronophthisis is a progressive tubulo-interstitial renal disorder with autosomal recessive inheritance and clinical features somewhat similar to CINF, this human disease gene has been assigned to HSA2q13 (Antignac *et al*. 1993), not to HSA3. The causative gene for human familial juvenile nephronophthisis has been identified as *NPHP1* encoding an SH3 domain protein (Hildebrandt *et al*. 1997). It is likely that the *CINF* gene is different from the *NPHP1* gene.

CINF diagnosis with microsatellites

Microsatellite loci *BM9019* and *INRA49* were examined to determine whether they were useful for diagnosis of CINF-carrier cattle. Each of the markers has several alleles in the Wagyu population ranging in size from 106 to 118 bp for *BM9019* and 153–161 bp for *INRA49*. All CINF-affected cattle in the 17 families subjected to the linkage analysis were homozygous for *BM9019* (116 bp) and *INRA49* (157 bp). The CINF-related 116 bp allele of *BM9019* was found at a frequency of 0·18 in 224 Wagyu chromosomes, while the *INRA49* 157 bp allele was the most common allele at a frequency of 0·53. We applied both microsatellite loci for identification of CINF-carriers to more than 2000 descendants of Sire A. Approximately 10% of these cattle could not be designated as normal or CINF-carriers, even if the family history was well known. Together, these observations suggest that it is difficult to identify CINF-carriers using *BM9019* and *INRA49* genotyping. A causative gene for CINF is required for the development of a reliable DNA test.

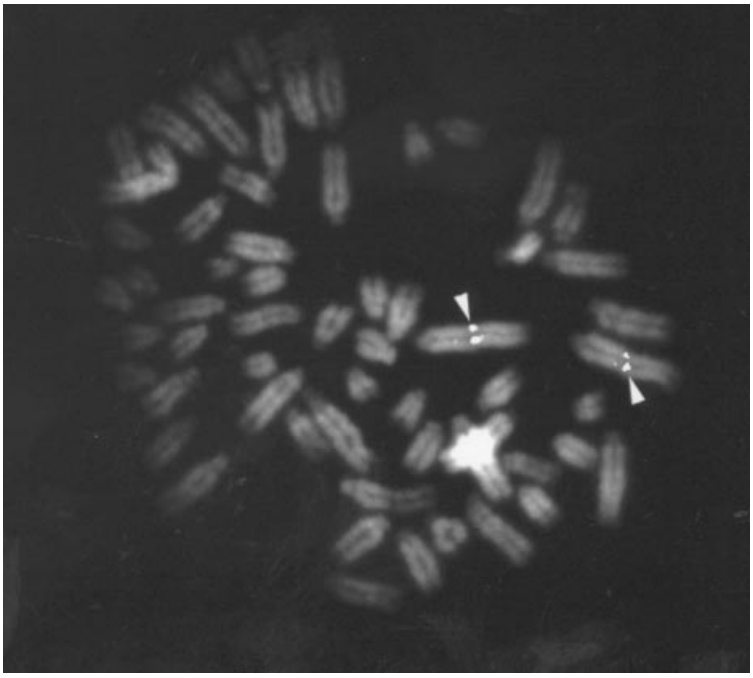

Fig. 3. Physical mapping of a cosmid containing BMS4009 on bovine chromosome 1q31–33 by FISH.

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