# Genetic Analysis of Resistance to Cadmium-Induced Testicular Damage in Mice (37380)

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Cadmium pollution has been a source of increasing concern in recent years (1). Cadmium is eliminated slowly and accumulates with age. The effects of cadmium exposure in man are not well known, but it may be involved in tumor formation and cardiovascular disease. Studies on experimental mammals serve both to empirically evaluate potentially harmful effects of cadmium and to elucidate the underlying mechanisms. The mammalian testis is differentially sensitive to $Cd^{2+}$, and necrosis follows administration of sublethal doses (2). Inbred strains of mice differ markedly in their sensitivity to $Cd^{2+}$-induced testicular damage (3, 4). The purpose of this study was to determine the genetic basis for these interstrain differences. Such a study is a prerequisite for biochemical or physiological investigations attempting to detect the basis of these interstrain differences. Also, if the inheritance is found to be simple, the gene or genes responsible for the physiological strain differences may be useful markers in a variety of other genetic studies.

In this paper we demonstrate that resistance to $Cd^{2+}$-induced testicular necrosis in inbred mice is controlled by a single autosomal recessive gene. Evidence for this conclusion derives from (a) a survey of inbred strains and $F_1$ hybrids, (b) segregation and linkage studies utilizing backcross and $F_2$ generations, and (c) analyses of 23 partially inbred *recombinant inbred* (RI) lines derived from the $F_2$ generation of a C57BL/6J × DBA/2J cross. In addition, we tested several congenic resistant strains in an effort to detect linkage between the $Cd^{2+}$ sensitivity gene and other marker genes.

*Materials and Methods.* All mice were obtained from either the Production Department or the research colonies of The Jackson Laboratory. This study included males from 10 to 12 wk old of 45 inbred strains and six $F_1$ hybrids. Eighteen strains were previously tested by Gunn, Gould and Anderson (3). In an effort to detect the linkage of the gene affecting $Cd^{2+}$ susceptibility, we also tested 13 congenic resistant strains of the type in which a gene from a susceptible strain has been placed on the genetic background of a resistant strain (C57BL/10Sn). If the gene conferring susceptibility to $Cd^{2+}$ were closely linked to one of the selected markers, it would probably be carried along with the marker, thus revealing linkage.

Male C57BL/6J × DBA/2J $F_1$ hybrids ($B6D2F_1$) were mated to either C57BL/6J females or $B6D2F_1$ females to produce backcross and $F_2$ progeny, respectively.

In an additional test for single gene inheritance, we used *recombinant inbred* (RI) lines (5) derived by brother–sister mating from the $F_2$ generation of a $B6D2F_1$ hybrid. These are denoted by BXD and a numeral suffix, *e.g.*, BXD-2.

Mice were injected subcutaneously with a single dose of 0.03 m*M*/kg of body weight of $CdCl_2$ (Fisher) in a volume of approximately 0.1 ml. Forty-eight hours later the mice were asphyxiated with $CO_2$; the testes were removed and fixed in 10% neutral formalin. After 2 days in fixative, each testis was cut along either the longitudinal or transversal axis for gross pathological evaluation. Samples of both resistant and susceptible testes were sectioned and stained with hematoxylin and eosin for microscopic evaluation. This procedure was followed for all specimens for which the gross diagnosis was considered doubtful.

*Results.* In general, there was no difficulty

in classifying individual mice as either resistant or susceptible on the basis of gross examination of fixed, sliced testes. Testes from resistant strains appeared uniformly white or cream-colored, while testes from susceptible strains were generally darker and exhibited easily visible black hemorrhagic areas. Microscopically, the findings were extensive edema, hyperemia, hemorrhages, necrosis, and destruction of spermatogenic tissue.

