Fine Mapping of the Region Including Hypogonadism (hgn) Locus on Rat Chromosome 10

Hiroetsu SUZUKI1), Eijiro NAKAMIYA1), Kumi DAIGO1), Kenichi SAITO1) and Katsushi SUZUKI1)

1) Department of Veterinary Physiology, Nippon Veterinary and Animal Science University, 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180–8602, Japan

(Received 21 November 2003/Accepted 19 April 2004)

ABSTRACT. The hypogonadic rat (hgn/hgn) shows male sterility, reduced female fertility, and renal hypoplasia, controlled by a single recessive gene located on rat chromosome 10. We developed a fine map around the hgn locus using 565 rat backcross progeny and a Rat/Hamster radiation hybrid panel. The hgn locus was linked to Aldoc (aldolase c) and whn (winged helix of nude), and located in a 0.34-cM region between D10Rat30 and D10Rat68. The distance of the region was approximately 840-kb on rat physical map. Neither loci responsible for male sterility nor renal hypoplasia has been mapped on the homologous regions of mouse chromosome 11 and human chromosome 17. Identification of the gene responsible for the hgn mutation would provide important information on urogenital development.

KEY WORDS: hypogonadism, linkage map, radiation hybrid map.

Male hypogonadic rats (hgn/hgn) show severe hypogonadism that inherited as an autosomal single recessive manner. The weight of an adult hgn/hgn testis is almost equal to that of a normal female ovary and about 1% that of a normal rat testis. These animals have small male reproductive accessory organs but no female reproductive organs [18]. Plasma testosterone is low and levels of gonadotropins are high, indicating that the cause of hypogonadism lies within the testes itself [5]. Testicular pathogenesis begins in the fetal period and progresses during postnatal testicular development [20]. Most germ cells degenerate in the testes before entering meiosis because of Sertoli cell dysfunction [13, 17]. hgn/hgn males show not only hypogonadism but also bilateral hypoplastic kidney (HPK) [19]. The HPK of hgn/hgn rat contains only one-quarter the number of nephrons that are found in the normal kidney [12]. Therefore, the HPK causes chronic progressive renal failure [14]. The phenotypes of this disorder resemble those of oligomeganephronia, a congenital renal hypoplasia reported in humans [2], in respect of the reduced number of nephrons with hypertrophy of individual nephrons [12]. This combination of HPK and hypogonadism makes it possible to distinguish hgn/hgn females from phenotypically normal ones in the HGN strain [19]. hgn/hgn females identified by the presence of HPK have hypoplastic ovaries at birth, reduced reproductive performance, and early reproductive senescence [11]. Therefore, the female hgn/hgn rat is a congenital animal model of premature ovarian failure (POF) in women [9, 11]. No other mutant animal showing a phenotype similar to that of the hgn/hgn rat has been reported. Therefore, the hgn/hgn rat would be a useful model for investigating the development of the mammalian reproductive and urinary organs. Our recent report revealed that the hgn locus was located in the region close to the D10Mit2 locus on rat chromosome 10 [15]; in the previous study we presented only a rough linkage map around the hgn locus, using 48 backcross progeny and 11 rat microsatellite markers. In the present study, we performed further linkage analysis using 565 rat backcross progeny to make a fine linkage map around the hgn locus. Radiation Hybrid (RH) mapping was also performed to determine the order of the markers mapped at the same positions in the linkage analysis.

All rats used in this study fed a certified commercial diet (CR-LPF: Oriental Yeast Co., Ltd., Tokyo, Japan) and were kept in a clean conventional animal room under controlled light (14L: 10D), temperature (22 ± 1°C), and humidity (55 ± 5%) [17]. The experimental procedure and care of animals were in accordance with the guideline of the Animal Care and Use Committee of Nippon Veterinary and Animal Science University. One hundred and twenty-six hgn/hgn females were selected from a population of the HGN inbred strain by laparotomy for the detection of HPK at weaning [19]. They were mated with the F1 (+/hgn) males obtained by mating between a BN (Brown Norway strain) male (+/+), a hgn/hgn female (HGN strain) [15]. As a result, 510 male backcross progeny were obtained. They were sacrificed at 21 days of age. Their phenotypes were determined by measuring their testicular weights with an electric balance [19, 20]. Their livers were excised and frozen at −20°C until DNA extraction. In a total of 388 out of the 510 male progeny, kidneys, adrenal glands, spleen, thymus, heart, lung, brain, and pituitary gland were also weighed. Student’s t-test was used for statistical analysis of the data. The testes were fixed in 4% neutral formalin, dehydrated with graded alcohol, embedded in paraffin, and sectioned at 3 µm. The sections were stained with hematoxylin and eosin and examined under a light microscope [20].

