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Mapping the adrenal lipid depletion gene of the AKR/J mouse strain

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SUMMARY

The pattern of inheritance of the autosomal recessive adrenal lipid depletion (*ald*) allele among the AKXL recombinant inbred mouse lines, derived from a cross between AKR/J and C57L/J, revealed that the *ald* locus is located on chromosome 1 near the dipeptidase-1 (*Dip-1*) locus. The linkage was confirmed in a backcross generation and the gene order *ln-Dip-1-ald* was established.

1. INTRODUCTION

The adrenal lipid depletion (*ald*) gene of the mouse is an autosomal recessive, found only in the high leukemic-AKR strain and its descendants (Arnesen, 1955). Homozygotes exhibit spontaneous adrenocortical lipid depletion at the time of sexual maturity in the normally lipid-rich adrenal cortex. Lipid depletion is essentially complete in males but incomplete and variable in females. Adrenals of the *ald/ald* mice are characterized by decreased corticosterone content (resting state), and by decreased corticosterone synthesis *in vivo* and *in vitro* in response to ACTH, but increased formation of mitochondria, pointing to increased energy metabolism, increased RNA synthesis, and increased activity of the phosphogluconate oxidation pathway (Molne, 1969). The reduced corticosterone response to exogenous ACTH was taken as evidence that the *ald* condition is not due to reduced ACTH secretion of the pituitary and that the primary site of gene action may be intrinsic to the adrenal gland. We report here the chromosomal location of the *ald* gene.

2. METHODS

Recombinant inbred (RI) lines were produced by continuous brother–sister mating beginning with the F_2 generation of the cross between AKR/J and C57L/J (Taylor, Meier & Myers, 1971). The lines are designated AKXL with a numeral suffix to denote distinct independently inbred RI lines, e.g. AKXL-4. Such lines have utility for the study of segregation, linkage, and pleiotropy (Bailey, 1971).

AKXL-lines 1, 4, 6, 8, 11, 12, 13, 14, 36, 37 and 38 were derived from the cross of C57L/J females with AKR/J males, while lines 16, 17, 18, 19, 21, 23, 24, 25, 28 and 29 were derived from the reciprocal F_1 hybrids. Lines 1 and 23 are now extinct.

In the development of three of the lines there were deviations from strict

brother–sister mating. At the outset one of the lines (AKXL-16) was established inadvertently from a mating between an F_2 and a C57L-backcross rather than two F_2's, resulting in an expectation of 5/8 autosomal contribution from the C57L/J progenitor strain. At the time the error was discovered, the decision was made that the line was too valuable to discard. In the development of AKXL-6 and AKXL-11 reproductive crises were encountered at F_7 and F_{10}, respectively, such that there were no fertile females. An attempt to rescue these lines was undertaken by mating an AKXL male to C57L/J females, and subsequently backcrossing to AKXL males. An F_6 AKXL-6 male was mated to a C57L/J ♀. A female from this mating was mated to an F_7 AKXL-6 son of the F_6 AKXL-6 male. A female from this mating was mated to its father. Brother–sister mating was then resumed. The expected fractional autosomal contribution of the C57L/J strain is 9/16. An F_{10} AKXL-11 male was mated to a C57L/J ♀ and a daughter was mated to the same male. Brother–sister mating was then resumed. The expected fractional autosomal contribution of the C57L/J strain is 5/8. The consequences of these deviations from brother–sister mating on the approach toward homozygosity and the probability of fixing a recombinant genotype are considered to be slight and will be disregarded in calculations.

After ten or more generations of sib mating ($F > 0{\cdot}89$), the lines were typed with respect to numerous known allelic AKR/J versus C57L/J differences, including the *ald* gene and the chromosome 1 markers leaden coat colour (*ln*) and dipeptidase-1 electrophoretic variant (*Dip-1*). Four to six sexually mature (> 10 weeks of age) males from each AKXL RI line were killed, the adrenals removed, excess fat trimmed, and frozen sections cut and stained with Oil Red O, mounted, and examined microscopically for the presence or absence of the adrenocortical lipid depletion phenotype. Pigmented AKXL RI lines were classified directly as *ln* or +, and pedigreed males of the albino (*c*/*c*) AKXL RI lines were outcrossed to C57L/J females, to progeny test for the leaden genotype. Retired breeding pairs were typed for *Dip-1* by starch-gel electrophoresis of kidney homogenates according to the method described by Chapman, Ruddle & Roderick (1971).

