

New mouse *dw* allele: Genetic location and effects on lifespan and growth hormone levels

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MORE THAN 50 years ago, Snell²⁵ reported the occurrence of a new autosomal recessive mutation called dwarf (*dw*) in a silver stock of mice maintained at Harvard. Shortly thereafter, Smith and MacDowell²⁴ showed that the mutation adversely affected pituitary function. Since the original report, more than 200 papers have been published on the *dw* mutant gene, verifying these observations and extending knowledge about the mutation's impact on diverse biological systems. In 1973, a pair of C3H/HeJ mice in the Animal Resources colonies of the Jackson Laboratory was observed to have a litter containing both normal and small-sized mice. We established a breeding colony from the original pair and their offspring and made the following observations. Mutants were identifiable by smaller body size and immature pinnae at two weeks of age. By 8 weeks of age, mutants appeared to be one-third the size of their normal littermates. Neither male nor female mutants reproduced in the absence of exogenous hormone therapy. The frequency of dwarfed young observed in mating pairs that produced dwarfed young was approximately 25 percent, indicating the new mutation was autosomal and recessive. Allelism tests between DW/J strain mice heterozygous for dwarf (*dw*) and C3H/HeJ strain mice heterozygous for the new mutation yielded both normal and dwarf mice. Therefore, the new dwarfing gene was a mutation at the *dw* locus and given the name dwarf-J (*dw^J*).

We report here observations on *dw^J/dw^J* mice relative to growth hormone deficiency and lifespan. In addition, we assign *dw* and another gene known to be linked to *dw*, weaver (*wv*), to chromosome 16.

Materials and Methods

Growth hormone analyses

Snell's dwarf (*dw/dw*) mice have been shown to be deficient in pituitary growth hormone (GH) by bioassay²⁴, by acryl-

amide gel electrophoresis¹⁵, and by immunoassay^{11,23}. To determine whether *dw^J/dw^J* mice had a similar deficiency in pituitary and serum GH, 5-10 week-old male and female dwarfs and normal littermates from both DW/J and C3H/HeJ strains were weighed, then killed by decapitation for serum collection. Pituitary glands were removed, homogenized in 0.05 M phosphate buffered saline, pH 7.2 and stored at -8°C until assayed for GH. Growth hormone was determined by radioimmunoassay (rat RIA kit, NIAMDD; lowest detectable value 0.5 ng/ml) of serially diluted pituitary extract after the homogenate had been centrifuged at 15,000 × *g* for 30 minutes at 2°C. Serum GH was determined in pooled samples from a different group of dwarf mice and their normal littermates.

Preliminary lifespan data

In view of conflicting reports that *dw/dw* mice live less than 110 days¹⁰, more than 8 months²², or at least 18 months²⁰, a small group of C3H/H3J-*dw^J/dw^J* mice (5♀ and 3♂) was set aside to gain longevity data. Husbandry procedures included placement of food pellets in the bottom of cages, use of longer sipper tubes for water, and addition to each cage of a normal BALB/cJ female mouse for extra warmth. Three normal female littermates, not previously assigned to other experiments, were available for lifespan data.

Genetic linkage

The dwarf males used in the linkage experiments were of three types: C3H/HeJ-*dw^J/dw^J*, DW/J-*dw/dw*, or (C3H/HeJ × DW/J)F₁-*dw^J/dw*. Because no attempt was made to use a specific type of dwarf male for a particular cross, the symbol *dw* is used for the *dw* or *dw^J* allele when presenting linkage data. Fertile dwarf males used in linkage experiments were produced, as follows: at the time of weaning (3-4 weeks), they were placed on a diet that contained 0.25 g thyroid powder (Sigma Chemical Co., T-1251) per 1000 g nonpelleted mouse chow (Old Guilford Diet 96; Emory Morse Co., Guilford, CT). Approximately 3 weeks later, a normal pituitary gland from a same strain or F₁ hybrid donor was grafted under a kidney capsule. The combination of these treatments yielded 8 to 10-week-old fertile dwarf males weighing between 15 and 20 g.