In the linkage analysis, we typed a total of 565 backcross males, including 55 males obtained previously [15]. The hgn locus has been reported to locate in the region close to the D10Mit2 locus on the rat chromosome 10 [15]. Therefore, the rats were typed for the following rat microsatellite
markers in the close vicinity of D10Mit2, D10Rat159, D10Rat161, D10Rat69, D10Rat211, D10Arb9 (AldoC), D10Rat50, D10Rat68, D10Wox24 (Nos2: nitric oxide synthase 2), whn (winged helix of nude), D10Rat133, D10Got80, D10Arb27, and D10Rat29 [4]. Forward and reverse primers for each locus were purchased from Research Genetics Inc. (Huntsville, AL, USA) or synthesized by an outside supplier (TaKaRa, Kyoto, Japan). The primer sequences for whn were 5′-ACCGTACGTCAGGT-GTGAATGG-3′ and 5′-TTTGATTCCTAGGACCACCATGG-3′, which amplify microsatellite DNA of an intron of mouse whn genomic DNA [10]. Isolation of genomic DNA, polymerase chain reactions (PCRs), separation of PCR products, and gel staining were done as previously described [16]. Data analysis for linkage was carried out with Map Manager QTb [7]. The order of the loci was determined in a way that minimized the number of recombinant events. A Rat/Hamster radiation hybrid (RH) panel was purchased from Research Genetics Inc. (Huntsville, AL, U.S.A.). The PCR of RH mapping was basically similar as genotyping in linkage analysis except for using 25 μg genomic DNA of RH clone instead of that extracted from rat liver. The PCR products were separated by non-denatured 10–12.5% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining [16]. The presence or absence of each of 9 microsatellite markers, which located in 1cM-region including absence of each of 9 microsatellite markers, which located by ethidium bromide staining [16].

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We omitted whn from RH mapping analysis, since the product of whn from rat FR DNA showed smear. The positions of the makers on rat physical map of chromosome 10 were determined by the similarity search program for the genome database (BLAST) with their sequences against rat genome [6].

As in our previous report [15], the male rats of the backcross progeny were unambiguously classified into normal and affected groups according to their testicular size at 21 days of age, although it was impossible to identify HPK from normal ones by renal weight. Therefore, we used only the male rats of the progeny for the linkage analysis. Table 1 shows the average values of body weights and relative weights of each organ in 182 hgn/hgn and 206 +/hgn males of the backcross progeny at 21 days of age. The body weight of hgn/hgn rats was significantly smaller than that of +/hgn. The relative weight of testis of hgn/hgn rats was almost 10% that of +/hgn rats at 21 days of age. The histological appearance of the testes in the affected rats of the backcross progeny was basically similar to that of hgn/hgn rats of the HGN strain. However, compared with the latter, more seminiferous tubules remained in the affected testes of the backcross progeny (data not shown). This suggests that

<table>
<thead>
<tr>
<th>Locus</th>
<th>+/hgn (206)</th>
<th>hgn/hgn (182)</th>
</tr>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>36.70 ± 8.99</td>
<td>34.52 ± 8.63*</td>
</tr>
<tr>
<td>testis**</td>
<td>2101 ± 243</td>
<td>190 ± 43**</td>
</tr>
<tr>
<td>kidney**</td>
<td>5529 ± 493</td>
<td>4864 ± 612**</td>
</tr>
<tr>
<td>adrenals gland**</td>
<td>146 ± 40</td>
<td>149 ± 29</td>
</tr>
<tr>
<td>spleen</td>
<td>2976 ± 711</td>
<td>2635 ± 621**</td>
</tr>
<tr>
<td>thymus</td>
<td>3463 ± 607</td>
<td>2366 ± 547**</td>
</tr>
<tr>
<td>heart</td>
<td>5212 ± 621</td>
<td>5766 ± 853**</td>
</tr>
<tr>
<td>lung</td>
<td>10427 ± 2435</td>
<td>11718 ± 4031*</td>
</tr>
<tr>
<td>brain</td>
<td>39193 ± 7731</td>
<td>39365 ± 8.445</td>
</tr>
<tr>
<td>pituitary gland</td>
<td>47 ± 23</td>
<td>49 ± 20</td>
</tr>
</tbody>
</table>

