NOTE *Laboratory Animal Science*

Linkage Mapping of the Mouse Nephrosis (*nep*) Gene to Chromosome 15

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ABSTRACT. ICGN is a partially inbred strain of mice with nephrotic syndrome caused by spontaneous glomerular lesion. It has been reported that the albuminuria in ICGN mouse was controlled by at least a single autosomal recessive gene (*nep*). In this study, we mapped the *nep* locus by linkage analysis of backcross progeny between ICGN and MSM mice using DNA pooling method. The linkage analysis revealed that the *nep* locus was localized on the distal part of chromosome 15.

KEY WORDS: ICGN mouse, linkage mapping, nephrosis.

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ICGN is a partially inbred strain of mice with nephrotic syndrome caused by spontaneous glomerular lesion [11] and was derived from an outbred Yok:ICR colony at the National Institute of Health (Japan) in 1986. In Osaka University, the inbreeding of the ICGN mouse is in progress. The affected mice show a nephrotic syndrome which is characterized by albuminuria, hypoalbuminemia, hyperlipidemia and edema [14]. As far as we know, few animal models for human nephrotic syndrome caused by spontaneous idiopathic glomerular lesions have been reported. ICGN is a useful model for not only nephrosis but also chronic renal failure [6, 8]. Again there are very few animal models for spontaneous renal failure, which is believed to be a terminal event of most renal diseases. Suitable models for chronic renal diseases are always being sought by nephrologists because of the increase in chronic renal failure patients due to diabetes, hypertension and other modern chronic diseases. In fact, before we demonstrated with the aid of this model that hepatic growth factor (HGF) is effective as a cure for chronic renal failure[7], there were no other treatments for chronic renal failure except renal transplantation. Furthermore, we proved the existence of reciprocal balance between HGF and transforming growth factor β1 (TGF-β1) in chronic renal failure by using this strain of mice [9]. It therefore should be of value to identify the responsible gene for chronic renal failure in this strain of mice.

In a previous study using the backcross progeny between ICGN and Yok:ICR, it was demonstrated that the albuminuria in ICGN mouse was controlled by at least a single autosomal recessive gene, which was designated the *nep* gene [4]. The positional cloning of the responsible gene is thus a useful strategy for clarifying the mechanism of the renal disease. The linkage study presented here was carried out as the first step in this respect.

MSM, an inbred mouse strain of *Mus musculus molocci-nus* [2] was selected as the parent strain for obtaining the backcross progeny. The MSM strain was kindly donated by Dr. Moriwaki (National Institute of Genetics, Mishima, Japan). Matings were arranged between female ICGN and male MSM mice, and some F_1 hybrids were produced. F_1 hybrids were then backcrossed to the male ICGN mouse.

The urine was collected in glass tubes and urine samples were analyzed with SDS-PAGE as described by Laemmli [5] to detect the albumin fraction. The samplings of the urine were carried out in several times after weaning. The electrophoresis system used consisted of a mini-slab with a 12% gel, which was stained with a Silver Stain Plus kit (Bio-Rad, Hercules, CA.). The protein fraction that appeared at 66.1 kD was ascertained to be an albumin fraction and mice with urine that showed the 66.1 kD fraction were designated as a phenotype of nephrosis.

We could obtain 133 backcross progenies from the (ICGN $\times$ MSM)F_1 $\times$ ICGN cross and 90 from the ICGN $\times$ (ICGN $\times$ MSM)F_1 cross (Table 1). In the case of the (ICGN $\times$ MSM)F_1 $\times$ ICGN cross, the ratio of the numbers of nephrotic to non-nephrotic mice was almost 1:1. In the case of the ICGN $\times$ (ICGN $\times$ MSM)F_1 cross, however, the ratio was much removed from 1:1. For several mice, it could not be determined whether they were nephrotic or not, because faint albuminuria had been present throughout the observation period. And the quality of DNA extracted from several mice was not suitable for PCR. As a result, out of 133 offspring from the (ICGN $\times$ MSM)F_1 $\times$ ICGN cross, 59 nephrotic mice and 49 non-nephrotic mice were used for the linkage analysis.

