Mapping of the Dilute-Opisthotonus (dop) Gene on Chromosome 8 of the Rat

Kyoko OHNO1,2, Yasuhiko KANOU2, Sen-ichi ODA1, Noboru WAKASUGI1, Minoru INOUE2, and Hideki YAMAMURA2

1The School of Agricultural Sciences and 2Research Institute of Environmental Medicine, Nagoya University, Chikusa-ku, Nagoya-shi 464-01, Japan

Abstract: The rat dilute-opisthotonus (dop) autosomal recessive gene, causing ataxia and coat color dilution, was mapped on chromosome 8 by PCR-amplified microsatellite markers. To facilitate the linkage analysis, an intersubspecific cross with a Japanese wild rat strain was used. The recombination frequencies were 12.8% between Apoc3 and dop, and 32.1% between dop and Myo1v. The following order of three genes is proposed: Apoc3-dop-Myo1v. This mutation appears to be homologous to dilute-lethal (d') of the mouse in terms of clinical symptoms, coat color effect and chromosomal location of the gene loci.

Key words: ataxic mutant rat, dilute-opisthotonus (dop), gene mapping

The mouse dilute (d) locus on chromosome 9 has been characterized by more than 200 spontaneous and mutagen-induced autosomal recessive mutations. The original dilute coat color mutation shows a lightening of coat color, caused by clumping of the melanin pigment due to abnormal dendritic melanocytes [10]. The homozygotes of the dilute-lethal (d') allele have diluted coat color and suffer from neurologic symptoms that include clonic convulsions with opisthotonus and ataxia, which appear at 8–10 days of age and continue until the death of animals soon after weaning [13]. The molecular study of the mouse dilute gene indicates that it encodes a novel type of myosin heavy chain and it is suggested that an important role for the mouse dilute gene product is in the elaboration, maintenance or function of cellular processes of melanocytes and neurons [9].

In the rat, there are two reports on similar mutation. One is a blue dilution (d), which resembles the mouse dilute gene [2, 12], but the linkage of this gene has not been reported yet. The other is the dilute-opisthotonus (dop) used in this study, which was originally discovered in a breeding colony of Wistar rats and found to be controlled by an autosomal recessive gene [3]. The mutants can be distinguished from normal littermates at the age of 3 or 4 days by the lighter pigmentation and subsequent diluted coat color caused by irregular clumps of pigmentation. They start leaning to one side and the other around 12 days after birth and thereafter they are hardly able to walk. Around 3 weeks of age, the symptoms become more severe and they show opisthotonus and convulsive limb movement and die soon after showing great weakness and emaciation. This mutation is very similar to mouse dilute-lethal in terms

(Received 16 June 1995 / Accepted 10 August 1995)
Address Corresponding: K. Ohno, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-01, Japan
of clinical symptoms and coat color effect. A strain that propagated this congenital disease was developed to study the genetics and neuropathology underlying this deficiency.

The combination of using interspecific crosses, which involve a laboratory mouse strain and a distantly related species of Mus, and highly polymorphic DNA markers, such as restriction fragment length polymorphisms (RFLPs) and PCR amplified microsatellites, has been successfully used in mouse gene mapping [1]. Recently, an informative rat genetic linkage map was developed by using polymorphic microsatellite markers [14]. This is a great help for genetic studies with rat models for human diseases. Furthermore, a rat genetic linkage map and comparative maps for mouse or human homologous rat genes were reported [16]. It was proposed that the rat chromosome 8 is homologous to the mouse chromosome 9 and that the gene order on homologous chromosomes or chromosomal segments between the two species is fairly well preserved. If the rat dop gene is homologous to the mouse d' gene, it is expected that the dop locus is located on chromosome 8. We therefore tested several microsatellite markers on chromosome 8 and attempted to map the dop gene by using an interspecific cross.

**Mating experiment:** A male and a female that were known heterozygotes (+/dop) of the BN/Mai-dop +/+ strain were mated with normal (+/+) rats of the MITE strain. The BN/Mai-dop +/+ strain was developed by introducing the dop gene more than 4 successive backcrosses into BN/Mai inbred strain. The MITE strain is a normal inbred strain that was established from Japanese wild rats (Rattus norvegicus caraco) in order to provide new genetic variations [11]. For the purpose of progeny testing to determine which F1 hybrids carried the dop gene for generation of the F2 for mapping, all F1 hybrids (+/+ or +/dop) were backcrossed to known heterozygotes (+/dop) of the BN/Mai-dop +/+ strain. Those F1 hybrids carrying the dop gene were mated with each other to generate F2 offspring.