To confirm linkage C57L × AKR F_1 females were mated to an AKXL-1 male (*ln Dip-1*^{b} *ald*/*ln Dip-1*^{b} *ald*) to produce a testcross generation. The pigmented male progeny were typed for the three markers as described above.

3. RESULTS AND DISCUSSION

Table 1 shows the segregation of the three markers in 21 AKXL RI lines. Eight lines are fixed for the *ald* gene of AKR/J, while the 13 other lines are apparently fixed for the dominant normal allele from C57L/J. A significant deficiency of recombinant genotypes with respect to *Dip-1* and *ald* was evident ($P < 0{\cdot}01$). Of 21 lines typed for *Dip-1* and *ald* only three were recombinant types, AKXL-12, AKXL-24 and AKXL-29. Eight of 21 AKXL lines involved recombination between *ald* and *ln*, suggesting that the *ald-Dip-1* interval is shorter than the

Table 1. *Segregation of chromosome 1 markers in AKXL recombinant inbred lines*

AKXL line (or progenitor strain)	Loci*				
	ln		*Dip-1*		*ald*
1	L	×	A		A
4	L		L		L
6	L	×	A		A
8	A		A		A
11	L		L		L
12	L	×	A	×	L
13	A	×	L		L
14	A	×	L		L
16	L		L		L
17	A		A		A
18	L	×	A		A
19	L		L		L
21	L		L		L
23	A	×	L		L
24	A		A	×	L
25	A		A		A
28	L	×	A	×	L
29	A		A		A
36	L		L		L
37	L		L		L
38	L	×	A		A
(AKR/J)	(A)		(A)		(A)
(C57L/J)	(L)		(L)		(L)

* 'A' and 'L' are used here as generic symbols for alleles inherited from the AKR/J and C57L/J strains, respectively. The AKR/J genotype is $+$ *Dip-1*^{b} *ald*/$+$ *Dip-1*^{b} *ald*. The C57L/J genotype is *ln Dip-1*$^{a}+$/*ln Dip-1*$^{a}+$. Regions where crossovers have resulted in recombination in the different AKXL lines are denoted by an ×.

Table 2. *Segregation of chromosome 1 markers in testcross progeny from (C57L/J × AKR/J) F_1 ♀ × AKXL-1 ♂**

Hybrid gamete	Frequency
Parental	
$+$ *Dip-1*^{b} *ald*	12
ln Dip-1^{a} $+$	3
Crossover, *ln* – *Dip-1*	
$+$ *Dip-1*^{a} $+$	1
ln Dip-1^{b} *ald*	3
Crossover, *Dip-1* – *ald*	
$+$ *Dip-1*^{b} $+$	2
ln Dip-1^{a} *ald*	0
Double crossover	
$+$ *Dip-1*^{a} *ald*	0
ln Dip-1^{b} $+$	0
Total	21

* The genotypes of the testcross parents were $+$ *Dip-1*^{b} *ald*/*ln Dip-1*^{a} $+$ ♀ × *ln Dip-1*^{b} *ald*/*ln Dip-1*^{b} *ald* ♂.

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ln-Dip-1 and *ald-ln* intervals (Table 1). Since the distance between *ln* and *Dip-1* is about 11 cM (Chapman *et al.* 1971), the gene order *ln-ald-Dip-1* seemed unlikely as this would require eleven recombinations in the *ln-Dip-1* interval. Thus the gene order *ln-Dip-1-ald* was preferred. The test cross data shown in Table 2 confirmed the preceding interpretation. Among 21 progeny there were four crossovers between *ln* and *Dip-1* and two crossovers between *Dip-1* and *ald*. No double crossovers need be postulated if the gene order is *ln-Dip-1-ald* (Table 2).

