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Linkage of *aconitase-1* and *major urinary protein-1* loci in male rats

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Abstract. A new major urinary protein allele *Mup-1*^{c} with "null" activity was detected in males of the COP strain. The (BN × COP)F1 × COP backcross had significant segregation distortion of the *Mup-1* and *Aco-1* alleles that indicated a linkage between the genes, at a map distance of 13 ± 4 cM. The loci reside on the linkage group II of the rat with the *b* locus. According to our data and the results published previously, the map distance and the orientation of genes is *b* − 8 ± 4 − *Mup-1* − 13 ± 4 − *Aco-1*. These genes form a syntenic group in both the mouse and the rat.

Keywords. Urinary protein; aconitase; linkage; rat.

1. Introduction

The polymorphism of the major urinary protein (*Mup-1*) in the urine of male rats was detected by van Zutphen *et al* (1981), and its linkage to brown coat color (*b*) was reported by Nikaido *et al* (1982). The urinary protein was found in a very low concentration in the urine of female rats as well (Kondo and Yamada 1983). Major urinary proteins in the mouse are synthesized in the liver (Knopf *et al* 1983) by two divergently oriented genes (Clark *et al* 1984).

The polymorphism of aconitase (E.C.4.2.1.3) in the rat kidney was described recently by Adams *et al* (1984). The enzyme was screened on cellulose acetate and two phenotypes representing the slow and fast forms of the enzyme were found. No linkage to cytoplasmic aldehyde dehydrogenase-2 (*Ahd-2*), immunoglobulin kappa chain (*Igk-1*) and seminal vesicle protein-1 (*Svp-1*) was detected. The aconitase locus (*Aco-1*) on the mouse chromosome 4 is linked to *b* and to *Mup-1* (Nadeau and Eicher 1982). The *Aco-1* on chromosome 9 in man is in the same syntenic group (Cook *et al* 1978).

It was logical to assume that *Aco-1* would be linked to *Mup-1* in the rat because both markers are linked on chromosome 4 in the mouse and the rodents have several conserved linkages. Indeed, we report in this article the linkage of *Aco-1* and *Mup-1* and the finding of a new major male urinary protein allele of the *Mup-1* locus.

2. Materials and methods

2.1 *Animals*

The rat colony was maintained on tap water and solid pelleted food *ad libitum*, and illumination was provided from 7 to 19 hr. The COP strain was obtained from the

National Cancer Institute, National Institutes of Health, Bethesda, MD, and the Brown Norway (BN) rats were purchased from the Charles Rivers Breeding Laboratories, Wilmington, Maine. The origin of the other rat strains was described previously (Stolc *et al* 1980).

The COP strain carries the *Aco-1*^{b} marker, and the BN strain carries the *Mup-1*^{a} and *Aco-1*^{a} markers. The genotypes were used according to Bender *et al* (1984). Our result indicated that the COP strain carries the *Mup-1*^{c} marker. The segregation of the glyoxalase-1 (*Glo-1*), and hooded (*h*) alleles was also studied as the COP and BN strains carry different traits. The segregation of the alleles was studied in 139 rats of the (BN × COP)F1 × COP backcross. The linkage of *Aco-1* to the *Mup-1* locus was analyzed in 78 males of the (BN × COP)F1 × COP backcross.

2.2 *Biological material*

Experimental rats were transferred to a container with a lid and taken out after several seconds. They marked the container with urine almost immediately after the lid was closed. Urine was taken out with plastic pipets and stored at −70°C. The isoelectrofocusing was performed using thin polyacrylamide gels and electrode buffers at pH 3 and 10. The material was purchased from Serva (Westbury, NY). Five μl of urine were delivered on gels and electrophoresis was performed at 4 watts till the voltage reached 1700 volts. The proteins in the gels were then denatured in 15% trichloroacetic acid and stained with SERVA blue dye according to the manufacturer's recommendation.