All of the markers used in this study were microsatellite markers. The primers for these markers were purchased from Research Genetics, Inc. (Huntsville, AL). PCR amplifications and gel electrophoresis were carried out according to a previously reported procedure [15]. In order to rapidly determine the chromosome carrying the *nep* gene, we employed the pooled-SSR (simple sequence repeat) analysis for the first step [16]. Spleen DNAs from individual mice

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Table 1. Segregation of nephrotic and non-nephrotic mice (MSM × ICGN, ICGN)

Female × Male	Litter (Mean Size*)	No.of Progeny	Nephrotic M	Nephrotic F	Nephrotic Total	Non-nephrotic M	Non-nephrotic F	Non-nephrotic Total	Not judged M	Not judged F	Not judged Total
(ICGN × MSM)F_1 × ICGN	16 (8.31)	133	31	30	61	26	30	56	4	12	16
ICGN × (ICGN × MSM)F_1	12 (7.50)	90	8	11	19	35	19	54	12	5	17

*: The litter size was examined at a few days after birth.

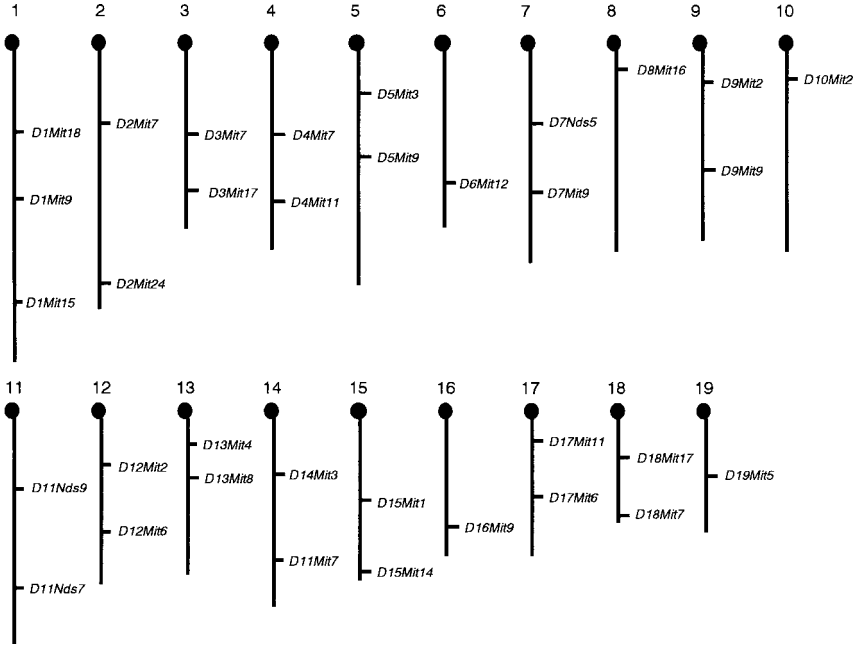

Fig. 1. Chromosomal locations of 34 selected markers for pooled-SSR analysis. Locations of individual SSR loci and length of individual chromosomes are taken form the microsatellite map distributed by the MIT Genome Center. Centromeric ends are indicated by knobs.

were standardized to 40 μg/m*l* in distilled water, after which the same amounts of individual DNAs were pooled. The nephrotic pool was obtained from 25 individual DNAs, and the non-nephrotic pool from 23 individuals. On the basis of a report of Taylor *et al.* [16], 34 loci about 20 cM apart from each other were selected, and the genotypes of both DNA pools were determined for each locus (Fig. 1). Of these 34 loci, two (*D15Mit1* and *D15Mit14*) which are located on chromosome 15 seemed to show a linkage relationships with the *nep* locus (Fig. 2). Next, 108 backcross offspring were typed for 14 markers on chromosome 15 (Fig. 3). The linkage relationships among *nep* and microsatellite loci were analyzed by means of Gene-link [10]. These 14 loci showed a significant linkage relationships with the *nep* locus. No recombinant was found among the *nep* and eight of the genetic marker loci, *D15Mit14*, *D15Mit15*, *D15Mit16*, *D15Mit39*, *D15Mit40*, *D15Mit77*, *D15Mit79* and *D15Mit149*.