The results of testing the 18 inbred strains previously analysed by Gunn, Gould and Anderson (3) confirmed their findings in detail. Strains AKR/J, CBA/J, C57BR/cdJ, C57L/J, C58/J, DBA/1J, DBA/2J, RF/J, SWR/J, and 129/J proved to be susceptible, whereas strains A/J, A/HeJ, BALB/cJ, C3H/HeJ, C3HeB/FeJ, C57BL/6J, and C57BL/10J proved to be resistant. Among the SJL/J mice, 14 were susceptible and 6 were resistant. This was the only case with variability within an inbred strain and this had been noted previously (3). Eighteen additional strains were classified as susceptible: AU/SsJ, BDP/J, BUB/BnJ, CBA/CaJ, CBA/H-T6J, C57BL/KsJ, CE/J, LP/J, LT/ReJ, MA/J, NZB/BnJ, P/J, PL/J, ROP/Gn, RIII/2J, SEA/GnJ, SM/J, ST/bJ, WC/ReJ, and WH/ReJ. Seven other strains were found to be resistant: HRS/J, I/LnJ, LG/J, PRO/Re, SEC/1ReJ, WB/ReJ, and WK/ReJ. The strain distribution pattern reflects known relationships among inbred strains (6). For example, sublines of the same strains were either both resistant, *e.g.*, A/HeJ and A/J, C3H/HeJ and C3HeB/FeJ, C57BL/6J and C57BL/10J, or both susceptible, *e.g.*, DBA/1J and DBA/2J.

The clear distinction between resistant and susceptible strains, with the exception of SJL/J, suggested that a single major gene might account for the difference. To further evaluate this hypothesis several $F_1$ hybrids were tested. The results are presented in Table I. The six standard $F_1$ hybrids available from the Production Department of The Jackson Laboratory provided all of the desired combinations: (i) susceptible $\times$ susceptible ($AKD2F_1$), (ii) resistant $\times$ susceptible ($LAF_1$, $C3D2F_1$, and $B6D2F_1$) and (iii) resistant $\times$ resistant ($CAF_1$, $B6AF_1$). The results demonstrated dominance or partial dominance of susceptibility over resistance, since all the $F_1$ hybrids involving a susceptible parent, either male or female, were susceptible. These results exclude X- or Y-linked inheritance. To critically analyze the single gene hypothesis, backcross (C57BL/6J $\times$ $B6D2F_1$) and $F_2$ mice from the $B6D2F_1$ were tested. The ratio of susceptible to resistant mice in the $F_2$ generation (22:10), and in the backcross to the resistant parent (10:14) conformed to the expected Mendelian ratios of 3:1 and 1:1, respectively. No significant association between the segregating coat color genes, dilute (*d*) and brown (*b*), and cadmium susceptibility was observed in the $F_2$ generation.

Since all of the congenic resistant strains resembled the resistant C57BL/10Sn partner, it can be concluded that the gene conferring cadmium susceptibility is neither identical to nor closely linked to the genes listed in Table II.

The results of testing 23 BXD RI lines are presented in Table III. BXD- lines 5, 6, 16, 27, and 29 appeared to be genetically

TABLE I. Results of Testing Six $F_1$ Hybrid Strains for $Cd^{2+}$-Induced Testicular Damage.

| $F_1$ hybrid designation | Female parent | Male parent | Mating type[a] | No. of positive/total |
|---|---|---|---|---|
| $AKD2F_1$ | AKR/J | DBA/2J | S $\times$ S | 4/4 |
| $LAF_1$ | C57L/J | A/J | S $\times$ R | 4/4 |
| $B6D2F_1$ | C57BL/6J | DBA/2J | R $\times$ S | 10/10 |
| $C3D2F_1$ | C3H/HeJ | DBA/2J | R $\times$ S | 4/4 |
| $B6AF_1$ | C57BL/6J | A/J | R $\times$ R | 0/4 |
| $CAF_1$ | BALB/cJ | A/J | R $\times$ R | 0/4 |

[a] S denotes a susceptible strain; R, a resistant strain.

TABLE II. Results of Testing C57BL/10Sn Congenic-Resistant Strains for $Cd^{2+}$-Induced Testicular Damage.

