A New Mutant Strain of Gunn-LA Wistar Rats with Genetic Deficiencies in Bilirubin and Androsterone Uridine Diphosphate-Glucuronosyltransferases

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Hooded Gunn rats which have low-activity of 4-nitrophenol uridine diphosphate-glucuronosyltransferase (GT) and defect in bilirubin GT were crossed with albino LA Wistar rats with a defect in androsterone GT. From F₂ and F₃ progeny were selected Gunn-LA Wistar rats which have defective bilirubin and androsterone GTs and low-active 4-nitrophenol GT. These rats had either hooded or albino coat color. In order to establish a uniform genetic background, albino male Gunn-LA Wistar rats were crossed with heterozygous albino female Gunn-LA Wistar rats in terms of bilirubin GT. The offsprings were all albino and were classified to Gunn-LA Wistar rats and heterozygous Gunn-LA Wistar rats by assaying their hepatic GT activities or by the appearance of jaundice. The linkage relationships between GT and coat color genes are discussed.

Keywords — Gunn-LA Wistar rat; uridine diphosphate-glucuronosyltransferase; isoenzyme deficiency; bilirubin; androsterone; 4-nitrophenol; genetic linkage; coat color

Introduction

Genetic deficiencies of hepatic uridine diphosphate (UDP)-glucuronosyltransferase (GT) are known in several mutant rat strains. Gunn¹⁾ reported that a mutant strain of Wistar rats shows hereditary hyperbilirubinemia and that the jaundice is inherited as an autosomal recessive trait. The mutant strain of Gunn rats lacks the ability to glucuronidate bilirubin.²⁾ Gunn rats are a good animal model for human Crigler-Najjar syndrome.³⁾ Scragg *et al.*⁴⁾ reported the absence of bilirubin GT protein in Gunn rats. In contrast, Chowdhury *et al.*⁵⁾ provided evidence that Gunn rats have functionally defective bilirubin GT protein. Cloning of bilirubin GT gene has not been reported. The details of the defective mechanism of bilirubin GT activity in Gunn rats remain to be elucidated. It has been reported that Gunn rats also inherit low GT activities toward 4-nitrophenol^{4,6)} and digoxigenin monodigitoxoside.^{7,8)} However, the low 4-nitrophenol GT activity is activated to the normal level by addition of *N*-nitrosodiethylamine⁵⁾ or *N*-nitrodiethylamine.⁹⁾

Wistar rats are mixed colonies with high (normal)-activity (HA) and low-activity (LA) of hepatic androsterone GT.^{10–12)} LA Wistar

rats have normal GT activities toward bilirubin and 4-nitrophenol¹³⁾ and the genetic expression of low androsterone GT activity is inherited in an autosomal recessive fashion.¹⁴⁾ Androsterone GT is absent in LA Wistar rats,¹⁵⁾ probably due to the deletion of androsterone GT gene.¹⁶⁾

Crossbreeding between Gunn and LA Wistar rats, followed by brother-sister inbreeding, provided Gunn-LA Wistar rats with defective bilirubin and androsterone GTs and low-active 4-nitrophenol GT.¹⁷⁾ Gunn rats used in this study have black pigment on their heads and a black stripe on their backs (hooded), whereas LA Wistar rats were albino. Gunn-LA Wistar rats were either hooded or albino.

In this paper, albino male Gunn-LA Wistar rats were crossed with albino female LA Wistar rats heterozygous in terms of bilirubin GT, to establish an albino inbred strain of Gunn-LA Wistar rats with a uniform genetic background.

Materials and Methods

Chemicals — [1,2-³H]Androsterone (53.3 Ci/mmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. Androsterone and bilirubin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 4-

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Nitrophenol, digitonin and Triton X-100 were purchased from Wako Pure Chemical Ind. Ltd., Tokyo, Japan. UDP-Glucuronic acid disodium salt and UDP-glucuronic acid trisodium salt were obtained from Boeringer, Mannheim, Germany and Yamasa Syoyu Co., Ltd., Tokyo, Japan, respectively. *N*-Nitrodiethylamine was prepared as described previously.¹⁸⁾ All other reagents were of analytical grade.