**PCR and gel electrophoresis:** DNA was extracted from the liver and dissolved in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. PCR was performed using 96-well microtiter plates in a type PTC-100 Programmable Thermal Controller (MJ Research, Inc.). The reaction volume was 50 μl containing 1–10 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% w/v gelatin, 200 μM dNTPs, 1 unit of Taq DNA polymerase (Perkin Elmer) and 10 pmol/μl of each primer. Two types of primer were used in this experiment. The primers R102 and R109 were designated to define (GT)27 microsatellite in the Apoc3 (Apolipoprotein C-III) locus and (TG)21 microsatellite in the Mylcv (Myosin light chain, alkali, cardiac ventricles) locus on the chromosome 8 respectively. Nucleotide sequences of the R102 primers are GATTTGAAGCGATTGTCCCA and GTCTAGCTGGCCACAGGAG, and those of the R109 primers are CTCTACCTGTCTAGGGCTGG and GACAGAGCCACTGCTCA [14]. Temperature and time cycles were: 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C (R102) or 60°C (R109) and 30 sec at 72°C, followed by a final elongation step of 10 min at 72°C. PCR products were resolved by 4 % agarose gel electrophoresis (Nusieve/SeaKem 3:1 agarose, FMC bioproduct) and stained by ethidium bromide.

**Linkage analysis:** The distances of the three loci were calculated from the recombination values [6] derived from the phenotypes of the F2 progeny.

In the mating experiment, all of 26 F1 hybrids: 14 males and 12 females, showed agouti coat color and no ataxia. Four males and 6 females of these F1 hybrids were detected as heterozygotes (+/dop) by backcross mating. Matings of these F1 carriers produced 205 F2 progeny: 101 males and 104 females. The segregation ratio in the F2 progeny was 155 normal: 50 affected (27 males and 23 females), in good agreement with the expected ratio of 3:1 for an autosomal recessive trait. All affected rats showed diluted coat color, ataxia and opisthotonus.

Both microsatellite markers of Apoc3 and Mylcv loci were polymorphic between parental strains. Fig. 1 shows the electrophoretic pattern that indicates the length variation of the PCR product. The length of the PCR product of BN/Mai-dop +/+ strain was about 10 basepairs shorter than that of MITE strain in both microsatellite markers. For convenience of description, the allele of BN/Mai-dop +/+ strain was denoted by the symbol “B” and that of MITE strain by the symbol “M”. Two bands, denoted by the symbol “BM”, were observed in all F1 hybrids. All F2 progeny were examined in phenotype of the two microsatellite markers. The result of the linkage test between dop and two
Fig. 1. Electrophoretic patterns of PCR-amplified microsatellite markers in (a) Apoc3 and (b) Mylclv. The fast band of BN/ fMai-dop/+ strain is “B” type (lane 2), the slow band of MITE strain is “M” type (lane 3), and the two bands of (BN/ fMai-dop/+ × MITE) F1 hybrids are “BM” type (lane 4). Lane 1 shows the DNA marker of φX174 digested by HaeIII.

Microsatellite markers in F2 progeny is shown in Table 1. From the data, the recombination values were calculated as 12.8 ± 2.7% between dop and Apoc3, and 32.1 ± 3.7% between dop and Mylclv. The result of the linkage test between Apoc3 and Mylclv is shown in Table 2, and recombination value of 38.1 ± 3.3 can be calculated. The proposed order of these three loci is: Apoc3-(12.8 cM)-dop-(32.1 cM)-Mylclv on chromosome 8.