On the basis of previously published negative linkage tests and of much unpublished negative linkage information kindly provided by Priscilla Lane, the Jackson Laboratory, we chose the following genes (chromosomal assignment) for testing linkage to *dw*: *Id-1* (chr 1), *Amy-1* (chr 3), *Gpd-1* (chr 4), *Mpi-1* and *Mod-1* (chr 9), *Np-1* (chr 14), *Gdc-1* and *Gpt-1* (chr 15), and *Ce-2*, *Pgk-2* and *Apl* (chr 17). A listing of full

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gene names can be found in *Mouse News Letter*¹⁷. The following translocations were tested for linkage to *dw*: *Rb(4.6)-2Bnr*, *T(10;18)18H*, *T(2;3)24H*, *T(X;16)16H*, and *T(8;16)17H*. In addition, we tested for linkage of *wv*, a gene linked to *dw*^{12,13}, to the *my* locus (chr 3) and to translocations *Rb(4.6)2Bnr*, *Rb(5.15)3Bnr*, *T(10;18)18H*, *T(9;17)138H*, *T(16;17)17H*, and *T(2;16)28H*. Because positive linkage was found only in crosses segregating for translocations involving chr 16, only these data are presented.

PHA-stimulated blood cultures²⁶ or bone marrow cells were used to obtain chromosome preparations from females. G-banding of chromosomes was accomplished as in Eicher and Washburn⁷. Meiotic chromosome preparations were obtained from the testis using a technique similar to that described by Evans et al.⁹.

1. *Dwarf linkage tests*. Two translocations, *T(X;16)16H* (*T16H*) and *T(8;16)17H* (*T17H*), were used to determine whether *dw* was located on chr 16. The breakpoint in chr 16 for *T16H* is 16B5⁷ and for *T17H*, 16C2 (unpublished). Thus, the *T16H* breakpoint is located in the middle of chr 16 and the *T17H* breakpoint in the distal third.

The *T16H* translocation is a unique X-autosomal translocation because in *T16H/+* females the normal X chromosome appears to be the inactive X. Thus, females heterozygous for the X-linked gene *Ta* (tabby) and *T16H* appear phenotypically *Ta/Ta* if they are *Ta T16H/+*, or *+/+* if they are *+T16H/Ta+*. The *Ta* gene is located within 1 centiMorgan of the *T16H* breakpoint and thus provides an excellent gene marker for *T16H*¹⁶. Males carrying the *T16H* translocation have very small testes and are sterile. In addition, a quadrivalent, formed from the pairing of the translocated chromosomes (*X*¹⁶ and *16*^X) with chr 16 and the Y chromosome, can be seen in meiotic first metaphases.

Two different matings involving *dw* and *T16H* were made. In cross 1, *Ta T16H/+ + dw* females were mated to dwarf males. The resulting (*Ta T16H +/+ + dw*)F₁ females were backcrossed to dwarf males and their offspring classified for *Ta*, thus *T16H*, and *dw*. In cross 2, *T16H +/+ Ta* females were mated to dwarf males. The (*T16H +/+ dw*)F₁ females were backcrossed to dwarf males. In this cross, the presence of *T16H* was determined in females using G-banded chromosomes from blood cultures and in males using meiotic chromosomes from the testis. Testis size was noted in all males from crosses 1 and 2. In all cases, small testis size correlated with the presence of the *T16H* translocation as determined by

the presence of a quadrivalent in first metaphase plates.

For linkage of *T17H* and *dw*, *T17H/+* females were mated to dwarf males and their (*T17H +/+ dw*)F₁ female offspring (presence of *T17H* determined in G-banded chromosomes from blood cultures) mated back to dwarf males. The presence of *T17H* in female offspring was determined from G-banded chromosomes prepared from blood cultures or bone marrow and in male offspring by the presence of a quadrivalent configuration in first metaphase plates prepared from the testis.

All dwarf offspring in crosses involving *T16H* or *T17H* were placed on the thyroid supplemented diet at weaning and mitotic or meiotic chromosomes prepared after they were 6 weeks of age.

