TM Rats: A Model for Platelet Storage Pool Deficiency

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Abstract: TM rats have a light brown hooded coat pattern resembling that of Fawn hooded (FH) rats which are a model of platelet storage pool deficiency (SPD). We examined whether the TM strain has the same platelet SPD as the FH strain. TM rats had a prolonged bleeding time and a low blood serotonin level, although their blood coagulation time and platelet counts were normal. The light coat color of the TM strain was judged to be associated with the red-eyed dilution gene as in the FH strain, but not pink eye dilution as in the RCS rat strain. Platelet SPD seen in TM rats may be a pleiotropic effect of the red-eyed dilution gene proposed in FH rats. Despite these similarities, the genetic background of the TM strain was obviously different from that of the FH strain. The TM strain, developed independently of the FH strain, will therefore be used as a model of platelet SPD.

Key words: platelet storage pool deficiency, red-eyed dilution, TM rats

TM rats have a light brown hooded coat pattern (Fig. 1) akin to Fawn hooded (FH) rats [24]. The light coat color of TM rats is inherited in a simple Mendelian fashion as a recessive trait and the effective gene is named tentatively dilution (d) [30]. This rat strain, called the Testor Moriyama (TM) strain was given as the Long Evans strain by the Aichi Cancer Center to a psychiatry hospital, Moriyama-soh (Aichi, Japan). The strain has been utilized as a reference animal for examining coat color genes of albino rats by a test cross. After that the strain was established as an inbred strain of TM by Dr. Masahiko Nishimura (Hamamatsu University School of Medicine). At present, the TM strain is maintained at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University (TM/Kyo) and at Institute for Experimental Animals, Hamamatsu University School of Medicine (TM/Ham). FH (fawn hooded) rats, which have a fawn color hooded coat pattern resembling that of TM rats, are known to have a prolonged bleeding time due to platelet storage pool deficiency [14]. Both the prolonged bleeding time and the light coat color seen in FH rats may be the result of the pleiotropic effects of a recessive gene, red-eyed dilution (r), on chromosome 1 [16, 23]. Herein, we examined the platelet SPD and color genes in the TM rats.

First we examined the bleeding time of the TM/Kyo rat strain and the following 9 inbred rat strains maintained at the Institute of Laboratory Animals, Faculty of Medicine Kyoto University: ACI/NKyo, BN/

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fMaiKyo, IS/Kyo, RCS, SHR/Kyo, W/Kyo, WAG/RijKyo, WKS/Ms and WKYO/Kyo. Under ether anesthesia, the tail was cut at a site of 2 mm from the tip, and immediately immersed in physiological saline. As shown in Fig. 2, both males and females of the TM strain required more than 900 sec for cessation of bleeding, while in all rats of other strains bleeding stopped between 30 and 451 sec (152.1 ± 101.3; Mean ± S.D.).

Blood coagulation time, platelet count and the blood serotonin level of TM rats with a prolonged bleeding time and BN rats with a normal bleeding time were compared. Blood coagulation time was determined by a modification of Everson’s method [7, 25]. The platelet count was determined by a direct method with a Coulter Counter Type S-Plus VI. The blood serotonin level was measured by HPLC with an L-6000 a High Performance Liquid Chromatograph. As shown in Fig. 3, the blood coagulation time and the platelet count were normal in all rats in each strain [25, 27] and did not differ significantly between TM rats and BN rats.

Blood serotonin levels were much lower in TM rats than in BN rats. These hematological data for TM rats were similar to those for FH rats [24, 28]. Since TM rats had a prolonged bleeding time and decreased blood serotonin level despite normal blood coagulation time and platelet count, they were suspected of having platelet SPD as in FH rats.

