Hermaphrodism and sex reversal associated with the *dominant hemimelia* mutation in XY mice

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Abstract: After two generations of backcrossing six different Y-consomic strains onto a C57BL/6J inbred mouse strain with a *dominant hemimelia* (*Dh*) mutation, a small percentage of *Dh*/+ males containing a Y chromosome from the AKR/J or RF/J strain showed hermaphrodism; they lacked the right testis and had an ovary and a uterus instead. Approximately 15% of *Dh*/+ females considered to be phenotypically normal had the *Sry* gene from the AKR/J or RF/J strain; they were actually sex-reversed XY females. Backcrossing of Y chromosomes from BALB/cA, C3H/HeJ, C57BL/6J, DH/Sgn, and DDD/Sgn onto the C57BL/6J strain with *Dh* did not result in hermaphrodism or sex reversal in adult mice. Subsequent linkage mapping analysis revealed that at least one C57BL/6J-derived homozygous allele at a locus on chromosome 13 was required for hermaphrodism and sex reversal. This condition was genetically distinct from known inherited sex-reversal conditions. It therefore offers a novel opportunity to investigate the genetic basis of sex determination in mammals.

Keywords: *Dominant hemimelia* (*Dh*), hermaphrodism, linkage mapping, sex reversal

Introduction

The *dominant hemimelia* (*Dh*) mutation arose spontaneously in a crossbred male mouse about 50 years ago in England.[1] *Dh* causes visceral abnormalities and skeletal malformations of varying degrees of severity.[2] Visceral abnormalities include a small stomach, short intestine, hydropic kidneys, and congenital absence of the spleen. Skeletal malformations are identified in the trunk caudally from the thorax, particularly in the hindlimbs.[2,3] In general, abnormalities induced by *Dh* are expressed more severely in *Dh*/*Dh* than in *Dh*/+. *Dh*/*Dh* mice rarely survive; most of them die shortly after birth because of their visceral defects.[2]

We have previously shown that F$_1$-*Dh*/+ males from a cross between inbred DDD strain females and DH-*Dh*/+ males die during the neonatal period.[4] Affected F$_1$-*Dh*/+ males exhibit growth retardation

and problems with urination and defecation, which seem to be caused by the presence of a rectovesical fistula formed between the rectum and urinary bladder in conjunction with an imperforate anus.[5] The lethal phenotype does not occur in the reciprocal cross between DH-*Dh*/+ females and DDD males. Genetic mapping of the loci underlying the lethality has revealed that it is caused by a combination of three independent gene loci, i.e., the DH allele at the *Dh* locus on chromosome 1, the DDD allele at the *Grdhq1* locus on the X chromosome, and a putative Y-chromosome-linked (hereafter designated as "Y-linked") locus in some inbred strains.[6] Several Y-consomic strains with *Dh* were analyzed to further substantiate the involvement of the Y-linked gene in this lethality.[7] It was found that among *Mus musculus musculus* Y chromosomes (Y^{MUS}), those from C57BL/6J and BALB/cA cause lethality but those from C3H/HeJ and CAST/EiJ do not. Similarly, among *Mus musculus domesticus* Y chromosomes (Y^{DOM}), those from AKR/J, DDD, SJL/J, SWR/J, and TIRANO/EiJ cause lethality but that from RF/J does not. These results indicate that two distinct Y chromosomes with functional differences coexist in inbred mouse strains.[8]

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During the course of transferring six different Y chromosomes with the *Dh* mutation onto the genetic background of C57BL/6J strain, a standard inbred mouse strain, I noted that two *Dh*/+ males containing the Y chromosome from AKR/J lacked the right testis and had an ovary and a uterus instead, suggesting that *Dh* caused hermaphrodism. Furthermore, some of the backcrossed *Dh*/+ phenotypically normal females had two ovaries and the *Sry* gene from the AKR/J strain, thus suggesting that they were actually XY females; *Dh* also caused male-to-female sex reversal. The genetic basis underlying sex determination is not yet completely understood. Therefore, it has been suggested that one of the most powerful approaches to understanding the molecular basis of the sex determination pathway is to identify and analyze inherited sex-reversal conditions.[9] Here, I genetically analyzed this hermaphrodism and sex reversal.

Materials and methods

Mice and genetic cross. The *Dh* mutation has been maintained in an inbred DH/Sgn strain (hereafter called "DH"; DH includes both *Dh*/+ and +/+, which respectively are referred to here as DH-*Dh*/+ and DH-+/+). As a rule, DH-+/+ females are mated with DH-*Dh*/+ littermate males. The inbred strains DH and DDD/Sgn (DDD) are maintained at the National Institute of Agrobiological Sciences, Tsukuba, Japan. The inbred strains BALB/cA (BALB), C3H/HeJ (C3H), and C57BL/6J (B6) were purchased from CLEA Japan (Tokyo, Japan) and the inbred strains AKR/J (AKR) and RF/J (RF) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA).

