Biochemical Polymorphisms in Long-Evans Cinnamon (LEC) Inbred Strain of Rat

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ABSTRACT. Long-Evans Cinnamon (LEC) rat is an inbred strain of mutant which has been proposed to be an animal model for human Wilson's disease. In the present study, 28 genetic marker proteins were analyzed by electrophoresis in sublines of LEC inbred strain held separately by four facilities. As expected, the 27 markers were homozygous and identical in all of examined animals. We further found a novel variant of esterase-2 (ES-2) showing an intermediate mobility between profiles of ES-2A and ES-2C types. —KEY WORDS: biochemical marker, inbred strain, LEC rat.


Long-Evans Cinnamon (LEC) rats is an inbred strain of mutant which was established from a closed colony of the Long Evans rats at the Center for Experimental Plants and Animals, Hokkaido University, Sapporo, Japan [8]. The mutant rat develops a necrotizing hepatic injury and finally hepatoma [5, 6]. Recently, both excess hepatic copper accumulation and deficiency of serum ceruloplasmin activity were found in LEC rat and, thereby, the rat was proposed to be an useful animal model for human Wilson's disease [4]. The genetic authenticity and uniformity of the inbred strain of LEC mutant are important for considering genetic and environmental factors, both of which are associated with the abnormal phenotype of LEC rat. Therefore, is it essential to carry out genetic surveillance of the inbred strain of LEC mutant periodically. In addition, sublines of LEC inbred strain held separately by different facilities should be monitored genetically.

In this paper, we screened 28 marker proteins in the sublines of LEC inbred strain held separately by four facilities and found a novel variant of esterase-2 (ES-2).

The rats used in this study were provided by four facilities, the Center for Experimental Plants and Animals, Hokkaido University (LEC/Hok); Institute for Animal Experimentation, Sapporo Medical College (LEC/Sme); Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd. (LEC/Ok); Institute for Animal Experimentation, University of Tokushima School of Medicine (LEC/TJ). The examined animals were in the generation shown in Fig. 1. Rats were anesthetized with ether, and the tissues were removed after bleeding. The tissues (liver, kidney, testis, and small intestine) were homogenized in 2 vol. of distilled water with Polytron homogenizer at 4°C. The homogenates were centrifuged

![Fig. 1. Pedigree of sublines of LEC rat strain. The value of generation in LEC/Hok represents the passing generation since the finding of mutant rat. The values of generation in other rats represent the passing generation since the separation into sublines.](image1)

![Fig. 2. Electrophoretic patterns of ES-2 on polyacrylamide gel. Lanes 1 and 6, control rat with ES-2A; lanes 2 and 5, control rat with ES-2C; lanes 3 and 4, LEC/Ok rat with ES-2C' variant.](image2)

<table>
<thead>
<tr>
<th>Subline</th>
<th>Aconl Acp2 Ahdl2 Ahdlc Akpl Alpl Amyl Cld Cdl Est Es2 Es3 Es4 Es6 Es7 Es8 Es9 Es10 Fh Gc Gdcl Gsi1 Haol Hbgb Hbb Lapl Pep3 Pep3 Pga Snp1</th>
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<tbody>
<tr>
<td>LEC/Hok</td>
<td>a b a a a a b b c d b a b a c b a b a b a b a b a b a b a b a b a</td>
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<tr>
<td>LEC/Hok</td>
<td>a b a a a a b b c d b a b a c b a b a b a b a b a b a b a b a b a</td>
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<tr>
<td>LEC/TJ</td>
<td>a b a a a a b b c d b a b a c b a b a b a b a b a b a b a b a b a</td>
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<tr>
<td>LEC/Ok</td>
<td>a b a a a a b b c d b a b a c b a b a b a b a b a b a b a b a b a</td>
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<td>LEC/Sme</td>
<td>a b a a a a b b c d b a b a c b a b a b a b a b a b a b a b a b a</td>
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<td>LEC/Sme</td>
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at 30,000 × g for 20 min at 4°C. The supernatants, sera and red blood cells were stored at −70°C until use.

Aconitase-1 (ACON-1), acid phosphatase-2 (ACP-2), aldehyde dehydrogenase-2 (AHD-2), aldehyde dehydrogenase-c (AHD-c), kidney alkaline phosphatase-1 (AKP-1), serum alkaline phosphatase-1 (ALP-1), amylase-1 (AMY-1), red cell catalase-1 (CS-1), fumarate hydratase-1 (FH-1), glycerophosphate dehydrogenase-1 (GDC-1), hydroxoyacid oxidase-1 (HAO-1), hemoglobin beta-chain (HBB), urinary peptidase-1 (PG-1), phosphoglucomutase dehydrogenase (PGD), and seminal vesicle protein-1 (SVP-1) were analyzed with cellulose acetate membrane (Helena, Titan-III) electrophoresis. Esterase-1, 2, 3, 4, 6, 7, 8, 9, and 10 (ES-1, 2, 3, 4, 6, 7, 8, 9, and 10), and group-specific component (GC) were analyzed with polyacrylamide gel electrophoresis. Glutathione S-transferase-1 (GST-1), leucine amino peptidase-1 (LAP-1), and peptidase-3 (PEP-3) were analyzed with starch gel electrophoresis. Staining procedures were performed according to protocols described previously [3].

The LEC sublines provided by different facilities were examined for 28 biochemical marker loci by using electrophoresis (Table 1). Twentyseven marker loci except for Es-2 were found to be homozygous in all of animals and identical among the sublines, showing low or no degree of genetic variability among the sublines. Matsumoto et al. [7] previously analyzed the genetic background in a variety of sublines of Spontaneously Hypertensive Rat (SHR), Stroke Prone SHR (SHRSP) and Wistar Kyoto (WKY) inbred strain, and demonstrated high degree of genetic variability among the sublines. These suggest that LEC rat was separated with higher level of inbred proceeding into different facilities than SHR, SHRSP, and WKY rats did. Long-Evans Agouti (LEA)/Hok inbred strain which was originated from the same closed colony as LEC rat did, was different from LEC/Hok inbred strain in 5 marker loci (data not shown).

It has been reported that Es-2 locus has five alleles, Es-2a, Es-2b, Es-2c, Es-2d, and Es-2e alleles defined by fast banded pattern, no visible enzyme activity, very fast banded pattern, slow banded pattern, and very slow banded pattern respectively, in serum sample [9]. Furthermore, Bender et al. [2] reported the distribution of the five alleles in the rat inbred strains. In LEC/Otk subline, we detected a new banded pattern, which showed an intermediate mobility between profiles of ES-2A and ES-2C types (Fig. 2). The new variant was tentatively designated as ES-2C' type. Recently, serum samples of ES-2C and ES-2C' types were reported to show the same

mobility by treating with sialidase [1]. This shows that the difference in mobility between ES-2C and ES-2C' types is due to the difference in addition of sialic acid into ES-2. The LEC/Otk rat derived from the LEC/Hok rat which was in more advanced generation and possessed higher level of inbreeding than other sublines did (Fig. 1). Therefore, on the assumption that the LEC/Otk rat has the higher level of genetic similarity to the LEC/Hok rat compared with other sublines, we strongly suppose that the occurrence of ES-2C' type is caused by a different environmental factor in Otsuka facility from that of other facilities. However, we cannot exclude the possibility that the difference in the addition of sialic acid into ES-2 is caused by the difference in genetic background among LEC sublines.

In order to exactly monitor genetic uniformity in the inbred strain of LEC mutant, the increase in the number of loci tested and the development of exquisite gene screening methods to detect polymorphism will be required.

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REFERENCES