To examine the inheritance of prolonged bleeding time traits in TM rats, the (BN × TM)F₁ and the (BN × TM)F₁ × TM backcross were produced. The bleeding time was normal in all of the F₁ hybrids examined. Of 64 backcross progenies, bleeding time was prolonged in 36 and normal in 28. This does not disagree with the expected 1:1 segregation ratio ($\chi^2$=1.00), suggesting that the prolonged bleeding time is inherited in a Mendelian fashion as a recessive trait. The gene symbol Pbt was therefore tentatively applied to the effective gene. With the following 13 genetic markers Aco1, Ahd2, d, Est1, Es3, Fh, Gc, h, Itbb, Map1, Pepc, Rti1A and Svp1, the location of the gene encoding prolonged

Fig. 1. Appearance of TM rat.

Fig. 2. Bleeding time of rats from 10 inbred strains. ○= Male, ●= Female.

Fig. 3. Coagulation time, platelet counts and serotonin concentrations in TM and BN rats.
bleeding time in TM rats was examined. Typing of the genetic markers was judged by the standard methods previously reported [1, 2, 6, 8, 10, 18–20, 22, 29]. As shown in Table 1, a significant linkage was found between Pbt and the light coat color gene d ($\chi^2$=49.78), and a weaker linkage was found between Pbt and Hbb ($\chi^2$=7.87) on chromosome 1. No linkage was observed between Pbt and any of the other 11 markers on different chromosomes. In the backcross progeny, bleeding time was prolonged in all rats with a light coat color, although bleeding time was judged as prolonged in 4 in 32 rats with a non-light coat color. It is therefore possible that prolonged bleeding time is a pleiotropic effect of the d gene like that of the r gene in FH rats [23]. A similar finding for platelet SPD and the r gene has also been reported in FH rats [23]. The four rats may demonstrate the traits of bleeding time prolonged for other reasons. Considering the distance between the d gene and Hbb, the d gene may be allelic with the r gene (red-eyed dilution gene; r-Hbb$\approx$26.6 cM) or the p gene (pink eye dilution gene; p-Hbb$\approx$30 cM) on chromosome 1. We crossed TM rats with FH/Wjd rats

<table>
<thead>
<tr>
<th>Locus symbol</th>
<th>Chromosome No.</th>
<th>Progenitor's genotype</th>
<th>Genotype</th>
<th>Prolonged bleeding time</th>
<th>Total</th>
<th>$\chi^2$ for linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>d (probably same as r)</td>
<td>1</td>
<td>dd</td>
<td>DD</td>
<td>Dd</td>
<td>Prolonged</td>
<td>Normal</td>
</tr>
<tr>
<td>Hbb</td>
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<td>bb</td>
<td>aa</td>
<td>ab</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Spp1</td>
<td>3</td>
<td>aa</td>
<td>bb</td>
<td>ab</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Acol</td>
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<td>bb</td>
<td>aa</td>
<td>ab</td>
<td>11</td>
<td>14</td>
</tr>
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<td>aa</td>
<td>ab</td>
<td>20</td>
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<tr>
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<td>bb</td>
<td>aa</td>
<td>ab</td>
<td>11</td>
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</tr>
<tr>
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<td>bb</td>
<td>aa</td>
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<td>17</td>
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</tr>
<tr>
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<td>9</td>
<td>18</td>
</tr>
<tr>
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<td>20</td>
<td>uu</td>
<td>nn</td>
<td>nu</td>
<td>21</td>
<td>16</td>
</tr>
</tbody>
</table>

* P<0.01, ** P<0.005. a): Recombination frequency $= 32.803 \pm 3.757\%$. b): Recombination frequency $= 6.250 \pm 1.936\%$. 
Table 2. Eye and Coat color phenotypes of TM, FH, RCS, [TM × FH]F₁, and [TM × RCS]F₁.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype*</th>
<th>Eye color</th>
<th>Coat color of the hood</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td>aabBCChhPPrr</td>
<td>the same color as FH</td>
<td>the same color as FH</td>
</tr>
<tr>
<td>FH</td>
<td>aabBCChhPPrr</td>
<td>red</td>
<td>fawn</td>
</tr>
<tr>
<td>RCS</td>
<td>aabBCChhPPrr</td>
<td>pink</td>
<td>tan (lighter than fawn)</td>
</tr>
<tr>
<td>[TM × FH]F₁</td>
<td>aabBCChhPPrr</td>
<td>the same color as FH</td>
<td>the same color as FH</td>
</tr>
<tr>
<td>[TM × RCS]F₁</td>
<td>aabBCChhPpPr</td>
<td>black</td>
<td>black</td>
</tr>
</tbody>
</table>