Animals — Gunn rats used in this study were supplied by Sankyo Co., Shizuoka, Japan.⁹⁾ Male Gunn rats (genotype HaHa/jj, dominant and recessive autosomal traits for androsterone GT and bilirubin GT, respectively) were crossed with female LA Wistar rats (haha/JJ),¹⁴⁾ followed by brother-sister inbreeding to provide F₁ and F₂ offsprings.¹⁷⁾ Gunn-LA Wistar rats (haha/jj) were selected by assaying GT activities toward bilirubin, androsterone and 4-nitrophenol in liver microsomes obtained by partial hepatectomy.¹⁷⁾ Male Gunn-LA Wistar rats (haha/jj) were crossed with heterozygous female Gunn-LA Wistar rats (haha/Jj). Fifty percent of the litters are expected to be Gunn-LA Wistar rats, which were selected by determining hepatic GT activities or by the appearance of jaundice.

Preparation of Microsomal Fractions — Animals (150–430 g, 60–100 d of age) were allowed free access to food and water before being hepatectomized. The liver was homogenized in ice-cold 0.25 M sucrose–0.1 M Tris-HCl buffer, pH 7.4, with a Teflon-glass homogenizer. Microsomal fractions were obtained by differential centrifugation as described previously.⁹⁾ The microsomal pellets were resuspended in 0.25 M sucrose–0.1 M Tris-HCl buffer, pH 7.4. The microsomal protein was determined by the method of Lowry *et al.*¹⁹⁾ with bovine serum albumin as standard.

Enzyme Assay — GT activities toward androsterone and 4-nitrophenol were assayed as described previously.¹⁷⁾ The standard incubation medium contained 0.17 mM [³H]androsterone (13 nCi) or 0.36 mM 4-nitrophenol, 2 mM UDP-glucuronic acid, 10 mM MgCl₂ and 0.1 M Tris-HCl buffer, pH 7.4 in a total volume of 0.5 ml. To activate GT activities, 0.02% Triton X-100 or 0.02% Triton X-100–10 mM *N*-nitro-

diethylamine¹⁷⁾ was added to the incubation medium. The latter combined activators stimulated low 4-nitrophenol GT activity to the normal level in Gunn rats.⁹⁾ Bilirubin GT activity was assayed by the method described by Heirwegh *et al.*²⁰⁾ The incubation was carried out for 20 min at 37 °C.

Results and Discussion

In a previous study on the genetic linkage of GT genes,¹⁷⁾ we reported a new mutant strain of Gunn-LA Wistar rats, which has defects in bilirubin and androsterone GTs and low-activity of 4-nitrophenol GT. These rats were raised by one outcross of Gunn rats with LA Wistar rats, followed by brother-sister inbreeding.

Gunn rats were of Wistar origin and were originally albino.¹⁾ Since Gunn rats have been maintained by crossbreeding with various rat strains, it is possible that Gunn rat colonies are not genetically identical except for the jaundiced locus. Gunn rats used in this study were initially obtained from Albert Einstein College of Medicine, New York and were crossed with Wistar-Imamichi rats at Sankyo Co., Shizuoka, Japan. They have hooded coat color similar to Long-Evans rats. The occurrence of hooded Gunn rats has been reported previously.^{21,22)} LA Wistar rats are albino and all the F₁ hybrids between Gunn and LA Wistar rats had hooded coat color. In F₂ generation, the albino and hooded rats were segregated in the approximate ratio of 1:3,¹⁷⁾ which is compatible with Mendel's First Law, the Principle of Segregation. About 7% of the F₂ progeny were Gunn-LA Wistar rats with albino or hooded coat color.

