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Linkage of the Structural Gene for Uroporphyrinogen I Synthase to Markers on Mouse Chromosome 9 in a Cross Between Feral and Inbred Mice

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The Ups *locus has been mapped to mouse chromosome 9 in a three-point cross. The observed gene order is centromere–*Ups*–15–*Mpi-1*–22–*Mod-1*. *Ups *is unlinked to* Lv*, which encodes the previous enzyme in the heme biosynthesis pathway. Feral mice collected at Skive, Denmark, have been characterized at several biochemical loci; multiple differences from inbred strains make this a useful stock for linkage analysis.*

KEY WORDS: heme; porphobilinogen deaminase; Skive; isozymes.

INTRODUCTION

Uroporphyrinogen I synthase (porphobilinogen deaminase; EC 4.3.1.8) is the third of eight enzymes in the biosynthetic pathway leading to heme production. These enzymes are ubiquitous among mammalian tissues, and genetic deficiency of any one of them results in human disease (Meyer and Schmid, 1978). Until now, the linkage relationships among the structural genes for this pathway in higher organisms have not been studied. The *Lv* locus in the mouse, encoding the second enzyme in the pathway, porphobilinogen synthase, has been assigned to mouse chromosome 4 (Hutton and Coleman,

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1969). The recent identification of a variant at the mouse *Ups* locus encoding uroporphyrinogen I synthase (Meisler and Carter, 1980) provided the opportunity to investigate the linkage relationship between the *Lv* and the *Ups* loci.

Structural variants of uroporphyrinogen I synthase are rare in mouse and human populations (Meisler and Carter, 1980). A basic variant of the human enzyme was observed in 4 individuals among 951 surveyed. No variants were found among 17 inbred strains examined, but an acidic variant was detected in feral mice *Mus musculus musculus,* collected in Denmark. We have used this acidic variant to determine the chromosomal location of mouse *Ups*, in a cross between the feral mice and a subline of inbred C57BL/6J mice.

Isoelectric focusing followed by staining for enzyme activity was used to identify structural variants. The method reveals the presence of multiple UPS isomers in mammalian cells. The isomers are produced by the sequential binding of one to four molecules of the acidic substrate, porphobilinogen, by the monomeric enzyme (Anderson and Desnick, 1980; Meisler *et al.,* 1981; Jordan and Berry, 1981). The difference in isoelectric points of the human and mouse isozymes in hybrid cell lines made it possible to assign the human *Ups* locus to chromosome region 11q13 → ter (Meisler *et al.,* 1980, 1981). In the present work, the chromosomal location of the mouse locus was investigated.

MATERIALS AND METHODS

Animals. Feral *Mus musculus musculus* (Skive) used in this study were collected by J. T. Nielsen, University of Aarhus, at Skive, Denmark. They were sent to RPMI in 1973, where they were established in the laboratory as a random-breeding stock. They have been maintained as a closed colony since that time. A congenic strain, C57BL/6J.*le rd Gus*^{h}, was established by Dr. E. Russell, the Jackson Laboratory, Bar Harbor, Maine. The stock was sent to RPMI in 1973. It has been maintained as an inbred strain since that time. In this article, it is designated C57BL/6J-1e.

Three F_1 litters were obtained from a cross between a Skive female and a C57BL/6J-1e male. Six F_1 females, heterozygous at *Ups*, were mated with C57BL/6J-1e males to produce the backcross generation which was analyzed. The segregating markers used in this study are listed in Table I.

YBR/Ki mice were purchased from the Kirschbaum Memorial Mouse Colony, Rootstown, Ohio. The PAC strain is the c line derived from a colony of mice that were trapped near Philadelphia, Pa., by Dr. James Connor. He established several inbred strains from these populations. PAC is one of those lines sent to RPMI in 1974 at F_{20}. This line has been maintained as an inbred strain at RPMI using brother–sister matings. All other inbred mice were purchased from the Jackson Laboratory. In most cases, individual mice from each strain were typed. CD 1 mice were obtained from the Charles River Breeding Lab, North Wilmington, Mass.

Table I. Loci Examined in Backcross Animals

Locus	Chromosome	Alleles	
		C57BL/6J-1e	Skive
Ups	—	a	b
A	2	a	A
1e	5	1e	+
Gpi-1	7	b	a
Mod-1	9	b	a
Mpi-1	9	b	a

Erythrocytes from MOL/JA, an inbred strain derived from *M.m. molossinus* by Dr. T. Roderick, Jackson Laboratory, were obtained from Dr. J. Hilgers, Netherlands Cancer Institute.