Each value represents the average with the standard deviation. Significant smaller than that of +/hgn as p<0.05 and p<0.01, respectively. #, ##: Significantly larger than that of +/hgn at p<0.05 and p<0.01, respectively. Number in parenthesis represents the number of animals examined.
eny, including 271 hgn/hgn and 294 +/hgn males, is shown in Fig. 1A. The segregation ratio of hgn/hgn and +/hgn did not deviate significantly from the expected 1:1 ratio (χ²=0.94, P>0.20). No recombinant event was observed between hgn, D10Rat69, whn, and D10Arb9(Aldoc). One recombination was observed between hgn and D10Rat68, and one recombination was observed between hgn and D10Rat30 (in the same position as D10Rat161 and D10Rat211). Therefore, the hgn locus was located in a 0.34-cM region between D10Rat30 and D10Rat68. One recombination was observed between hgn and D10Rat68, and one recombination was observed between hgn and D10Rat30 (in the same position as D10Rat161 and D10Rat211). Therefore, the hgn locus was located in a 0.34-cM region between D10Rat68 and D10Rat30. The order and distances of the microsatellite markers revealed in this experiment are basically identical with those of linkage maps reported by others [4, 8]. BLAST search with the sequences of the markers revealed that their positions (parentheses attached to locus names) on rat physical map of chromosome 10 [6]. The positions of D10Rat161, D10Rat211, and D10Rat30 mapped on the same position in the linkage map were inconsistent with those presumed from the rat physical map obtained from NCBI [6]. We supposed that the inconsistency might be derived from the incompleteness in the draft sequences of rat genome, since the physical map includes gaps and incorrect segments of chromosome. Our RH map (Fig. 1B) and another fine linkage map around the region reported by our group [1] support our fine linkage map presented here. Consequently, integration of the linkage map with the RH map suggests that the hgn locus is located in a 22.6cR-region between D10Rat68 and D10Rat30. Although the region includes unsettled segment of chromosome, in which the order of loci is opposite to that reported in the homologous region of mouse chromosome 11 and human chromosome 17 (Fig. 1C; shown by thick arrow), the distance of the region was estimated as a 840-kb on the physical map (Fig.1C).Comparison of the region with mouse homologous region on chromosome 11 indicates that the region includes 16 genes with known or inferred functions, and 8 segments with unknown function. It has been reported that Spag5 encoding a protein associated with the mitotic spindle apparatus is abundantly expressed in testis. However recent report revealed that the knockout mice of Spag5 had normal fertility [21]. At present, neither loci responsible for male sterility nor renal hypoplasia has been mapped on the homologous regions of the mouse chromosome 11 and human chromosome 17 [6]. Therefore, it is strongly postulated that the gene responsible for male sterility is located in the 22.6cR-region between D10Rat68 and D10Rat30.

Fig. 1. (A): Fine linkage map of the area around the hgn locus on rat chromosome 10, showing the location of hgn in relation to other loci. The hgn locus is located in the 0.34-cM region between D10Rat30 and D10Rat68. The number in parenthesis attached with locus name represents the position (kbp) of the locus on physical map. (B): Radiation hybrid map showing the order of the markers close to the hgn locus. (C): Rat physical map showing the positions of genes with known or inferred functions in the 840-kb region including hgn locus. The homologous genes of mouse are shown in the parentheses attached to locus ID number. Thick arrow indicates that the order of the loci in the region is opposite to those shown in mice and human. Thick line shows the distance of the hgn-region restricted in each map.
for hgn is one of the genes, of which function have not yet been settled. In order to narrow the distance of the region including hgn locus, we will generate new polymorphic markers in the region to type the recombinants in the backcross progeny. To identify the mutated gene hgn, we will examine the expression of the candidate genes in the embryonic organs in which the effects were shown in hgn/hgn rats. Our efforts to identify the mutated gene hgn would lead to discover new information important for development and function of urogenital organs.

ACKNOWLEDGMENTS. We are grateful to Prof. T. Kunieda (Faculty of Agriculture, Okayama University) for his advice and the generous gifts of whn primers. We thank all the members of our laboratory for their time-consuming efforts to maintain the mutant strains. This work was supported in part by a Grant-in-Aid for Scientific Research to H. Suzuki (NO.12760204) and to K. Suzuki (NO.13660309) from the Ministry of Education, Science and Culture of Japan.

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