In the mouse, arrangement of the gene loci on chromosome 9 is as follows: centromere-Apoc3 (Apolipoprotein C-III)-(13 cM)-d.(32 cM)-Mylc (Myosin light chain, alkal, cardiac ventricles)-telomere [8]. The rat chromosome 8 is homologous to the mouse chromosome 9 and the gene order on chromosomal segments is well preserved in the two species. Since the position of the dop locus

<table>
<thead>
<tr>
<th>Locus</th>
<th>Phenotype</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Total</th>
<th>( \chi^2(p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoc3</td>
<td>MM</td>
<td>50</td>
<td>1</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>94</td>
<td>12</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>11</td>
<td>37</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>155</td>
<td>50</td>
<td>205</td>
<td>70.273 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Phenotype</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Total</th>
<th>( \chi^2(p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mylclv</td>
<td>MM</td>
<td>55</td>
<td>5</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>75</td>
<td>23</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>27</td>
<td>22</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>155</td>
<td>50</td>
<td>205</td>
<td>19.281 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

\( ^a \) F2 progeny were obtained from the intercross between (BN/fMai-dop/+ × MITE) F1 carriers. \( ^b \) Expected ratio is (Normal, MM):(Normal, BM):(Normal, BB):(Abnormal, MM):(Abnormal, BM):(Abnormal, BB)=3:6:3:1:2:1.  

Table 2. Linkage analysis between Apoc3 and Mylclv locus in the F1 progeny

<table>
<thead>
<tr>
<th>Locus</th>
<th>Phenotype</th>
<th>Mylclv</th>
<th>( \chi^2(p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>29</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>58</td>
<td>98</td>
</tr>
</tbody>
</table>

\( ^a \) F2 progeny were obtained from the intercross between (BN/fMai-dop/+ × MITE) F1 carriers. \( ^b \) Expected ratio is (MM, MM):(MM, BM):(MM, BB):(BM, MM):(BM, BM):(BM, BB):(BB, MM):(BB, BM):(BB, BB)=1:2:1:2:4:2:1:2:1.
between the two microsatellite markers was very similar to that of the \( d \) locus in the mouse, these two loci are thought to be homologous (Fig. 2). Additional studies are required to elucidate more detailed linkage with other markers on chromosome 8. As in the mouse, the interspecific or intersubspecific crosses exploit the genetic diversity inherent between wild species and common laboratory strains. Most genes or DNA sequences are polymorphic in an interspecific cross and can be placed relative to other genes in a single interspecific cross [1]. Because the MITE rat strain is supposed to have a considerably different genetic background from existing laboratory rat strains, it would be useful for linkage studies (Serikawa T., personal communication).

Molecular study on the mouse \( d \) locus revealed that the spontaneous \( d^{202} \) mutation is caused by an interstitial deletion that removes a single coding exon of the dilute gene and a functional null allele of the dilute gene [15]. The predicted dilute amino-acid sequence indicates that the dilute gene encodes a novel type of myosin heavy chain, with a tail region that has elements of both type I and type II myosin heavy chains [9]. The transcripts of the dilute gene are very abundant in neurons of central nervous system, cephalic ganglia, and spinal ganglia. And it is suggested that an important role for the dilute gene product is in normal neuronal function. However, neither the peripheral nor the central nervous systems of dilute-lethal mouse display striking histopathological deficiencies.

Studies in other organisms revealed that MYO2 of \( Saccharomyces cerevisiae \) [7], chicken brain myosin-V [4], and myoxin (MYH12) of the human [5] are homologous to the mouse dilute gene. MYO2 mutations appear to affect the microtubule-based kinesin that is involved in the transport of cellular components in the axons of neuronal cells. Myoxin associates with various classes of intermediate filaments and may function in axoplasmic transport along the neurofilaments. Then myosin-V associates with Golgi-derived cytoplasmic vesicles that are eventually transported out to the cell periphery.

The rat is one of the most commonly used laboratory animals for biomedical research because its larger size facilitates experimental interventions and because it is more suitable for behavioral observations. Molecular study at the \( d \) locus in the mouse is progressing, but neuropathological and physiological defects of the dilute lethal in the mouse are not clear. Therefore, the dilute-opisthotonus rat might be an interesting alternative model to study genetical, neuropathological and physiological properties of neurologic diseases.

**Acknowledgments**

The authors thank Dr. T. Serikawa, Kyoto University, for kindly providing the primers and advice. We are also grateful to Dr. S. Wakana, Central Institute for Experimental Animals, for his invaluable advice. This study was supported by Grant in Aid No. 07670708 from the Japanese Ministry of Education, Science and Culture of Japan.
References