Blood was collected in the EDTA-containing vacutainers from rats that were under light ether anesthesia. The rats were then killed by cervical dislocation. Erythrocytes were lysed in 10 mM Hepes buffer, pH 7·4, containing 5 mM magnesium chloride. Glyoxalase activity was determined as described previously (Stolc *et al* 1980). The kidneys were homogenized in 5 volumes (w/v) of glass distilled water and centrifuged at 1,000 g for 10 min at 4°C. Protein concentration in the supernatant was determined by the method of Lowry *et al* (1951) and aliquots containing the same amount of proteins (≈ 100 μg/3 μl) were delivered on agarose gel. Thin-layer agarose isoelectrofocusing gels were purchased from Isolab (Akron, Ohio) and electric power was applied at 12 watts till the final voltage of 1200 volts was reached. The electrode buffers were 0·5 M acetic acid and 0·5 M NaOH for the pH range of 3–10. Aconitase activity was detected using the staining method described by Harris and Hopkinson (1976). Briefly, the gels were incubated at 25°C in a mixture composed of *cis*-aconitic acid, 75 mg; magnesium chloride, 286 mg; NADP, 5 mg; MTT, 5 mg; PMS, 1·25 mg; isocitrate dehydrogenase, 2 units; and Tris/HCl buffer, 0·5 M. The final pH was adjusted to 8·0, and the volume to 25 ml.

3. Results

3.1 *Urinary proteins*

The isoelectrophoretic pattern of urinary proteins detected in the urine of BN, COP and (BN × COP)F1 male and female rats is shown in figure 1. The

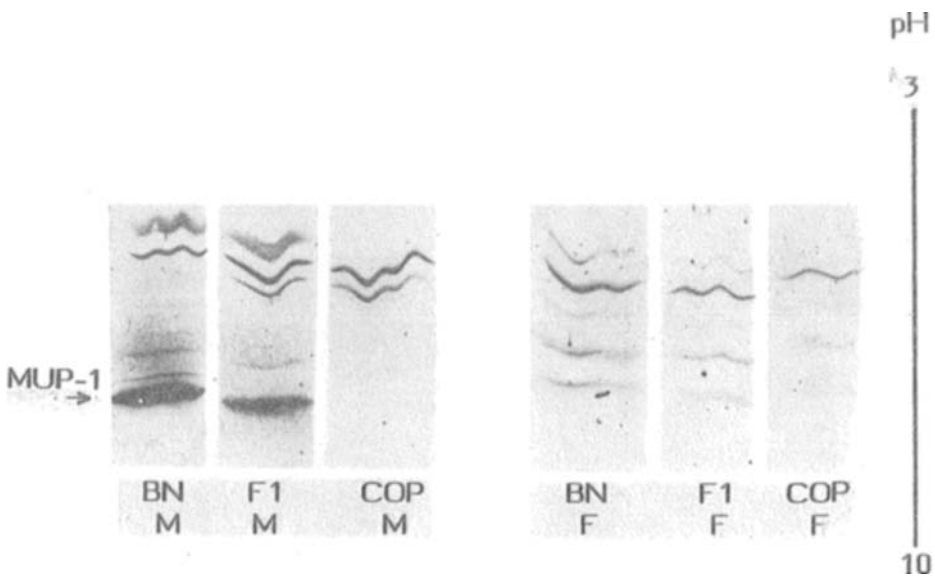

Figure 1. Isoelectrofocusing of urinary proteins. The position of the major male urinary protein (MUP-1) is shown by the arrow. M = males, F = females, F1 = (BN × COP)F1 hybrid.

isoelectrofocusing resolved 5 to 7 protein bands. One of the bands was clearly present in abundant amounts in male urine. As shown in figure 1 the quantity and pattern of male and female urinary proteins were different. In addition, there was a striking difference in the urinary protein patterns between the males of the COP and BN strains. The BN males lacked a band that was present in the COP males, and in contrast, the COP males were deficient in two urinary proteins including the major male urinary protein in comparison to BN males. Because the BN rats carry the *Mup-1*^{a} allele, the absence of the major protein in the urine of COP male rats indicated a "null" allele. Hence, the COP male rats carry the *Mup-1*^{c} allele. The (BN × COP)F1 hybrids showed a combined pattern, indicating that the "active" urinary protein alleles had incomplete dominance over the "null" alleles. The extremely low concentration of the major male urinary protein in the urine of females precluded genetic analysis. In addition, only the presence or absence of the major male urinary protein was detected in the urine of the backcross males due to technical problems with different batches of the isoelectrofocusing gels.