Males of these strains were backcrossed onto a DH strain background with the *Dh* mutation for a minimum of 10 generations, and a series of Y-consomic strains were established. Each mouse of these Y-consomic strains was either *Dh*/+ or +/+. Y-consomic strains—DH-Chr Y^{AKR}-*Dh*/+, DH-Chr Y^{B6}-*Dh*/+, DH-Chr Y^{C3H}-*Dh*/+, DH-Chr Y^{DDD}-*Dh*/+, DH-Chr Y^{DH}-*Dh*/+ (identical to DH-*Dh*/+), and DH-Chr Y^{RF}-*Dh*/+—were also backcrossed onto a genetic background of B6 strain, a standard inbred mouse strain, for several generations. According to the guidelines for nomenclature of mouse and rat strains (Mouse Genome Informatics, MGI, http://www.informatics.jax.org/), when DH-Chr Y^{AKR}-*Dh*/+ is backcrossed twice onto a B6 strain

with *Dh*, the resultant mice should be designated C57BL/6J-Chr Y^{AKR}-*Dh*/+ N(2); the rule is to include the full host-strain name in the nomenclature of the consomic strain. However, for convenience here, I have abbreviated the name of the strain to "B6".

Examination of the nucleotide sequences of the *Sry* gene revealed that the Y chromosomes from B6, BALB, C3H, and DH were Y^{MUS}, whereas those from AKR, DDD, and RF were Y^{DOM}.[6),8),10]

All mice were maintained in a specific pathogen-free facility with a regular light cycle of 12 h light/ 12 h dark and controlled temperature and humidity. Food and water were freely available throughout the experimental period. Experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Agrobiological Sciences.

Identification of sex and *Dh* genotype. Initially, mice were classified at weaning as male or female by the appearance of the external genitalia. At autopsy, mice were categorized into one of three phenotypes on the basis of the gross anatomy of their gonads: male, hermaphrodite or female.[11] Mice with the male phenotype were defined as having bilateral testes. Hermaphrodites were defined as having one testicle and a contralateral ovary (including one mouse that had a right testis but lacked a left one). Mice with the female phenotype were defined as having bilateral ovaries. All hermaphrodites had been classified as male at weaning. The sex of all *Dh*/+ mice considered to be phenotypically female was determined by PCR typing of the *Sry* gene.[12] The presence of the *Dh* genotype was determined by the presence of hindlimb malformations and confirmed by the absence of the spleen.

Histology. Testes, ovaries, and uterus were fixed in 10% neutralized formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Linkage mapping. Linkage mapping was performed on N_2 mice, considered to be either hermaphrodites or sex-reversed XY females, to localize putative chromosomal regions responsible for the occurrence of hermaphrodism and sex reversal. Initially, 85 informative microsatellite markers, which had been selected out of a 410-microsatellite set (MapPairs, Research Genetics, Huntsville, AL, USA), were genotyped in 20 affected mice. After eight more microsatellite markers were selected for chromosomes 13 and 18, nine newly produced N_2

Table 1.   Incidence of hermaphrodism and sex reversal in backcross progeny

Mice	Classification of adult mice by phenotypic sex and *Dh* genotype						
	Males		Hermaphrodites		Females		
	+/+	*Dh*/+	+/+	*Dh*/+	+/+	*Dh*/+	
							(No. of *Sry* positive phenotypically normal *Dh*/+ females)
(B6 × DH-Chr Y^{AKR}-*Dh*) F$_1$	13	17	0	0	18	21	(*)
B6-Chr Y^{AKR} N(2)	246	42	0	12	270	98	(15)
B6-Chr Y^{AKR} N(3)	66	5	0	1	60	31	(4)
B6-Chr Y^{AKR} N(5)	46	1	0	0	48	14	(4)
B6-Chr Y^{DDD} N(2)	49	13	0	0	53	17	(0)
B6-Chr Y^{RF} N(2)	44	14	0	1	51	14	(1)
B6-Chr Y^{B6} N(2)	15	6	0	0	18	4	(0)
B6-Chr Y^{C3H} N(2)	20	6	0	0	11	2	(0)
B6-Chr Y^{DH} N(2)	65	11	0	0	59	22	(0)

Mice were investigated at age of 31 to 80 days. * not examined.

mice were further typed for microsatellite loci on chromosomes 13, 17, and 18.

Linkage between a marker locus and the occurrence of hermaphrodism or sex reversal was evaluated by simple tests for association.[13],[14] In mice considered to be hermaphrodites or sex-reversed XY females, the statistical significance of a departure from the expected 1:1 ratio of homozygous (B6/B6) to heterozygous (B6/DH) genotypes was assessed at each marker locus by a binomial test. The probability (P) that x of the n hermaphrodites or sex-reversed mice had a homozygous genotype was calculated by the binomial formula:

$$P(X = x) = \binom{n}{x} p^x (1-p)^{n-x},$$

$$\text{where } \binom{n}{x} = {}_nC_x = \frac{n!}{x!(n-x)!}$$

Under the null hypothesis of no linkage, the probability (p) that hermaphroditic or sex-reversed mice have a homozygous (B6/B6) genotype is 0.5. Linkage was statistically significant when P < 0.0001.[15]

Results

Dh causes hermaphrodism in combination with B6-derived homozygous alleles at autosomal loci and the Y chromosome from AKR or RF strains.   After two generations of backcrossing Y-consomic strains onto the B6 strain with *Dh* I

found two adult B6-Chr Y^{AKR}-*Dh*/+ N(2) externally normal males were hermaphrodites; they lacked the right testis and had an ovary and a uterus instead. The left testis was in the regular position. To examine whether this hermaphrodism had occurred by chance the following test backcrosses were performed.