Figure 4(a) shows the linkage map of mouse chromosome 15 including the *nep* locus. At the same time, a linkage map of the marker on the chromosome 15 as previously reported is shown in Fig. 4(b). A comparison of these maps shows no difference in the order of the markers, except for *D15Mit37* and *D15Mit95*, but a considerable increase in nonrecombinant markers on our map. For a detailed mapping of the *nep* gene, a larger number of backcross progenies will have to be examined. Moreover, of several mice in our study it could not determined whether or not they were nephrotic because of faint albuminuria. It has been known that the nephrosis of the ICGN mouse was remarkably exacerbated as a result of feeding of a high protein diet (data not shown). For further detailed mapping, therefore, the feeding of a high protein diet to the backcross progenies may be effective.

The renal basement membrane contains a barrier against leakage of the plasma protein into the urine. It has been reported that structural abnormalities in the glomerular cap-

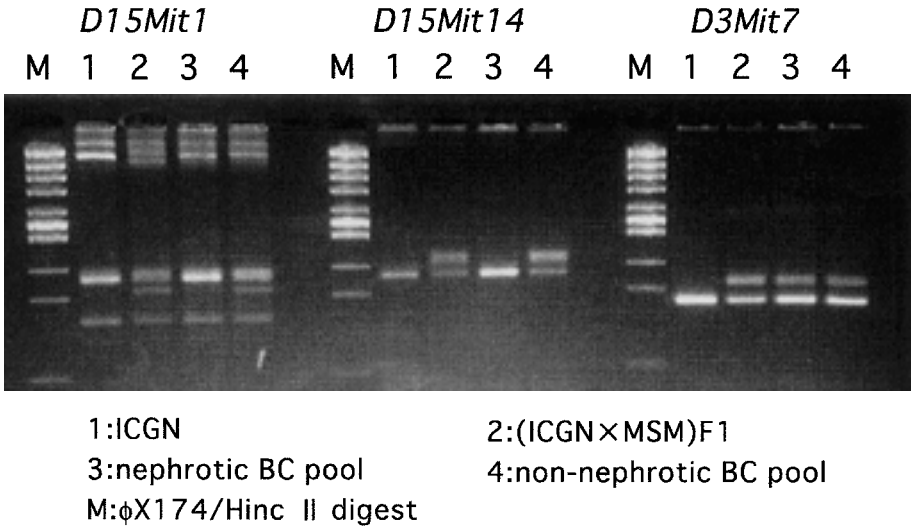

Fig. 2. The results of SSR analysis with three primer sets. In the case of the *D3Mit7*, which was not linked to *nep* gene, band pattern from nephrotic backross (BC) pool was almost identical with that from non-nephrotic BC pool. Namely, intensity of the PCR fragment originated form ICGN allele was stronger than that from MSM allele. When markers linked with *nep* were used (*D15Mit1*, *D15Mit14*), intensity of the PCR fragment from ICGN allele became stronger and that from MSM became weaker in neprotic pool.

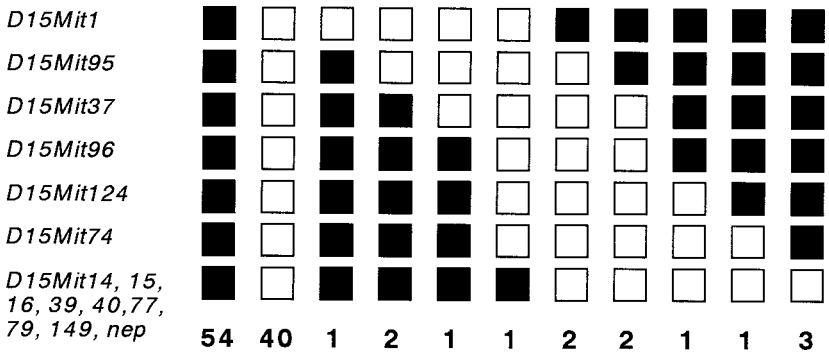

Fig. 3. Haplotypes inherited from F_1 parent for 108 backcross offspring from the cross (ICGN × MSM) F_1 × ICGN. The open boxes represent the alleles derived form ICGN, and the closed boxes those derived from MSM.