Sample Preparation. Erythrocyte lysates were prepared as previously described (Meisler and Carter, 1980). Livers were homogenized in 3 vol of 0.25 M sucrose in 50 mM Tris–HCl, *p*H 7.5, with a Polytron homogenizer (Brinkman). Kidneys were homogenized in 2 vol distilled H_2O. All homogenates were centrifuged at 28,000*g* for 30 min on the day of electrophoretic analysis.

Electrophoresis and Staining. Isoelectric focusing was carried out on PAG plates (*p*H 4.0–6.5) (LKB). The staining method for UPS is based on the fluorescence of the product (Meisler and Carter, 1980). Isozymes of glucose phosphate isomerase-1 (GPI-1), mannose phosphate isomerase-1 (MPI-1), and soluble malate dehydrogenase-1 (MOD-1) were separated by electrophoresis on Titan III cellulose acetate plates (60 × 75 mm) (Helena Laboratories, Tex.) in 25 mM Tris, 200 mM glycine buffer, *p*H 8.5. Electrophoresis was carried out at 200 V for 90 min, except that 120 min was required for MOD-1. GPI-1 was stained with fructose-6-phosphate as substrate in a 2% agar overlay (Carter and Parr, 1967). MPI-1 was stained with the substrate mannose-6-phosphate in a 2% agar overlay (Nichols *et al.*, 1973). MOD-1 was detected by the method of Holmes (1979).

RESULTS

Segregation of Ups *Alleles.* UPS phenotypes were determined by isoelectric focusing of hemolysates followed by staining for enzyme activity. Four or more enzyme isomers are visible in each hemolysate (Fig. 1). It is convenient to classify phenotypes on the basis of the major erythrocyte isomers (designated a and b in Fig. 1) since the minor components are not well resolved by this *p*H gradient. C57BL/6J-1e mice have the UPS A phenotype (Fig. 1, Lane 1), while the Skive mouse in our cross was UPS B (Fig. 1, Lane

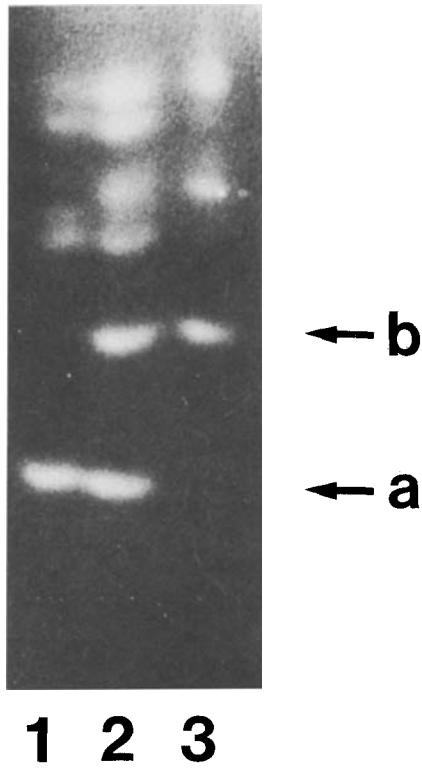

Fig. 1. Uroporphyrinogen I synthase phenotypes. Twenty-five-microliter samples of erythrocyte lysates were focused on 11-cm PAG plates, *p*H 4.0–6.5, followed by staining to detect UPS activity. Lane 1, C57BL/6J-1e; Lane 2, (Skive × C57BL/6J-1e)F_1; Lane 3, Skive. Multiple bands of activity are encoded by a single locus, as discussed in the Introduction. (A faint band in the position of the b allele product is occasionally observed in C57BL/6J mice. In such samples the intensity of the b-like band is always much less than that of the a band, which distinguishes the pattern from that of heterozygotes.)

3). Both sets of parental isomers are present in F_1 heterozygotes, in apparently equal amounts (Fig. 1, Lane 2). Backcrosses of heterozygous females to C57BL/6J-1e males produced mice of two phenotypes, UPS A and UPS A/B; the ratio of the two types (24:30) did not differ significantly from 1:1 ($\chi^2 = 0.67$; $P \cong 0.45$). Comparable numbers of males and females were present among the homozygotes and heterozygotes in the backcross generation. The results are consistent with the segregation of two codominantly expressed alleles at a single autosomal locus. This locus is designated *Ups*, with the alleles Ups^a producing the UPS A phenotype common to C57BL/6J and other inbred strains and Ups^b found in feral *M. m. musculus* (Skive).[4]