2. *Weaver linkage test*. Two translocations were used to determine whether *wv* was located on chr 16: *T17H* and *T28H*. The chr 16 breakpoint for *T28H* is 16C3²¹, thus more distally located than *T17H* (16C2). Weaver females were mated to *T17H/+* or *T28H/+* males. *T17H* carrying male offspring were identified by the presence of a quadrivalent in a meiotic chromosome preparation obtained from one testis and *T28H* carriers from analysis of G-banded chromosomes from PHA stimulated blood cultures. Proven *T17H* (*T17H +/+ wv*) or *T28H* (*T28H +/+ wv*) carrier males were crossed back to weaver females. All offspring were classified for *wv* and *T17H* or *T28H* (females in both crosses and males in the *T28H* cross by G-banded chromosome preparations from blood cultures and males in the *T17H* cross by analysis of meiotic chromosome preparations). In all matings involving *wv* as well as isolated litters containing *wv/wv* individuals, food pellets were placed in the bottom of the cages and extra shavings added so that these mutant mice could obtain food and water.

Results

Growth hormone analyses

The weight and GH data obtained from groups of dwarf and normal mice of the DW/J and C3H/HeJ strains are given in Table I. The body weights of C3H/HeJ-*dw^J/dw^J* mice 5–10 weeks of age were approximately one-third the weights of their normal littermates of either sex. The same relationship of weights existed for *dw/dw* and normal mice of the DW/J strain. Pituitary gland weights of dwarf mice were approximately one-third to one-fifth that of the normal mice of either strain. Pituitary GH was undetectable in DW/J-*dw/dw* males

Table I. Radioimmunoassayable growth hormone found in pituitaries and serum of dwarf and normal littermate mice

| Strain | Genotype | Sex | n | Age, weeks | Body weight $\bar{X} \pm \text{SEM}$, g | Pituitary | | n | Serum, ng GH/ml |
|---------|--------------------------------------|-----|----|------------|--|---------------|-----------------------------------|---|--------------------|
| | | | | | | weight, mg | (μg GH/mg) wet wt. | | |
| DW/J | <i>dw/dw</i> | ♂ | 11 | 5–10 | 6.7 ± 0.2 | 0.36 | <0.00008 | 8 | ND* |
| | <i>+/-</i> | ♂ | 4 | 5–10 | 31.0 ± 2.2 | 1.70 | 4.25 | 5 | >40 |
| | <i>dw/dw</i> | ♀ | 12 | 5–10 | 7.2 ± 0.2 | 0.54 | <0.00008 | 7 | ND |
| | <i>+/-</i> | ♀ | 4 | 5–10 | 24.4 ± 1.0 | 2.22 | 3.15 | 4 | 22 |
| C3H/HeJ | <i>dw^J/dw^J</i> | ♂ | 12 | 5–9 | 6.6 ± 0.5 | 0.37 | 0.00062 | 6 | ND |
| | <i>+/-</i> | ♂ | 4 | 5–9 | 21.1 ± 2.5 | 1.58 | 3.00 | 3 | 22 |
| | <i>dw^J/dw^J</i> | ♀ | 20 | 5–9 | 6.5 ± 0.2 | 0.33 | <0.00008 | 6 | ND |
| | <i>+/-</i> | ♀ | 4 | 5–9 | 18.7 ± 2.1 | 1.38 | 2.30 | 4 | 15 |

* ND = not detectable; all pituitary gland pools were in a concentration of 6 mg/ml, thus the minimum detectable GH was (0.5 ng GH/ml)/(6 mg pit./ml) or 0.083 ng GH/mg

and females and in C3H/HeJ-*dw^J/dw^J* females. A very small amount of GH was detected in C3H/HeJ-*dw^J/dw^J* males—approximately 0.02 percent of the GH concentration found in pituitaries of +/- males.