Several bands with aconitase activity were detected in the rat kidney using isoelectrofocusing gels (figure 2). The two middle bands were identical with the fast and slow moving aconitase isozymes described by Adams *et al* (1984). Screening of several inbred rat strains showed the same aconitase phenotypes as were published previously (Adams *et al* 1984). The ACI, BDIV, DA and WF strains had the ACO-1B, ACO-1B, ACO-1B and ACO-1A phenotypes, respectively. Thus the band in the aconitase region 1 (ACO-1) represents the slow moving aconitase isozyme and is the product of the *Aco-1*^{a} allele, and the band in the region 2

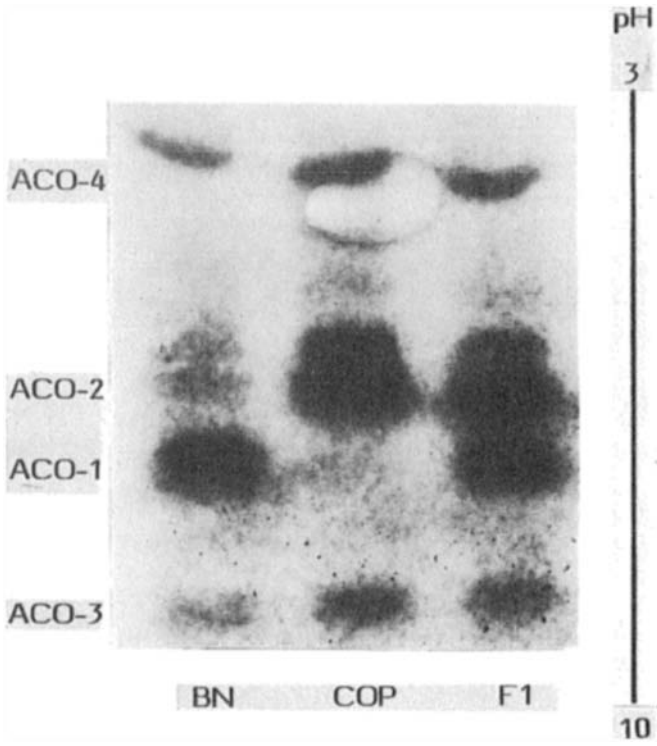

Figure 2. Isoelectrofocusing of aconitase isozymes in the kidney. The aconitase activity in the zone ACO-1 is identical with the "slow" isozyme and is a product of the *Aco-1*^{a} allele, and the aconitase band in the zone ACO-2 is identical with the "fast" isozyme and is the product of the *Aco-1*^{b} allele. The aconitase activity in the zones ACO-3 and ACO-4 was also detected. F1 = (BN × COP)F1 hybrid.

(ACO-2) represents the fast moving aconitase isozyme and is the product of the *Aco-1*^{b} allele. Our results thus confirm the previous observation that BN rats carry the *Aco-1*^{a} trait and COP rats carry the *Aco-1*^{b} trait. The (BN × COP)F1 male and female hybrids showed the ACO-1AB phenotype, hence the alleles were expressed in codominant fashion.

3.2 *Segregation of alleles*

Mup-1 alleles segregated close to a 1 : 1 ratio in the (BN × COP)F1 × COP backcross (chi-square = 2·51, $P > 0{\cdot}1$). There were 32 males with the MUP-1AC phenotype and 46 males with the MUP-1C phenotype. The aconitase autosomal alleles segregated according to normal Mendelian inheritance in the (BN × COP)F1 × COP backcross. The data showed 44 males and 33 females with the ACO-1B phenotype and 34 males and 28 females with the ACO-1AB phenotype. The data did not differ significantly from the expected 1 : 1 ratio (chi-square = 1·57 and 0·58, $P > 0{\cdot}25$).