Biochemical evidence indicates that the minor UPS isomers visible on the gels are stable complexes of the same monomeric enzyme with variable numbers of substrate molecules (Anderson and Desnick, 1980; Meisler *et al.*, 1981; Jordan and Berry, 1981). If this is correct, then all isomers should

[4] The original allele designations, Ups^1 and Ups^2 (Meisler and Carter, 1980) have been changed to conform to the recommendations of the Committee on Standardized Genetic Nomenclature for Mice (1979).

cosegregate as a unit in the backcross. In agreement with this expectation, minor acidic isomers segregated with the Ups^b allele and could be detected in $Ups^{a/b}$ heterozygotes in the backcross generation but not in the $Ups^{a/a}$ individuals (Fig. 2).

Linkage Analysis. The *Ups* alleles did not cosegregate with the visible markers Agouti or light ear (Table II). The human UPS locus is known to be syntenic with the β-globin genes (Meisler *et al.*, 1980). Since mouse GPI-1 is linked to the mouse globin gene complex region, and since a GPI-1 variant is segregating in this cross, we analyzed GPI-1 in the backcross animals. No linkage was observed (Table II). It subsequently became clear that the human β-globin and UPS loci are located on different arms of chromosome 11 (Meisler *et al.*, 1981), so that linkage in mice would not be anticipated (Lalley *et al.*, 1978).

Significant linkage was observed in a three-point cross with the chromosome 9 markers *Mod-1* and *Mpi-1*. The frequencies of parental and recombi-

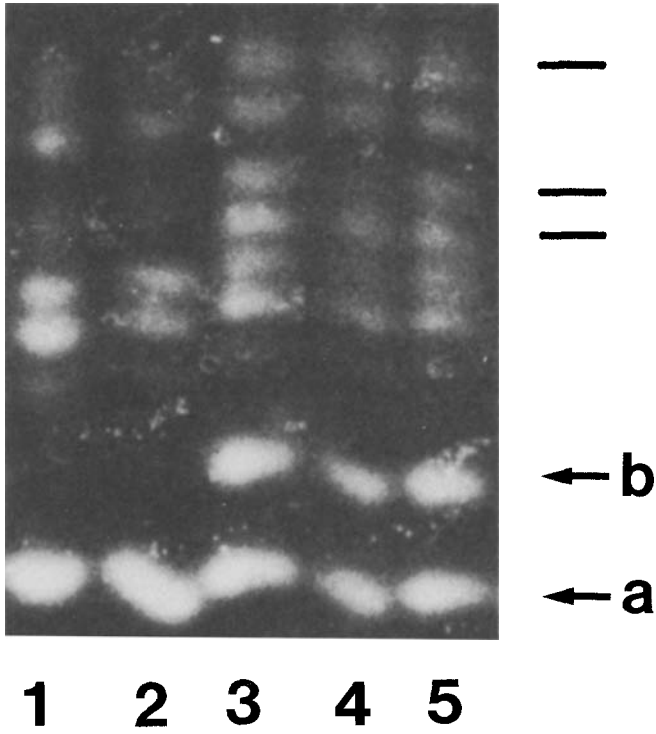

Fig. 2. Cosegregation of UPS isomers in the backcross generation. Five individuals are shown, with UPS phenotypes A (Lanes 1 and 2) and A/B (Lanes 3–5). In addition to the major b allele product, the heterozygotes contain three minor isomers (indicated by markers) which cosegregate with the major band.

Table II. Segregation of Alleles at *Ups*, with Alleles at the *Agouti*, *1e*, and *Gpi*-1 Loci in Progeny of the Cross (Skive × C57BL/6J-1e)F_1 × C57BL/6J-1e

Locus	Chromosome	Frequency of recombination with *Ups* ± SE
Agouti	2	24/54 = 0.44 ± 0.067
1e	5	23/54 = 0.43 ± 0.067
Gpi-1	7	31/54 = 0.57 ± 0.067

Table III. Segregation of Alleles at Three Linked Loci on Chromosome 9 in the Backcross Generation