In the first test backcross of ♀B6 × ♂(B6 × DH-Chr Y^{AKR}-*Dh*/+) F$_1$-*Dh*/+, 177 surviving N$_2$ mice were obtained from a total of 39 litters. Of these mice, 83 were phenotypically normal males (67 +/+ and 16 *Dh*/+), one was a hermaphrodite (1 *Dh*/+), and 93 were phenotypically normal females (65 +/+ and 28 *Dh*/+); they were investigated 80 days after birth. The number of mice was therefore apparently skewed in favor of +/+. The survival rate was then examined in the second test backcross of ♀B6 × ♂(B6 × DH-Chr Y^{AKR}-*Dh*/+) F$_1$-*Dh*/+. In a total of 36 litters, 291 live pups were born but only 184 of them successfully survived to weaning (survival rate 63.2%); these 184 N$_2$ mice comprised 80 phenotypically normal males (73 +/+ and 7 *Dh*/+), three hermaphrodites (3 *Dh*/+), and 101 phenotypically normal females (77 +/+ and 24 *Dh*/+); they were investigated 80 days after birth. In the third test backcross, sex was determined by *Sry* typing on the day of birth. The 115 newborn pups comprised 25 ♂+/+, 23 ♂*Dh*/+, 33 ♀+/+ and 34 ♀*Dh*/+ (chi-squared testing revealed that this was not significantly different from the expected ratio of 1:1:1:1).

Table 2. Gonadal abnormalities in hermaphrodites

Origin of Y chromosome	Mouse no. (backcross generations)	Characteristic abnormalities of the gonad	
		Left	Right
AKR	AKP21 (2)	Testis (spermatogenesis was confirmed)	Ovary and uterus
AKR	AKP22 (2)	Testis (spermatogenesis was confirmed; Fig. 1A)	Ovary (Fig. 2A) and uterus
AKR	AK183 (2)	Small testis	Testicular agenesis. Uterus-like structure developed
AKR	AK219 (2)	Small testis	Ovary (Fig. 2B) and uterus
AKR	AK231 (2)	Testicular agenesis (seminal vesicle malformed)	Small testis
AKR	AK280 (2)	Small testis	Ovary (Fig. 2C) and uterus (seminal vesicles absent)
AKR	AK604 (2)	Testis (Fig. 1B)	Ovary and uterus
AKR	AKM1 (2)	Testis	Ovary and uterus
AKR	AKAM59 (2)	Testis	Ovary (Fig. 2D) and uterus
AKR	AKAKF24 (2)	Testis	Ovary and uterus (seminal vesicles absent)
AKR	AKAM79 (2)	Testis (Fig. 1C)	Ovary (Fig. 2E) and uterus
AKR	AKMA1 (2)	Testis	Ovary and uterus (seminal vesicles absent)
RF	RFRM211 (2)	Testis	Ovary (Fig. 2F) and uterus
AKR	AK142 (3)	Small testis	Extremely small testis. Uterus-like structure developed

This result supported the high rate of death in $Dh/+$ mice of both sexes during the neonatal period. These 115 newborn pups were not used for subsequent investigation.

In total, 668 adult B6-Chr Y^{AKR} N(2) mice including those from the first and second test backcrosses, were obtained and analyzed. These mice comprised 288 phenotypically normal males (246 $+/+$ and 42 $Dh/+$), 12 hermaphrodites (12 $Dh/+$), and 368 phenotypically normal females (270 $+/+$ and 98 $Dh/+$) (Table 1); they were investigated 31 to 80 days after birth.

In addition, one hermaphrodite was also identified in B6-Chr Y^{AKR}-$Dh/+$ N(3) and another one in B6-Chr Y^{RF}-$Dh/+$ N(2) (Table 1). Hermaphrodites were not identified in B6-Chr Y^{DDD}-$Dh/+$ N(2), B6-Chr Y^{B6}-$Dh/+$ N(2), B6-Chr Y^{C3H}-$Dh/+$ N(2), or B6-Chr Y^{DH}-$Dh/+$ N(2). The absence of hermaphrodites in (B6 × DH-Chr Y^{AKR}-$Dh/+$) F_1-$Dh/+$ (Table 1) suggested that B6-derived homozygous alleles at autosomal loci were required for hermaphrodism. None of the backcrossed $+/+$ males developed as hermaphrodites.

Gonadal abnormalities in hermaphrodites. More detailed information on the hermaphrodites is presented in Table 2. Of the 14 hermaphrodites identified, 11 had an ovary and a uterus on the right side. A uterus-like structure developed in two, and there was agenesis of the left testis in one. In all except one of the hermaphrodites a testis developed only on the left side, and an ovary as well as a uterus developed on the right side (Fig. 1). In the remaining one there was a small testis in the right. Histologically, sperm were seen in the left testis in some but their presence was not confirmed in all hermaphrodites. Histological abnormalities were observed in all of the ovaries (Fig. 2). In general, the ovaries were atrophied and primordial follicles were absent. The uterus was also generally atrophied (data not shown).