| Strain abbr | "Donor" strain | Known introduced gene(s) | No. of backcross generations | No. of positive/total |
|---|---|---|---|---|
| B10.D2/o | DBA/2J | *H-2*$^{d}$, *Hc*$^{o}$ | 5 | 0/4 |
| B10.D2(55N) | DBA/2J | *H-11*? (not *H-11*$^{a}$) | 9 | 0/4 |
| B10.D2(57N) | DBA/2J | *H-8*$^{b}$ | 7 | 0/4 |
| B10.D2(58N) | DBA/2J | *Hbb*$^{d}$, *H-1*$^{a}$, *Mlv-1*$^{a}$ | 8 | 0/6 |
| B10.129(5M)/o | 129/J | *Hbb*$^{d}$, *H-1*$^{b}$, *c* | 10 | 0/4 |
| B10.129(6M) | 129/J | *H-12*$^{b}$, *H-2*$^{bc}$ | 6 | 0/4 |
| B10.129(7Ma)[a] | 129/J | ? | 10 | 0/4 |
| B10.129(7Mb)[a] | 129/J | ? | 10 | 0/4 |
| B10.129(7Mc)[a] | 129/J | ? | 10 | 0/4 |
| B10.129(9M) | 129/J | *H-10*$^{b}$ | 6 | 0/4 |
| B10.129(10M) | 129/J | *H-11*$^{b}$ | 7 | 0/4 |
| B10.129(12M) | 129/J | *H-12*$^{b}$ | 6 | 0/4 |
| B10.129(13M) | 129/J | *H-3*$^{b}$ | 10 | 0/4 |
| B10.129(21M) | 129/J | *H-4*$^{b}$, *p* | 10 | 0/4 |

[a] These congenic lines may differ from C57BL/10Sn and each other by unidentified, weak histocompatibility loci.

fixed for the C57BL/6J recessive allele conferring resistance. BXD- lines 2, 8, 12, 13, 20, 21, and 28 were uniformly susceptible. However, we cannot definitely conclude that all of the latter are fixed for the susceptibility allele of DBA/2J since susceptibility is dominant. Other BXD lines were evidently still segregating at the time of their testing. Although these results are difficult to evaluate statistically because different generations were tested in different lines, the results tend to support the single gene inheritance inferred from the backcross and $F_2$ data.

*Discussion.* All of the results presented are consistent with the hypothesis that resistance to $Cd^{2+}$-induced testicular damage is determined by a single, recessive, fully penetrant gene. We propose to tentatively designate the locus *cadmium resistance* with the gene symbol *cdm* for the recessive allele conferring resistance, the dominant allele for susceptibility being regarded as wild type (+). At present, the single-gene, two allele model has only been established for strains C57BL/6J (*cdm/cdm*) and DBA/2J (+/+). Nonetheless, the consistency of the $F_1$ hybrid results involving strains A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57L/J, and DBA/2J suggest that the validity of the model may be more general. Additional evidence is as follows: strains LT/Re, SEA/GnJ, and PRO/Re were derived from crosses of BALB/c × C58, BALB/c × P, and C57BL/6 × 129 (*i.e.*, resistant × susceptible), respectively. In each case, these derived strains resemble one or the other of the progenitor strains, *i.e.*, in two cases the susceptible strain and in one case the resistant strain.

The strain distribution pattern for *cdm* is unlike that of any other known polymorphism. Thus, *cdm* apparently is not another manifestation of some other known gene. Whether the *cdm* allele is polymorphic in feral mice or is a mutation that occurred in domestic mice is not known. The limited distribution of the *cdm* gene among inbred strains suggests that the latter possibility may be correct. BALB/cJ, A/J, A/HeJ, C3H/HeJ, and C3HeB/FeJ are all descendants of the Bagg-albino stock (7). HRS/J and SEC/1Re are descended from BALB/c. Thus, the occurrence of the *cdm* allele in the Bagg-albino stock could account for all of the *cdm* bearing strains except C57BL/6J, C57BL/10J, LG/J, and WB/Re. The facts that *cdm* is limited in distribution and recessive suggest that *cdm* is a mutant from wild

TABLE III. Results of Testing 23 BXD RI Lines for $Cd^{2+}$-Induced Testicular Damage.

| | Generations beyond $F_2$ of test animals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $F_2+5$ | | $F_2+6$ | | $F_2+7$ | | $F_2+8$ | |
| BXD- | S | R | S | R | S | R | S | R |
| 2 | | | 1 | 0 | | | 6 | 0 |
| 3 | 3 | 1 | | | | | | |
| 4 | | | 1 | 3 | | | | |
| 5 | | | 0 | 2 | | | 0 | 5 |
| 6 | | | 0 | 4 | | | 0 | 3 |
| 7 | 3 | 0 | 1 | 6 | | | | |
| 8 | 8 | 0 | | | | | | |
| 11 | | | | | | | 5 | 0 |
| 12 | 2 | 0 | 5 | 0 | | | | |
| 13 | | | | | | | 7 | 0 |
| 14 | | | 2 | 0 | 2 | 0 | | |
| 16 | 0 | 3 | 0 | 5 | | | 0 | 4 |
| 17 | 1 | 1 | | | | | 3 | 3 |
| 18 | | | 9 | 3 | | | | |
| 19 | | | | | 2 | 4 | | |
| 20 | 3 | 0 | 6 | 0 | | | | |
| 21 | 6 | 0 | 7 | 0 | | | | |
| 22 | | | 3 | 1 | | | 1 | 4 |
| 23 | 0 | 3 | 2 | 1 | | | | |
| 24 | 1 | 0 | 0 | 2 | | | 0 | 2 |
| 27 | 0 | 2 | 0 | 6 | | | | |
| 28 | | | 8 | 0 | | | | |
| 29 | 0 | 5 | 0 | 4 | | | | |