Gunn rats have been usually maintained by matings of male homozygotes (jj) and female heterozygotes (Jj), which give rise to theoretically 50% homozygotes and 50% heterozygotes. The cross between male homozygotes or heterozygotes and female homozygotes results in lower fertility, probably due to the high level of bilirubin present in the tissues of homozygous mothers.³⁾ On the basis of these data, matings of male Gunn-LA Wistar rats (haha/jj) and heterozygous female Gunn-LA Wistar rats (haha/Jj) appear to be a good choice in order to breed

TABLE I. Genetic Expression of Hepatic GT Activities in the F₃ Generation from Crosses between Male Gunn-LA Wistar Rats and Female LA Wistar Rats with Homozygous and Heterozygous Dominant Genotypes in Terms of Bilirubin GT

Animal	No	Bilirubin (Digitonin)	Androsterone (Triton)	4-Nitrophenol		Genotype
				(Triton)	(Triton + NEA)	
Type 1						
F ₂ parent						
Father	1	ND	0.17	2.80	11.93	haha/jj
Mother	1	0.37	0.10	7.28	12.60	haha/JJ
F ₃ offspring						
Male	4	0.48 ± 0.13	0.18 ± 0.01	6.29 ± 0.32	13.88 ± 0.94	haha/Jj
Female	5	0.47 ± 0.17	0.12 ± 0.02	6.27 ± 1.38	13.75 ± 1.27	
Total	9	0.47 ± 0.15	0.15 ± 0.04	6.28 ± 0.99	13.81 ± 1.06	
Type 2						
F ₂ parent						
Father	1	ND	0.08	0.93	10.54	haha/jj
Mother	2	0.12, 0.36	0.09, 0.08	3.54, 3.45	11.71, 12.26	haha/Jj
F ₃ offspring						
Male	2	ND	0.14, 0.14	1.80, 1.64	12.02, 14.21	haha/jj
Female	6	ND	0.10 ± 0.02	1.34 ± 0.25	12.88 ± 1.23	
Total	8	ND	0.11 ± 0.02	1.43 ± 0.28	12.94 ± 1.20	
Male	5	0.44 ± 0.11	0.15 ± 0.03	5.92 ± 1.36	15.06 ± 1.83	haha/Jj
Female	3	0.50 ± 0.16	0.08 ± 0.01	5.21 ± 1.35	12.66 ± 1.02	
Total	8	0.46 ± 0.12	0.13 ± 0.04	5.66 ± 1.31	14.16 ± 1.94	

GT activity is expressed as nmol/min per mg of protein. Each value is the mean ± S.D. Parenthesis indicates the activator(s) used for the enzyme assay. No: number of animals. ND: not detectable. Triton: 0.02% Triton X-100. NEA: 10 mM *N*-nitrodiethylamine.

TABLE II. Breeding of Albino Male Gunn-LA Wistar Rats and Heterozygous Albino Female Gunn-LA Wistar Rats in Terms of Bilirubin GT

Animal	No	Bilirubin (Digitonin)	Androsterone (Triton)	4-Nitrophenol		Genotype
				(Triton)	(Triton + NEA)	
Parent						
Father	3	ND	0.14 ± 0.05	1.88 ± 0.83	11.25 ± 0.89	haha/jj
Mother	3	0.35 ± 0.04	0.11 ± 0.01	6.42 ± 1.83	13.63 ± 1.76	haha/Jj
Offspring						
Male	12	ND	0.15 ± 0.02	2.18 ± 0.35	12.04 ± 1.20	haha/jj
Female	5	ND	0.09 ± 0.04	1.85 ± 0.50	12.43 ± 2.25	
Total	17	ND	0.13 ± 0.04	2.08 ± 0.42	12.16 ± 1.51	
Male	5	0.44 ± 0.08	0.13 ± 0.03	6.08 ± 1.35	13.51 ± 2.91	haha/Jj
Female	4	0.77 ± 0.24	0.09 ± 0.02	5.89 ± 0.89	13.66 ± 2.17	
Total	9	0.59 ± 0.23	0.11 ± 0.03	5.99 ± 1.10	13.57 ± 2.45	

GT activity is expressed as nmol/min per mg of protein. Each value is the mean ± S.D. Parenthesis indicates the activator(s) used for the enzyme assay. No: number of animals. ND: not detectable. Triton: 0.02% Triton X-100. NEA: 10 mM *N*-nitrodiethylamine.