Class	Genotype	Numbers	Total	Percentage recombinants
Parental	*Mod-1^b Mpi-1^b Ups^a*	14		
	Mod-1^b Mpi-1^b Ups^a		36	
	Mod-1^a Mpi-1^a Ups^b	22		
	Mod-1^b Mpi-1^b Ups^a			
Single crossover *Mod-1–Mpi-1*	*Mod-1^a Mpi-1^b Ups^a*	7		
	Mod-1^b Mpi-1^b Ups^a		10	18.5
	Mod-1^b Mpi-1^a Ups^b	3		
	Mod-1^b Mpi-1^b Ups^a			
Single crossover *Mpi-1–Ups*	*Mod-1^b Mpi-1^b Ups^b*	5		
	Mod-1^b Mpi-1^b Ups^a		6	11.1
	Mod-1^a Mpi-1^a Ups^a	1		
	Mod-1^b Mpi-1^b Ups^a			
Double crossovers	*Mod-1^a Mpi-1^b Ups^b*	0		
	Mod-1^b Mpi-1^b Ups^a		2	3.7
	Mod-1^b Mpi-1^a Ups^a	2		
	Mod-1^b Mpi-1^b Ups^a			

Frequency of recombination ± SE

Mod-1–Ups 20/54 = 0.37 ± 0.07
Mod-1–Mpi-1 12/54 = 0.22 ± 0.06
Mpi-1–Ups 8/54 = 0.15 ± 0.05

nant animals are presented in Table III. There were 20/54 recombinants between *Ups* and *Mod-1* and 8/54 recombinants between *Ups* and *Mpi-1*. The resulting gene order and map distances are centromere–*Ups*–15–*Mpi-1*–22–*Mod-1*. The frequency of double recombinants was 2/54 = 0.037; the expected frequency is 0.033.

Additional Electrophoretic Variants in Skive Mice. By comparisons with known alleles in various inbred strains, the Skive population was found to carry the following alleles: *Adh-1*^{a}, *Dip-1*^{b}, *Es-2*^{a}, *Es-3*^{b}, *Es-10*^{b}, *Gpd-1*^{b}, *Gus*^{b}, *Id-1*^{b}, *Pgm-1*^{b}, *Pgm-2*^{b}, and *Tam-1*^{b}. These biochemical markers, which with the exception of *Gus*^{b} all differ from the C57BL/6J alleles, make Skive a useful stock for linkage analysis.

Strain Survey. In a previous study, 17 inbred strains were found to carry the *Ups*^{a} allele (Meisler and Carter, 1980). Hemolysates from 18 additional strains have now been tested. The following 16 strains carry the *Ups*^{a} allele: ABY/Sn, AU/SsJ, BDP/J, C57BL/10J, C57L/J, DBA/1J, DBA/2J, I/LnJ, LG/J, MA/MyJ, P/J, PAC, RF/J, Sm/J, YBR/Ki, and 129/SvJ. In addition, 13 individuals from the outbred CD-1 stock were also homozygous for the *Ups*^{a} *allele. The Ups*^{b} allele has been identified in two inbred strains, LP/J and MOL/JA, which is derived from *M. m. molossinus.*

DISCUSSION

Linkage of the *Ups* locus to two segregating markers, *Mod-1* and *Mpi-1,* enabled us to determine the approximate location of this new biochemical marker on mouse chromosome 9. *Ups* is located close to the *Lap-1* and *Thy-1* loci, approximately 20 cM distal to the centromere of chromosome 9. The locus is thus unlinked to *Lv* on chromosome 4, demonstrating that the enzymes of heme biosynthesis are not clustered in the mammalian genome.

Our estimate of the distance between *Mod-1* and *Mpi-1*, 22 ± 6 cM, is in good agreement with the 19 cM reported by Nichols *et al.* (1973) based on their observation of 27 recombinants among 142 backcross progeny.

Several examples of conserved chromosome regions have emerged from comparison of human and mouse linkage groups (Lalley *et al.*, 1978; Pearson *et al.*, 1979). However, in the present case no conservation is evident. The three mouse chromosome 9 loci studied here, *Mod-1*, *Mpi-1*, and *Ups*, are unlinked in the human genome (Chen *et al.*, 1973; Meisler *et al.*, 1980; Heyningen *et al.*, 1975). On the other hand, two markers which are close to *Ups* on mouse chromosome 9, *Lap-1* and *Thy-1*, have not yet been assigned to human chromosomes. Similarly, the mouse homologues of human ESA-4 and SA11, which are linked to human *Ups* on the long arm of chromosome 11, have not been mapped in the mouse genome. Our results suggest possible locations for these unmapped loci.

It has been evident for some time that crosses between inbred and feral mice could be useful for linkage analysis, due to the presence of unusual

biochemical markers in wild mouse populations (Chapman, 1978). This study provides an example of the utility of feral mice for expansion of the mouse linkage map.

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