type and that susceptibility is the normal state. This interpretation is supported by the observation that other mammals (*e.g.*, rat, rabbit, and hamster) are sensitive to $Cd^{2+}$-induced testicular damage (8). Resistance to $Cd^{2+}$-induced testicular damage probably should not be considered an adaptive trait. In this regard, preliminary studies suggest that mice of the *cdm/cdm* genotype may be more susceptible to acute $Cd^{2+}$ toxicity than *cdm*/+ and +/+ mice.

The mechanisms by which *cdm* homozygotes are protected from $Cd^{2+}$-induced testicular damage are unknown. Lucis and Lucis (9) made the important observation that two $Cd^{2+}$ resistant strains (C57BL/6J and BALB/cJ) had less testicular uptake of $^{109}Cd^{2+}$ than two susceptible strains (CBA/J and DBA/1J). Although it would be hazardous to draw conclusions from a correlation based on only four points, the magnitude of the difference (fourfold) is sufficient to explain the difference between the strains with respect to testicular necrosis. Since only low tracer doses of $^{109}Cd$ were used in the uptake studies, it is unlikely that the difference in uptake was the consequence of differential testicular damage rather than the cause. It is not known whether the differential testicular uptake of $Cd^{2+}$ in + and *cdm* strains is an intrinsic property of the testis or merely a reflection of altered $Cd^{2+}$ circulation. $Cd^{2+}$ is reported to be chiefly bound to a low molecular weight, high methionine containing protein called "metallothionein" (10). Shaikh and Lucis (11) isolated two major $Cd^{2+}$ binding proteins from rat liver. The primary effect of the *cdm* gene might be a quantitative or qualitative alteration of the cadmium binding proteins of one or more tissues.

The reason for the differential sensitivity of testes to $Cd^{2+}$ is uncertain. The concentration of injected $Cd^{2+}$ is much lower in the testes than in the liver, kidney, or at the site of cadmium injection (12). The high degree of vascularization of the testis may render it particularly sensitive (13). It is generally thought that the deleterious effects of cadmium are due to competitive interactions with zinc. Cadmium is known to inhibit various enzymes including carbonic anhydrase. Cadmium induced testicular interstitial cell tumors in mice and rats and chronic cadmium exposure may cause a number of human diseases. Studies aimed at elucidating the mechanisms of $Cd^{2+}$ effects in experimental mammals should help to evaluate and predict the biological hazards of environmental cadmium pollution.

The BXD recombinant inbred lines should be useful in discovering pleiotropic effects of the *cdm* gene, *e.g.*, distribution of $^{109}Cd$ to testes and other organs and the $LD_{50}$ of + and *cdm* mice of both sexes. Comparison of the strain distribution pattern of the *cdm* locus in the BXD lines with the strain distribution pattern of other mapped loci may reveal the chromosomal location of the *cdm* locus. These lines are presently being typed for gene loci determining isoenzymes, cell surface antigens, and other characteristics. Once the *cdm* gene is mapped it in turn could

be useful as a genetic marker. The strain distribution pattern of *cdm* among standard inbred strains furnishes additional biological definition for these strains and also provides additional data on which to base inferences about probable interstrain relationships (6).

*Summary.* Resistance to cadmium-induced testicular necrosis is determined by a single autosomal recessive gene (*cdm*) in inbred mice. Forty-five inbred strains, six $F_1$ hybrids, 14 congenic resistant strains, and 23 recombinant strains were tested for cadmium-induced testicular damage. The *cdm* gene is not closely linked with several gene loci.

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