Dh causes sex reversal in combination with B6-derived homozygous alleles at autosomal loci and the Y chromosome from the AKR or RF strains. Phenotypic analysis of mouse sex gave a ratio of ♂$+/+$: ♀$+/+$ in B6-Chr Y^{AKR} N(2) of nearly 1:1, whereas that of ♂$Dh/+$: ♀$Dh/+$
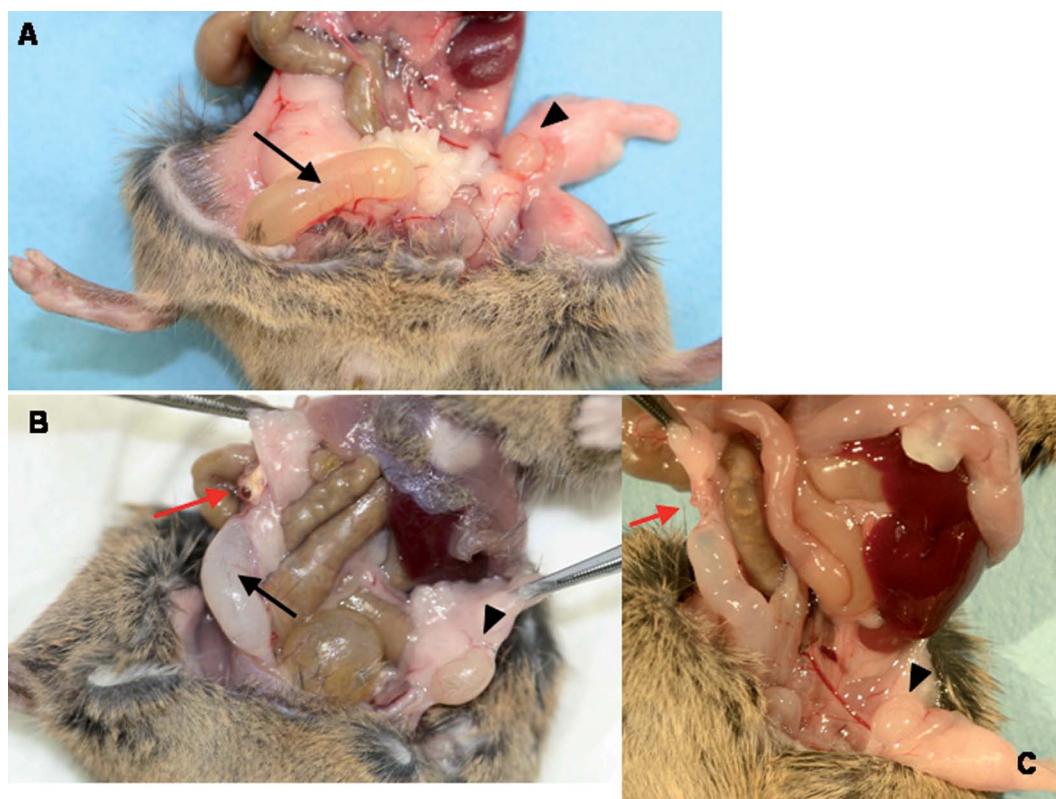
Fig. 1.   Incidence of hermaphrodism in B6-Chr Y^{AKR}-Dh/+ N(2) males. Arrowheads indicate left testis and arrows indicate uterus and/or ovary formed on the right side. Mouse numbers: (**A**) AKP22, (**B**) AK604, and (**C**) AKAM79.

(42:98) was not equal (Table 1). Genotypic sex was examined in these ♀Dh/+ mice by typing for the presence of the *Sry* gene. Unexpectedly, 15 of the 98 B6-Chr Y^{AKR}-Dh/+ N(2) phenotypic females were *Sry* positive, suggesting that they were actually sex-reversed XY females. In addition, 4 of 31 B6-Chr Y^{AKR}-Dh/+ N(3), 4 of 14 B6-Chr Y^{AKR}-Dh/+ N(5), and 1 of 14 B6-Chr Y^{RF}-Dh/+ N(2) mice that had been judged as phenotypically normal females were *Sry* positive (Table 1). They had an ovary and a uterine horn in the proper position on each side. Nucleotide sequences of the *Sry* gene were determined in two of the B6-Chr Y^{AKR}-Dh/+ N(2) sex-reversed XY females; both these females possessed the *Sry* gene from the AKR strain. All the phenotypically female Dh/+ littermates from the backcross progeny of B6-Chr Y^{DDD}-Dh/+ N(2), B6-Chr Y^{B6}-Dh/+ N(2), and B6-Chr Y^{C3H}-Dh/+ N(2), and B6-Chr Y^{DH}-Dh/+ N(2) were *Sry* negative; there was no incidence of sex reversal.

Fertility of sex-reversed XY females. Next, I tested the fertility of four B6-Chr Y^{AKR}- Dh/+ N(2) sex-reversed XY females. No pregnant sex-reversed XY females were identified after continuous mating. I examined the ovarian histology of two of the sex-reversed XY females (80 days old) (Fig. 3). These mice contained the *Sry* gene from the AKR strain. Like the ovaries formed in the hermaphrodites, these ovaries were severely atrophied and primordial follicles were absent.