illary basement membrane might play an important role in the onset and development of the disease in ICGN mice [12]. The nephrosis observed in the ICGN mouse resembles that seen in Alport's syndrome, which is a human inherited nephrosis. It is known that Alport's syndrome is caused by the abnormality of type IV collagen chains in renal basement membrane due to a mutation in either the *COL4A3* or the *COL4A4* gene [3]. Because the loci of these genes in mouse are located on chromosome 1 (Mouse Genome Database, 2000), the *nep* gene may not be a mutant of these genes. In a candidate gene search for the *nep* among known genes related to the renal components, the locus of integrin α5, the receptor molecule of the fibronectin which is one of the main components of the renal basement membrane as well as of type IV collagen, was mapped to mouse chromosome 15, distal to *D15Mit16* [1]. The possibility thus exists that the abnormality of this gene may be a cause of nephrosis in the ICGN mouse. And, the gene of aquaporin-2, which is a molecule involved in resorption in the renal uriniferous tubule, is also mapped to this region. The integrin α5 and aquaporin-2 genes thus seem to be one of the candidate genes. For the next step, these candidate genes will have to be examined in addition to the construction of the detailed linkage map.

Among the backcross progenies, several mice in this study could not be identified as to whether or not they were

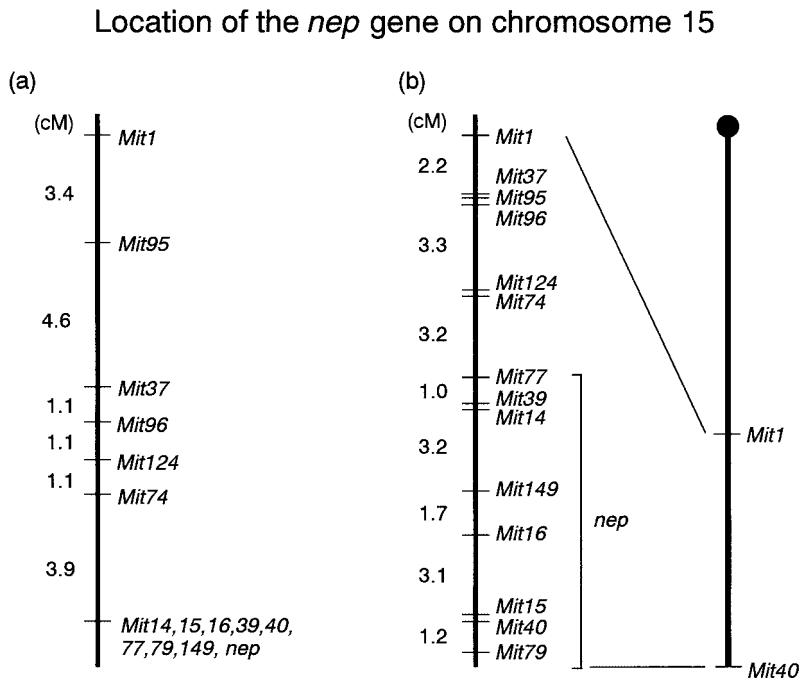

Fig. 4. Position of the *nep* locus on mouse chromosome 15. (a) Partial linkage map resulting from our study of mouse chromosome 15, showing the location of the *nep* locus in relation to the linked loci. (b) A linkage map of the selected markers on chromosome 15 according to the Mouse Genome Database (2000).

nephrotic. Among the ICGN × (ICGN × MSM)F_1 cross, the ratio of the numbers of the nephrotic mice to the non-nephrotic mice was far from 1:1 (Table 1). In addition, it was reported that there are sex-related differences in the incidence of nephrotic disease in (DBA/2 × ICGN)F_1 × ICGN [13]. Although it is certain that the main causative gene is the *nep* gene, multiple genes may control the nephrosis in ICGN mouse. As mentioned above, no recombinant was found among the *nep* and eight of the genetic marker loci. In one male out of 16 not judged progenies from the (ICGN × MSM)F_1 × ICGN cross, the recombination was occurred between *D15Mit77* and *D15Mit15*. It may be indicated that the support gene may be located near the *nep* locus.

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