The data in table 1 show significant segregation distortion of the *Aco-1* and *Mup-1* alleles in the (BN × COP)F1 × COP backcross, indicating a linkage between

the genes. The map distance was 13 ± 4 cM and the linkage was highly significant (chi-square = 43·13, $P < 0{\cdot}001$). The segregation of *Aco-1* and *Mup-1* alleles was normal (chi-square = 1·28 and 2·51). There were 28 rats with the MUP-1AC and ACO-1AB phenotypes, 40 rats with the MUP-1C and ACO-1B phenotypes and 10 were recombinant animals (4 with MUP-1AC, ACO-1B and 6 with MUP-1C and ACO-1AB phenotypes). The segregation analysis of other loci is shown in table 1. None of the other genes were linked to one another.

Table 1. Segregation analysis in the (BN × COP)F1 × COP backcross. The BN rats carry the *Aco-1*^{a}, *Glo-1*^{b}, *h*', and *Mup-1*^{a} markers, and the COP rats carry the *Aco-1*^{b}, *Glo-1*^{l}, *h*, and *Mup-1*^{c} markers.

Markers	Parental	Recombinant	chi-square	$P <$
Aco-1 : Glo-1	68	71	0·07	NS
Aco-1 . h	77	62	1·62	NS
Aco-1 : Mup-1	68	10	43·12	0·001
Glo-1 : Mup-1	40	38	0·05	NS
h Glo-1	63	76	1·21	NS
h : Mup-1	39	39	0·00	NS

NS = not significant.

4. Discussion

Rats and mice are phylogenetically closely related and they have in common several syntenic linkages (see Lalley and McKusick 1985). We discuss in this article that the mouse and the rat have the *b*, *Mup-1* and *Aco-1* genes linked to form a conserved group. The genes form part of the linkage group II of the rat and they are located on chromosome 4 of the mouse. The distance between the two terminal genes *b* and *Aco-1* is 21 cM in the rat and 15 cM in the mouse which is well in the range of experimental error. *Mup-1* is oriented between the two terminal *b* and *Aco-1* genes. *Mup-1* is controlled by 4 alleles in the mouse (Nikaido and Hayakawa 1984) and so far, three alleles were found in the rat. The assignment of aconitase to the linkage group II of the rat must be confirmed by additional studies because van Zutphen *et al* (1985) discussed recently its possible association with the albino and hemoglobin loci. Both genes are on linkage group I of the rat. The spurious linkage found by van Zutphen *et al* (1985) might be due to the affinity points on different chromosomes that exhibit preferential segregation at meiosis (Douglas 1966). The finding is also important for other studies in rat genetics because it shows that a map distance of 30 or 36 cM that was found between the *Aco-1* and *c* and *Hbb* loci need not represent true linkage.

Both markers, aconitase and the major urinary protein were investigated recently because of their significant association with pathological processes. Aconitase is a Krebs cycle enzyme and its importance in cancer biology was stressed by the finding that it is inhibited in tumor cells cocultivated with activated macrophages (Drapier and Hibbs 1985). The male and female urinary protein patterns indicate a regulatory influence of sex-related factors (Froehlich *et al* 1984).

The authors reported significant difference in the urinary protein pattern after castration. The proteins with a molecular weight of 19,000 in male rats and those with molecular weight 67,000 in female rats were the most affected. The administration of testosterone after the castration of rats significantly increased all urinary proteins, mainly the fraction with molecular weight of 19,000 (Schneider and Kugler 1984). In addition to steroids, excretion of the major male rat urinary proteins was also affected by growth hormone (Husman *et al* 1985). It was also found that the *Mup-1* locus controlled the major urinary proteins in females (Kondo and Yamada 1983).

The analysis of urinary proteins in the rat may help to develop models related to human kidney damage. Rats excrete large amounts of proteins in urine under physiological conditions. In man, the composition of urinary proteins vary, depending on the location of kidney damage (Free and Free 1978). Macromolecular proteinuria is a sign of glomerulopathy, in contrast to micromolecular proteinuria which is manifested during tubular insufficiency. Because pathological urinary protein patterns often precede the development of several diseases such as diabetes mellitus, hypertension, lupus erythematosus or transplanted kidney rejection, the study of urinary proteins may have preventive significance in man. Hence, further studies are warranted to develop valid rat models as they relate to human proteinuria.