Absence of hermaphrodism and/or sex reversal when *Dh* was backcrossed onto the BALB strain with the AKR Y chromosome. DH-Chr Y^{AKR}-Dh/+ N(2) were backcrossed twice onto the BALB background, i.e., BALB × (BALB × DH-Chr Y^{AKR}-Dh/+) F_1-Dh/+. Eighty of 91 liveborn pups survived to weaning. These 80 mice comprised 38 phenotypically normal males (21 +/+ and 17Dh/+) and 42 phenotypically normal females (22 +/+ and 20Dh/+); they were investigated 21 to 31 days after birth. There was no evidence of high postnatal death rates in the Dh/+ mice. Furthermore, there was no hermaphrodism in the 17 Dh/+ males and no sex reversal in the 20 Dh/+ females.
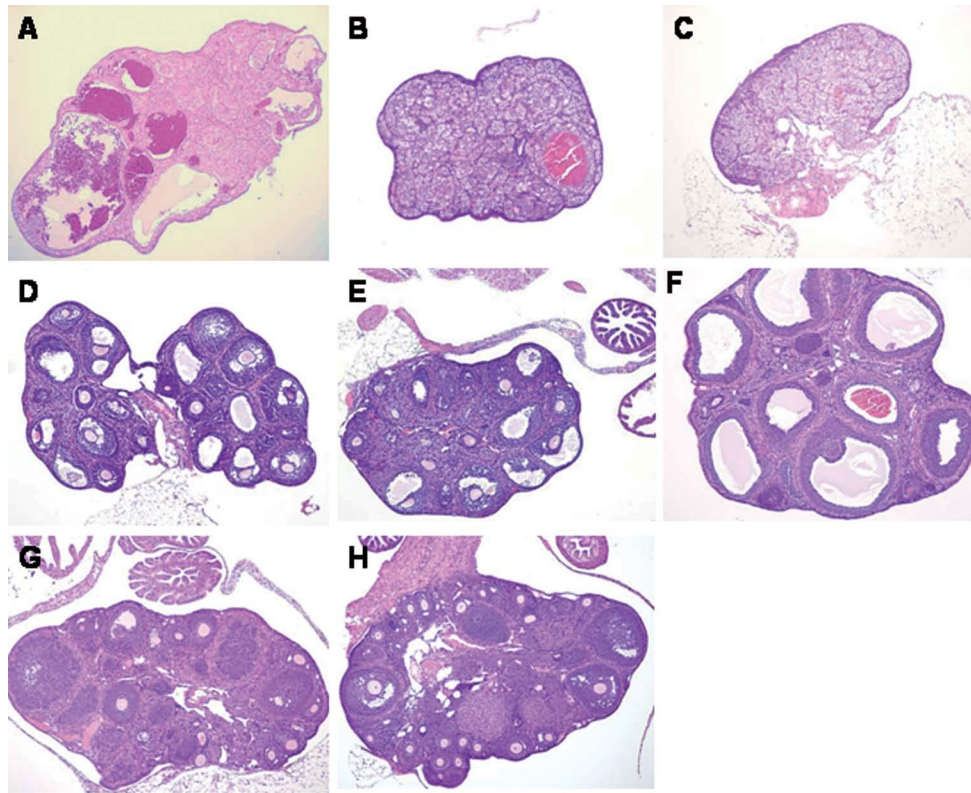
Fig. 2. (**A**-**C**): Ovarian histology of B6-Chr Y^{AKR}-*Dh*/+ N(2) hermaphrodites at 80 days of age. Mouse numbers: (**A**) AKP22, (**B**) AK219, and (**C**) AK280. Hyperplasia of luteinized cells was common to all. Atrophy of the ovary was noted in AK219 (**B**) and AK280 (**C**). A corpus luteum cyst was identified in AKP22 (**A**) and AK219 (**B**). (**D**-**F**): Ovarian histology of younger B6-Chr Y^{AKR}-*Dh*/+ N(2) and B6-Chr Y^{RF}-*Dh*/+ N(2) hermaphrodites. Mouse numbers: (**D**) AKAM59 at 31 days old; (**E**) AKAM79 at 35 days old, and (**F**) RFRM211 at 37 days old. Atrophy of the ovary was common to all. Atretic follicles degenerated at various stages. Primordial follicles were absent in all ovaries. (**G** and **H**): Ovarian histology of B6-Chr Y^{AKR}-*Dh*/+ N(2) normal-appearing females. At (**G**) 32 days old and (**H**) 45 days old. No marked were changes observed.

Linkage mapping of loci in the B6 genome responsible for the occurrence of hermaphrodism and sex reversal. The above data suggested that the hermaphrodism and sex reversal were caused by a combination of at least three gene loci, i.e., the *Dh* locus on chromosome 1, B6-derived homozygous alleles at one or more autosomal loci, and the Y-linked loci from AKR or RF. Therefore, linkage mapping analysis was performed in N$_2$ mice with hermaphrodism or sex reversal to identify the number and chromosomal location of gene loci responsible for these abnormalities in the B6 genome.