The data on serum GH levels, presented in the last column of Table I, were derived from different but similarly aged and processed mice from both strains. As one would predict from the pituitary GH data, the level of serum GH in dwarf mice was essentially undetectable, whereas normal mice of either strain were found to have 15 to >40 ng GH per ml serum.

Preliminary lifespan data

The eight *dw^J/dw^J* mice set aside for longevity observations lived an average of 790 days (individual ages at death were 227, 453, 861, 898, 919, 952, 984, and 1027). The three normal female littermate controls died between 608 and 821 days; one was observed to have a late-onset mammary tumor.

Linkage of *dw* and *wv* to chr 16

As can be seen in Table II, in two separate crosses the *dw* gene showed loose linkage to the *T16H* translocation (overall average of 29.4 percent recombination ± 4.2), indicating that *dw* was probably located on chr 16. Because the *T16H* breakpoint is centrally located within chr 16 (band 16B5), matings were established to test for linkage of *dw* to a more distal position on chr 16 by utilizing the *T17H* translocation (band 16C2). These results are given in Table II. In these matings *dw* and *T17H* recombined 18.2 ± 5.8 percent of the time, indicating *dw* was linked to *T17H* and confirming our conclusion using the *T16H* translocation that *dw* is located on chr 16.

Based on the reported linkage of *dw* and *wv* by Lane and Sweet^{12,13}, we established crosses that would determine whether *wv* was linked to translocations involving chr 16. As seen in Table III, loose linkage was obtained with *T17H* (28.5 ± 4.0). The distance from *T28H*, however, was much shorter (12.3 ± 4.0). Since *wv* appears to be more closely linked to *T28H* than to *T17H* and the *T28H* breakpoint on chr 16 is more distally located than *T17H*, *wv* is probably quite distally located on chr 16.

Discussion

Genetic and physiologic data confirm the relationship between *dw* and *dw^J* as alleles with similar phenotypic expression. The site of gene action for *dw* has been proposed to be the pituitary gland³, with GH¹⁵, prolactin⁴, and thyrotropin⁸ severely deficient. We chose to evaluate the status of GH as one example of the deficient tropic hormones in mutant and normal mice from both DW/J and C3H/HeJ inbred backgrounds. We found that both *dw/dw* and *dw^J/dw^J* mice had essentially no GH in either pituitaries or serum, whereas normal mice of both strains possessed significant quantities. Our findings of deficient GH in dwarf mice are in agreement with those of Lewis et al.¹⁵ and of Garcia and Geschwind¹¹. Furthermore, the deficient serum GH status of these mutant mice complements studies demonstrating the efficacy of exogenous GH treatment on growth of dwarf mice^{2,24}. On the other hand, Sinha, et al.²³ reported that pituitary GH concentration in *dw/dw* mice was one-seventh to one-twentieth of that found in normal littermates, and serum GH levels one-half to one-third of that found in normal littermates. Because such quantities of GH in dwarf pituitaries should have been detectable by all investigators, and

Table II. Linkage of *dw* to chromosome 16

| Cross | | Chr inherited from F ₁ parent | | | | Total | % recomb. \pm SE |
|--|---|--|-------------|-----------------------|----|-------|-----------------------|
| ♀ | ♂ | | | | | | |
| $\frac{Ta\ T16H +}{++\ dw} \times \frac{++\ dw}{++\ dw}$ | | <i>T16H</i> + | <i>dw</i> | <i>T16H dw</i> | ++ | 29 | 24.1 \pm 7.9 |
| | | 13 (all <i>Ta</i>) | 9 | 6 (all <i>Ta</i>) | 1 | | |
| $\frac{T16H +}{+\ dw} \times \frac{+\ dw}{+\ dw}$ | | 26 | 36 | 7 | 21 | 90 | 31.1 \pm 4.9 |
| | | Total | 39 | 45 | 13 | | |
| $\frac{T17H +}{+\ dw} \times \frac{+\ dw}{+\ dw}$ | | <i>T17H</i> + | + <i>dw</i> | <i>T17H dw</i> | ++ | 44 | 18.2 \pm 5.8 |
| | | 20 | 16 | 7 | 1 | | |