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References

Adams M, Baverstock P R, Watts C H S and Gutmann G A 1984 Enzyme markers in inbred rat *Rattus norwegicus* strains. Genetics of new markers and strain profiles. *Biochem. Genet.* 22: 611–629

Bender K, Adams M, Baverstock P R, den Bieman M, Bissbort S, Brdicka R, Butcher G W, Cramer D V, von Deimling O, Festing M F W, Gunther E, Guttmann R D, Hedrich H J, Kendall P B, Kluge R, Moutier R, Simon B, Womack J E, Yamada J and van Zutphen B 1984 Biochemical markers in inbred strains of the rat (*Rattus norvegicus*). *Immunogenetics* 19: 257–266

Clark A J, Hickman J and P' 'hop J 1984 A 45 kilobase DNA domain with two divergently orientated genes is the unit of organization of the murine major urinary protein genes. *EMBO J.* 3: 2055–2064

Cook P J L, Robson E B, Buckton K E, Slaghter C A, Gray J E, Blank C E, James F E, Ridler M A C, Insley J and Hulten M 1978 Segregation of ABO AK1 and ACONS in families with abnormalities of chromosome 9. *Ann. Hum. Genet.* 41: 365–370

Douglas L T 1966 Meiosis. I. Association of non-homologous bivalents during spermatogenesis in white mice. *Genetica* 37: 466–480

Drapier J C and Hibbs J B 1985 Aconitase, a Krebs cycle enzyme with an iron-sulfur cluster is inhibited in tumor-cells cocultivated with activated macrophages. *J. Leuk. Biol.* 38: 66

Free A H and Free H M 1978 *Urinalysis in clinical laboratory practice* 3rd printing (West Palm Beach, Fl.: CRC Press) p. 51

Froehlich H, Kugler P and Schiebler T H 1984 Sex differences of the protein pattern in the urine of castrated rats. *Z. Mikrosk.-Anat. Forsch.* 98: 86–106

Harris H and Hopkinson D A 1976 *Handbook of enzyme electrophoresis in human genetics* (New York: Elsevier)

Husman B, Norstedt G, Mode A and Gustafsson J A 1985 The mode of growth hormone administration is of major importance for the excretion of the major male rat urinary proteins. *Mol. Cell Endocrinol.* 40: 205–210

Knopf J L, Gallagher J F and Held W A 1983 Differential multihormonal regulation of the mouse major urinary protein gene family in the liver. *Mol. Cell Biol.* 3: 2232–2240

Kondo Y and Yamada J 1983 Male urinary protein-1 *Mup-1* expression in the female rat. *J. Hered.* 74: 280–282

Lalley P A and McKusick V A 1985 Report of the committee on comparative mapping. *Cytogenet. Cell Genet.* 40: 536–566

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275

Nadeau J H and Eicher E M 1982 Conserved linkage of soluble aconitase and galactose-1-phosphate uridyl transferase in mouse and man. Assignment of these genes to mouse chromosome 4. *Cytogenet. Cell Genet.* 34: 271–281

Nikaido H and Hayakawa J I 1984 A new allele at the *Mup-1* locus controlling an electrophoretic variant of major urinary proteins in mice. *J. Hered.* 75: 59–61

Nikaido H, Yamada J and Kondo Y 1982 Male urinary protein-1 (Mup-1) in the rat assigned to linkage group II. *J. Hered.* 73: 119–122

Schneider H and Kugler P 1984 Urinary protein pattern of rats after castration and treatment with sex hormones. *Z. Mikrosk.-Anat. Forsch.* 98: 873–892

Stolc V, Kunz H W and Gill T J III 1980 The linkage of glyoxalase-1 to the major histocompatibility complex in the rat. *J. Immunol.* 125: 1167–1170

van Zutphen L F M, Lagerwerf A, Bouw J and den Bieman M G C W 1981 Biochemical polymorphism in the rat genetics of three electrophoretic variants and characterization of inbred strains. *Biochem. Genet.* 19: 173–186

van Zutphen L F M, den Bieman M, Hedrich H J and Kluge R 1985 Assignment of *Lap-1* to linkage group I of the rat (*Rattus norvegicus*). *Biochem. Genet.* 23: 599–606