First, genome-wide linkage analysis was performed in 20 N$_2$ mice consisting of B6-Chr Y^{AKR}-*Dh*/+ N(2) and B6-Chr Y^{RF}-*Dh*/+ N(2) (Fig. 4). In an initial search ($n = 20$), significant linkage was applicable only to those marker loci with 19 or more

B6-derived homozygous alleles. The strongest association with abnormal phenotypes was identified on chromosome 13 (95% of N$_2$ were homozygous for B6 alleles), and there were other strong associations on chromosomes 17 and 18 (70% of N$_2$ were homozygous for B6 alleles). I then searched for additional microsatellite markers in widely spaced regions of chromosomes 13, 17, and 18. No markers were found on chromosome 17. Nine newly identified N$_2$ mice (either hermaphrodites or sex-reversed XY females) were then genotyped for all microsatellite marker loci on chromosomes 13 and 18 and for two loci on chromosome 17 (Table 3). In the final search ($n = 29$), statistical linkage was applicable only to those marker loci with 25 or more B6-derived homozygous alleles. There was significant linkage for loci on chromosome 13. Seven loci showed significant
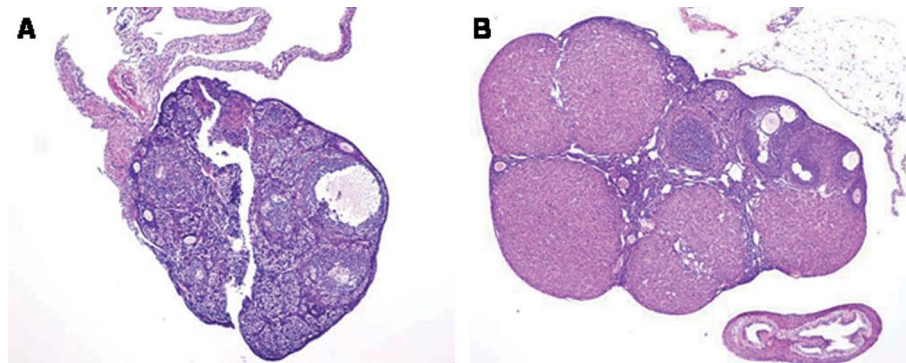
Fig. 3. Ovarian histology of two sex-reversed XY females [B6-Chr Y^{AKR}-*Dh*/+ N(2)] at 80 days of age. As a rule, ovaries were severely atrophied and primordial follicles were absent. (**A**) Sclerotic changes of the cortex were noted and developing follicles were absent. This ovary was considered to be anovualatory. (**B**) Cortex was markedly luteinized (plural corpora lutea); therefore, this ovary was considered to have ovulated once.
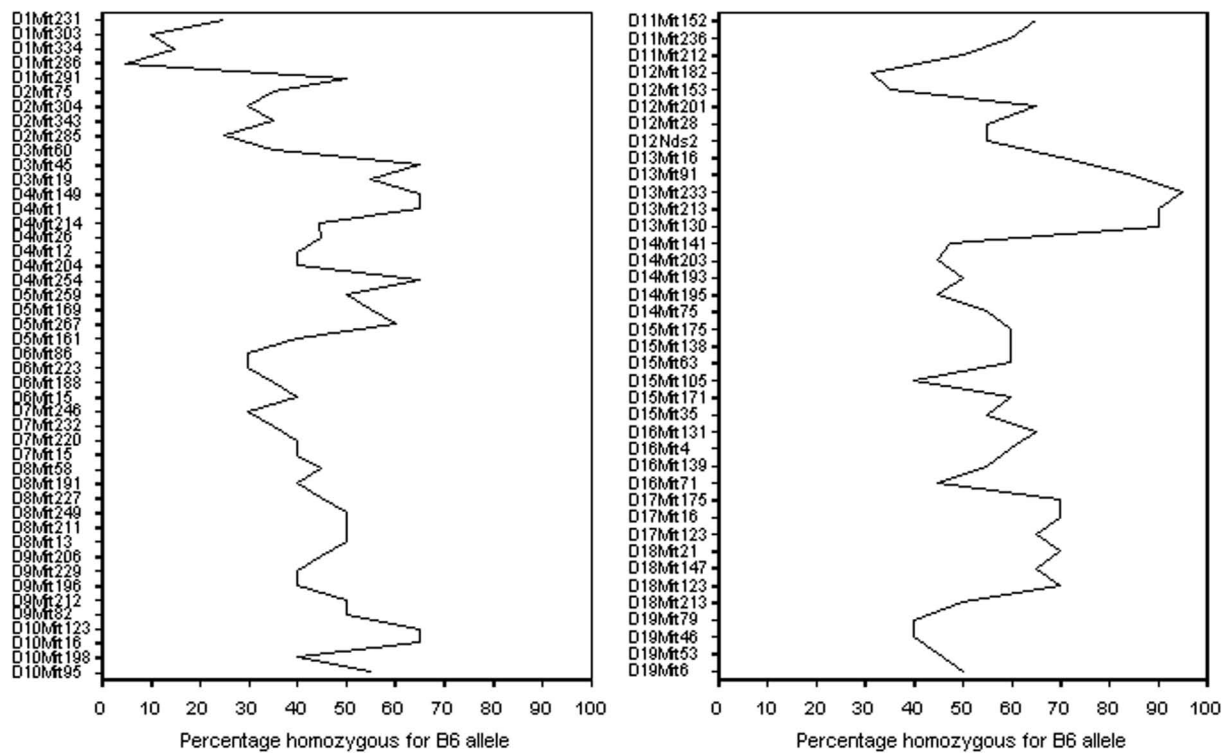

Fig. 4. Plot of percentages of mice homozygous for B6 alleles at each microsatellite marker locus in the initial genome-wide scan of 85 microsatellite marker loci.

linkage, and the strongest association was identified at four loci (27 of 29 N$_2$ were homozygous for the B6-derived allele). Because of the limited sample size, it was impossible to perform further mapping of the loci. Putative linkage was no longer supported for any loci on chromosomes 17 and 18.