*: The b locus in the TM strain was previously judged by a cross with BNF/MAiKyo (bb).

homzygous for the r gene [16] and with RCS rats homozygous for the p gene [16]. RCS rats were kindly provided by Dr. Kuriyama (see Acknowledgments). Table 2 shows the features of TM, FH, RCS and the offspring (F₁) of these crosses. TM and FH had the same fawn coat color and had the same red eye color. RCS had a tan coat color lighter than fawn and had pink eyes. The (TM × FH)F₁ progeny had a fawn coat color like the parents, while the (TM × RCS)F₁ progeny had a black coat. The (TM × FH)F₁ progeny had red eyes like the parents, while the (TM × RCS)F₁ progeny had black eyes. These findings suggest that the light coat color and red eye color of TM rats are encoded by a mutant gene of the r locus as in FH rats, but not the p gene as in RCS rats. This means that the tentative name d locus in TM rats should be deleted and r locus should be substituted for it.

Since the hematological data and the results of the genetic examination of the coat and eye colors suggested that the TM strain is the same as the FH strain or a substrain, we examined the microsatellite polymorphisms at 21 loci in three rat strains, TM/Kyo, FH/Wjd and FH/Ztk. The FH/Wjd strain is kept in Hamamatsu University School of Medicine, and the genomic DNA of FH/Ztk a kind gift from Dr. Hans P. Fortmeyer (see Acknowledgments). Out of the 21 microsatellite markers, 12 (57%) markers had different alleles in TM/Kyo and FH/Ztk, and 13 (62%) markers in TM/Kyo and FH/Wjd were different. On the other hand, only 2 (10%) markers in FH/Ztk and FH/Wjd were different. These findings indicate that the TM strain is clearly different from the FH strain, but it remains to be clarified whether the mutation for platelet SPD of TM is the same as that of FH.

Pigment mutations with SPD have also been reported in the mouse: pallid (pa, chromosome 2), cocoa (coa, chromosome 3), light ear (le, chromosome 5), ruby eye-2 (ru2, chromosome 7), mocha (mh, chromosome 10), beige (bg: same as the human gene of the Chediak-Higashi syndrome, chromosome 13), muted (mu, chromosome 13), pearl (pe, chromosome 13), pale ear (ep, chromosome 19) and ruby eye (ru, chromosome 19) [17]. Rat chromosome 1 has homologous relationships with mouse chromosome 7 or 19, and the region in which the r or Pht gene was mapped is homologous with the mouse chromosome 7 [31]. The comparative map suggests that the ru2 gene on the mouse chromosome is not a homologue for the r or Pht gene in the rat. Chediak-Higashi syndrome [3–5], Hermansky-Pudlak syndrome (HPS) [9, 12, 13, 15] and Wiscott-Aldrich syndrome [11] are human genetic diseases with the platelet SPD. In addition to platelet SPD, giant granules are seen in leukocytes of patients with Chediak-Higashi syndrome [21], and the platelet count is lowered in patients with Wiscott-Aldrich syndrome [11]. These changes were not seen in either the TM or FH strain, suggesting that the two human syndromes are different SPD types. The causative gene of HPS is localized on chromosome 10q23.1-q23.3 [9]. The region of distal 10q is syntenic to the region of mouse chromosome 19 that includes the ep gene and ru gene [9], suggesting that the r or Pht gene is not homologous with the HPS gene. Candidates of the human or mouse homologue for the Pht gene were therefore not found in the comparative gene map for rats, mice and humans.

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