Table III. Linkage of *wv* to chromosome 16

| Cross | | Chr inherited from F ₁ parent | | | | Total | % recomb. \pm SE |
|---|---|--|-------------|----------------|----|-------|-----------------------|
| ♀ | ♂ | | | | | | |
| $\frac{+\ wv}{+\ wv} \times \frac{T17H +}{+\ wv}$ | | <i>T17H</i> + | + <i>wv</i> | <i>T17H wv</i> | ++ | 130 | 28.5 \pm 4.0 |
| | | 49 | 44 | 13 | 24 | | |
| $\frac{+\ wv}{+\ wv} \times \frac{T28H +}{+\ wv}$ | | <i>T28H</i> + | + <i>wv</i> | <i>T28H wv</i> | ++ | 65 | 12.3 \pm 4.0 |
| | | 30 | 27 | 5 | 3 | | |

in the serum by Garcia and Geschwind and by us, it is difficult to explain the results of Sinha et al.²³.

The small group of dw^J/dw^J mice set aside for a preliminary estimate of lifespan lived an average of 790 ± 102 days, while the three normal littermates died between 608 to 821 days of age. In view of the small sample sizes, these lifespans have not been tested for differences; however, the values are similar to lifespan data reported for C3H/HeJ mice by Myers¹⁸. Fabris et al.¹⁰ reported that dw/dw mice (BALB/c background) had a shortened lifespan, deficient thymic and splenic tissue, and deficient immune responses, all of which could be cured with exogenous GH and thyroxine. Fabris and associates concluded that the dwarf mouse, characterized by endocrine deficiencies, represented a good model for premature aging. Although we have not collected longevity data for DW/J- dw/dw mice under our colony conditions, the lifespan of our C3H/HeJ- dw^J/dw^J mice is more consistent with the longevity data for dw/dw mice provided by Shire²² and by Schneider²⁰. We conclude that there is little support for the contention that mutations at the dwarf locus are any more likely to affect lifespan than are factors such as genetic background, environmental conditions, or endemic disease status of any given animal colony.

Linkage experiments designed to locate dw , and a locus linked to dw , wv , were successful. The dw locus was found to be linked to two separate translocations having chr 16 in common, thus assigning dw to chr 16. In addition, wv , reported linked to dw ^{12,13}, was found to be linked to $T17H$ and $T28H$ thus located on chr 16. Because wv was more closely linked to the $T28H$ rather than $T17H$ translocation, and the breakpoint on chr 16 for $T28H$ is more distally located than $T17H$, wv is probably distally located on chr 16. Because dw and wv are loosely linked, dw is probably more proximally located on chr 16 than wv . Further data generated from a three-point cross are needed to support or deny this hypothesis.

Two recent reports concerning the linkage of dw and wv are relevant to our findings. Davisson and Roderick⁵ reported that dw but not wv was linked to the $Rb(16.17)7Bnr$ translocation (recombination of 23.7 ± 5.6 percent for dw , and 45.7 ± 7.3 percent for wv) and suggested that both genes were carried on chr 16 or 17. Lane and Sweet reported that dw but not wv was linked to the gene mahoganoid (md), known to be located near the centromere end of chr 16¹⁴ (recombination between md and dw of 34.4 ± 1.9 percent and between md and wv of 44.6 ± 5.6 percent). These data support our conclusion that dw and wv are carried on chr 16 and that the order of loci is: centromere— dw — wv .

Summary

We have reported the finding of a new mutation at the dwarf locus, named dwarf-J, gene symbol dw^J , in the C3H/HeJ inbred mouse strain. The C3H/HeJ- dw^J/dw^J mice are like DW/J- dw/dw mice in that both homozygotes are virtually devoid of GH in either pituitary glands or serum. Lifespan of dw^J/dw^J mice was not reduced. Linkage experiments designed to assign the dw gene, together with another gene weaver (wv), were successful in that both were found to be on chromosome 16. The dw locus is probably more proximally located on chromosome 16 than the wv locus.

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