Except for the gonadal abnormalities, all hermaphrodites and sex-reversed XY females showed ordinary *Dh*/+ phenotypes, i.e., they were invariably asplenic and occasionally exhibited hydronephrosis. The spectrum of hindlimb malformations identified in the hermaphrodites and sex-reversed XY females

Table 3.   Result of linkage mapping of chromosomes 13, 17, and 18 in N_2 mice with hermaphrodism or sex-reversal phenotypes

Chromosome	Position (cM)	Locus	No. of hermaphrodites or sex-reversed XY females		P value	No. of control mice ($Dh/+$ males and $Dh/+$ females)	
			B6/B6	B6/DH		B6/B6	B6/DH
13	10	D13Mit16	21	8			
	16	D13Mit275	22	7			
	30	D13Mit91	24	5			
	36	D13Mit279	26	3	0.0000068		
	40	D13Mit254	27	2	0.00000076	56	69
	45	D13Mit233	27	2	0.00000076	58	67
	51	D13Mit106	27	2	0.00000076	57	68
	59	D13Mit213	26	3	0.0000068		
	61	D13Mit130	27	2	0.00000076		
	68	D13Mit262	25	4	0.000044		
17	17.4	D17Mit16	20	9			
	17.7	D17Mit175	20	9			
18	6	D18Mit21	19	10			
	12	D18Mit34	20	9			
	16	D18Mit147	19	10			
	24	D18Mit149	17	12			
	31	D18Mit123	18	11			
	37	D18Mit152	18	11			
	55	D18Mit213	14	15			

P values are given only for loci showing significant linkage.

was fully within the limits of those observed in inbred DH-$Dh/+$ mice. Therefore, the effect of Dh on the gonad seemed unlikely to be a secondary consequence of other malformations caused by Dh.

Discussion

Postnatal death of $Dh/+$ mice on B6 background.   The Dh mutation could not be completely transferred onto the B6 strain background because of the high postnatal lethality of $Dh/+$ on and after the second backcross generation regardless of the type of Y chromosome. Because no extensive lethality was observed in (B6 × DH-Chr Y^{AKR}-$Dh/+$) F_1-$Dh/+$ and $Dh/+$ mice from BALB × (BALB × DH-Chr Y^{AKR}-$Dh/+$) F_1-$Dh/+$, B6-derived homozygous alleles at autosomal loci must have been involved. Furthermore, since the number of surviving $Dh/+$ mice relative to $+/+$ mice decreased with an increase in the number of backcross generations (Table 1), more than one locus seemed to be involved. Nevertheless, analysis was performed on only a small subset of

$Dh/+$ mice because of the lethality. To increase the efficiency of such studies, it is crucial to establish $Dh/+$ mice on a B6 background by identifying the loci responsible for lethality and eliminating them.

Genetic similarities and differences between this sex reversal and other mouse sex-reversal conditions.   The hermaphrodism and sex reversal caused by Dh in combination with the B6-derived homozygous alleles at chromosome 13 and the AKR Y chromosome was genetically novel, i.e., it was unlike other mouse sex-reversal conditions.

Transfer of certain Y^{DOM} onto the B6 strain background results in abnormal testis development, i.e., hermaphrodism and sex reversal (B6-Y^{DOM} sex reversal).[16] The extent of phenotypic aberration in B6-Y^{DOM} sex reversal depends on the type of Y chromosome; for example, B6-Y^{AKR} and B6-Y^{RF} show only delayed testis cord formation or fetal hermaphrodism, and adult males have normal testes.[17,18] When the B6 strain contained the Y chromosome from *M. m. domesticus poschiavinus* (B6-Y^{POS}), XY

individuals developed either as females with two ovaries or as hermaphrodites, of which half had two ovotestes and half had an ovary and an ovotestis. None of the XY individuals developed normal testes in the fetal gonad.[16] B6-Y[POS] is a consequence of aberrant interaction between the *Sry* allele and B6-derived testis-determining autosomal (*tda*) genes located on chromosomes 2 (*tda2*), 4 (*tda1*), and 5 (*tda3*).[13] It is important to note that my hermaphrodites and sex-reversed mice had no substantial linkage with the *tda1* locus. *tda1* has been identified as a major locus causing B6-Y[POS] sex reversal, and it has been mapped to distal chromosome 4 (82 cM; the marker locus used here was *D4Mit254*, which is located at 82.5 cM). Similarly, no significant linkages were identified for *tda2* or for *tda3*. Conversely, chromosome 13 showed no evidence of linkage with B6-Y[POS].