Gunn-LA Wistar rats. Consequently, among F₂ progeny, male Gunn-LA Wistar rats were crossed with female rats (haha/Jj or haha/JJ) which have defective androsterone GT and high

bilirubin GT activities (Table I). In F_3 progeny, there were two types of genetic expression of GT activities. In type 1, all the offsprings showed a single defect in androsterone GT activity. Thus the mother of type 1 progeny had homozygous dominant genotype (JJ) in terms of bilirubin GT. In type 2, defects in bilirubin and androsterone GT activities as well as low 4-nitrophenol GT activity and a single defect in androsterone GT activity were segregated in the ratio of 1:1, which indicated that the mothers of type 2 progeny had heterozygous dominant genotype (Jj) in terms of bilirubin GT. Thus, these crosses provide a good means to distinguish the two genotypes in female rats and to maintain Gunn-LA Wistar rats.

Our Gunn-LA Wistar rats were not fixed genetically except for ha and j loci. To establish a uniform genetic background, we selected albino rats from F_2 and F_3 offsprings. Homozygous albino male Gunn-LA Wistar rats were crossed with heterozygous albino female Gunn-LA Wistar rats (Table II). All the offsprings were albino. Gunn-LA Wistar rats and heterozygous Gunn-LA Wistar rats were segregated in the ratio of 17:9. Further matings should bring the ratio to expected value of 1:1. We have been currently continuing these inbreedings to obtain the genetically homogeneous inbred strain of Gunn-LA Wistar rats. In a manner similar to Gunn rats, Gunn-LA Wistar rats exhibit some nervous symptoms such as wobbly gait and partial paralysis of the hind limbs.

It is known that rat coat colors are derived from several color genes such as gray, black, brown and albino as well as genes for color patterns such as uniform and restricted pigmentations.^{23,24)} It is well known that the hereditary transmission of rat coat colors follows Mendelian inheritance.^{23,24)} Wistar rats are homozygous at least for 3 genes: cc (absence of pigment), aa (black) and hh (white except for head and back stripe, hooded),²³⁾ their white coat color being due to cc genes. If cc genes are replaced by CC or Cc (C is a gene for full color and is dominant to c), hooded coat pattern makes its appearance.²⁴⁾ Gunn rats used in this study have been maintained by matings with Wistar-Imamichi rats, followed by brother-sister

inbreeding for more than 20 years and probably inherit CC, aa and hh genes similar to Long-Evans rats.²⁴⁾ In comparison with mouse genetics, a little is known about the chromosomal localization of rat genes. The c gene is assigned to rat chromosome 1.²⁵⁾ The h gene is linked to analbuminemia locus²⁶⁾ and appears to reside on rat chromosome 14.²⁷⁾ It was demonstrated that jaundiced gene (j) is not linked to c and h genes.^{28,29)} There are also no sex-linked inheritances of coat colors^{23,24)} and the GT isoenzymes.^{1,14)} These data together with our results provide evidence that gene locus for defective bilirubin GT does not reside on chromosomes 1, 14, X and Y, whereas defective androsterone GT gene is not located at least on chromosomes 1, X and Y. Six or more forms of GT isoenzymes exist in the rat liver.³⁰⁾ As previously reported,¹⁷⁾ androsterone GT and bilirubin GT genes are not linked with each other and are located on different chromosomes, whereas defective bilirubin GT and low-active 4-nitrophenol GT genes are closely linked on the same chromosome. However, further study is required to determine the chromosomal localization of the GT gene family. Gunn-LA Wistar rats with multiple GT deficiencies may be a good animal model for studying deficiency and multiplicity of GT isoenzymes.

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