Multiple opportunities for crossing over arise in the inbreeding process of developing an RI strain. With sib mating the probability of fixing a recombinant genotype (R), is related to the probability of recombination between the two loci in a single meiosis (r) by the equation, $R = 4r/(1+6r)$ (Haldane & Waddington, 1931). An estimate of the recombination frequency (r) can be obtained in terms of the observed proportion of lines that are crossover types (R_0), $r = R_0/(4-6R_0)$, and the large sample variance of the estimate is given by $V(r) = r(1+2r)(1+6r)^2/4N$, where N is the number of RI lines contributing to the estimate (E. L. Green, personal communication). Applying these relationships to the data in Table 1, we obtain the following estimates of recombination frequency: *ln-Dip-1*, $0{\cdot}30 \pm 0{\cdot}21$; *Dip-1-ald*, $0{\cdot}05 \pm 0{\cdot}03$. These are not significantly different from the respective values obtained in the backcross, $0{\cdot}19 \pm 0{\cdot}09$ and $0{\cdot}10 \pm 0{\cdot}06$. Note that the estimate obtained from the RI lines reflects the average recombination frequency in the two sexes, whereas the backcross data reflects recombination in females only. These results illustrate the relationship between the amount of information contained in the two sorts of data (RI and backcross) and the recombination fraction. For a given number of RI lines or backcross progeny tested, the relative efficiency of RI lines to backcross progeny is $4(1-r)/(1-2r)(1+6r)^2$. As the recombination frequency increases from zero to one-half, the relative efficiency drops from four to one-sixteenth. When r is one-ninth, the relative efficiency approximates unity. With both the RI and backcross data the distances estimated for the *ln-Dip-1* interval are somewhat greater than that obtained previously (Chapman *et al.* 1971). The combined data from the RI lines and the backcross give the following estimates of recombination frequency: *ln-Dip-1*, $0{\cdot}206 \pm 0{\cdot}080$; and *Dip-1-ald*, $0{\cdot}055 \pm 0{\cdot}028$.

The present study demonstrates that RI lines can be used to map loci as predicted by genetic theory. The *ald* locus is the first case of linkage detection through the use of AKXL RI lines. Obviously, as the AKXL RI lines are typed with respect to additional loci, the probability of detecting linkage will increase, and maps of individual chromosomes will become more detailed. At the present time the AKXL RI lines have been at least partially typed for more than thirty loci (Taylor & collaborators, unpublished data). There are at least 12 crossovers in the interval between *ln* and *ald* among the 21 AKXL lines that will help define the location of other loci in this interval that also segregate in the AKXL RI lines. This fact attests to the relative power of the RI approach for genetic fine structure. We can predict that there will be on average four crossover points per 100 cM of chromosome length in each RI line. The distribution of these points is not known,

but the expectation is that interference will not be intense since the recombinational events leading to multiple crossovers need not occur in the same gamete. For small intervals the frequency of triple and higher order crossovers will be negligible, so that the expected frequency of double crossovers is $24r^2/1+6r$, where r is the length of the interval in centimorgans. The two doubles observed (AKXL-12 and AKXL-28) are compatible with independence ($\chi^2_1 = 0{\cdot}81$, $P > 0{\cdot}35$). Much more data would be required to adequately test the predicted frequency of double crossovers.

Knowledge of the location of the *ald* locus may be useful for creating an *ald* congenic line so that the effects of the *ald* gene can be distinguished from the genetic background. An induced chromosome 1 inversion, In(1)1Rk (Davisson & Roderick, 1973), recombines with *ald* but not with *Dip-1*, indicating that the distal breakpoint of this inversion is located between *ald* and *Dip-1* (Roderick, personal communication).

The *ald* gene has been found only in the AKR strain and its descendants although a similar phenotype is found in DBA mice. The extent to which other inbred strains have been tested is obscure. However, we examined adrenals from males of strains MA/MyJ, PL/J, RF/J, SJL/J, ST/bJ and SWR/J with respect to the adrenal lipid depletion character and all were normal. These strains were chosen because to our knowledge they had not been previously tested, and other genetic information indicated that they might be to some degree related to AKR/J (Taylor, 1972). We cannot say whether the *ald* allele arose as a mutation within the AKR strain or was present in some of the non-inbred ancestors. The *ald* gene is not of recent mutational origin as it is present in different AKR sublines that have been separated for as much as 35 years (Arnesen, 1955; Metcalf, 1960).

The question of whether or not the *ald* gene predisposes to spontaneous leukemia in the AKR strain is unclear (Arnesen, 1956, 1974). The *ald* gene is not a sufficient cause of leukemia, but the possibility that it is a contributing factor to the disease is not ruled out. When life history studies of the AKXL RI lines are completed, it may be possible to evaluate the role of the *ald* locus in tumourigenesis.

It would also be of interest to know whether the *ald* gene is associated with any behavioural phenotype. The AKR/J strain is relatively docile, shy, inactive, and is not excitable. Casual observations of the AKXL RI lines reveal that both *ald* and + bearing lines vary widely with respect to these traits. However, future studies of the AKXL RI lines may reveal behavioural correlates of the adrenal lipid depletion phenotype.

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