C57BL/6J-*T*-associated sex reversal (B6-TAS)[17),19),20] is another sex-reversal condition. B6-TAS involves the T^{hp} or T^{Orl} allele at the *T* locus on chromosome 17, B6-derived homozygous alleles at one or more autosomal loci, and the AKR Y chromosome, inducing hermaphrodism and sex reversal. B6-TAS resembles the present condition with regard to the autosomal dominant mutation located outside the B6 genome, is involved with B6-derived autosomal loci and the AKR Y chromosome. Although B6-derived autosomal loci have not been mapped in this condition, Washburn *et al.*[21] speculated that they may be allelic with *tda1* or *tda2*.

According to Nagamine and Carlisle,[11] one of the alleles at the *kit* oncogene locus Kit^{W-42J} also exacerbates B6-Y[AKR] sex reversal. Like B6-TAS, this condition resembles the present one in that an autosomal dominant mutation is involved. However, the *kit* locus is located on chromosome 5; this condition therefore differs from the present one.

Phenotypic comparison between this sex reversal and the B6-Y[DOM] sex reversal condition. According to Eicher *et al.*,[16] when the gonads of XY B6-Y[POS] adult mice (from the N₄ to N₇ backcrossed generations) were analyzed, mice could be classified phenotypically as female, hermaphrodite, or male. However, an analysis on fetal gonads revealed that the testes of normal-appearing adult males might be actually ovotestes. I, too, observed these three adult phenotypes. Although I analyzed adult gonads, inclusion of ovarian tissues was not identified histologically in the left testes of the hermaphrodites. One cannot rule out the possibility

that ovarian tissues were missed in this histological analysis, because the presence of a small area of ovarian tissue in a fetal ovotestes is difficult to identify in an adult gonad, and the ovarian tissue present in the fetal ovotestes may have degenerated in the adult.[9),22] Therefore, the possibility that the left testes of the present hermaphrodites were actually ovotestes could not be excluded.

The findings in B6-Y[DOM] or B6-Y[POS] XY females are highly suggestive in regard to the fertility of sex-reversed XY females. With rare exceptions, B6-Y[POS] XY females are sterile.[16),23] According to Eicher *et al.*,[16] the ovaries of B6-Y[POS] XY females are histologically normal at age 4 weeks; however, because of subsequent rapid depletion of germ cells, XY females are no longer fertile by 8 weeks of age. According to Taketo-Hosotani,[23] in B6-Y[TIR] (Y[TIR] represents the Y chromosome from *M. m. domesticus tirano*, which is related to *M. m. domesticus poschiavinus*), the number of follicles in the XY ovary is smaller than that in the XX ovary at the age of about 1 month, and only a few follicles can be seen in XY ovaries by age 2 months. Nagamine *et al.*[24] found that the postnatal development of B6-Y[TIR] XY ovaries was normal up to age 6 weeks; however, after this stage, the corpora lutea in the XY ovary persist instead of degenerating. In light of these findings that the ovarian histology is relatively normal at a younger age and that persistence of corpora lutea lead to the hyperplasia of luteinized cells in the XY ovary (Fig. 3B), the results in B6-Y[POS] or B6-Y[TIR] were similar to my results in the ovaries of hermaphrodites or sex-reversed XY females. More detailed characterization of these ovaries is required for further discussion.

A novel gene locus involved in primary sex determination is located on chromosome 13. The locus (loci) that I identified on chromosome 13 may be a novel participant in the cascade of sex determination.

I used an MGI search to look for genes related to male-to-female sex reversal. However, no plausible candidate genes were located on chromosome 13; for example, *M33* (*Cbx2*) is located on chromosome 11,[25] *Ods* on chromosome 11,[26] *Fgf9* on chromosome 14,[27] *Wt1* on chromosome 2,[28] *Igf1r* on chromosome 7, *Insr* on chromosome 8, and *Insrr* on chromosome 8,[29] *Pod1* (*Tcf21*) on chromosome 6,[30] and *Dax1* on the X chromosome.[31] Furthermore, mouse chromosome 13 regions are syntenic to parts of hu-

man chromosomes 5, 9, and 15; however, according to Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM), there have been no reports of human mutations resulting in hermaphrodism or sex reversal.

Hermaphrodism or sex reversal was not observed in B6-Chr Y^{BALB}-$Dh/+$ N(2), B6-Chr Y^{C3H}-$Dh/+$ N(2), B6-Chr Y^{B6}-$Dh/+$ N(2), B6-Chr Y^{DH}-$Dh/+$ N(2), and B6-Chr Y^{DDD}-$Dh/+$ N(2). It seems therefore possible that there are functional differences among Y chromosomes in the ability to cause the production of abnormal gonads. However, it is not prudent to conclude that these Y chromosomes are incapable of inducing aberrations in primary sex determination. Fetal gonad analysis is required to validate this assumption, because the gonadal phenotype assigned to an adult could differ from that assigned to genetically identical fetuses.[22]

The genetic basis underlying sex determination is not yet completely understood. Therefore, it has been suggested that one of the most powerful approaches to understanding the molecular basis of the sex determination pathway is to identify and analyze inherited sex-reversal conditions.[9] The hermaphrodism and/or sex reversal condition described here was genetically distinct from previously reported sex reversal conditions.[16),17),24)] It therefore offers a novel opportunity to investigate the genetic basis of sex determination in mammals.

Acknowledgments

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