Effect of the Y chromosome on testis weight in mice

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ABSTRACT. We investigated the effect of the Y chromosome on testis weight in (B6.Cg-A^y × Y-consomic mouse strain) F_1 male mice. We obtained the following results: (1) Mice with the *Mus musculus domesticus*-type Y chromosome had significantly heavier testis than those with the *M. m. musculus*-type Y chromosome. (2) Variations in *Usp9y* and the number of CAG repeats in *Sry* were significantly associated with testes weight. The A^y allele was correlated with a reduced testis weight, and the extent of this reduction was significantly associated with a CAG repeat number polymorphism in *Sry*. These results suggest that Y chromosome genes not only influence testis weight but also modify the effect of the A^y allele in mediating this phenomenon.

KEY WORDS: A^y allele at the agouti locus, *Sry* CAG repeat number polymorphism, testis weight, *Usp9y*, Y-consomic strains

Testis weight is an important reproductive trait in males, because of its direct connection with spermatogenic ability [14]. This highly heritable quantitative trait varies widely among inbred mouse strains [36]. Many studies have extensively explored the genes or loci that control testis weight in mice [21, 22, 32, 36, 40], thus revealing many autosomal and X-linked genetic effects. In contrast, Y-linked genetic effects have not yet attracted significant attention. Several studies have addressed the relationship between testis weight and the Y chromosome, the results of which were rather controversial [7, 15–17, 22, 36]. We suggest that these controversies arose due to the implementation of experiments that did not thoroughly control the autosomal and/or X-linked genetic variations. Therefore, we investigated the effect of the Y chromosome on testis weight in Y-chromosome-consomic (Y-consomic) mouse strains by making the potential genetic effects other than Y chromosome uniform [36]. We investigated 17 Y-consomic strains that were established in the inbred mouse strain DH/Sgn (DH). In the current study, we further investigated the effect of the Y chromosome on testis weight in genetic backgrounds that differed from those used in the previous study. It is most feasible to produce F_1 mice using the Y-consomic strains as sires to alter their genetic backgrounds. Therefore, we produced and analyzed F_1 mice between B6.Cg-A^y females and Y-consomic strain males. Because A^y is an autosomal dominant allele and homozygous A^y/A^y mice are lethal *in utero* [9], the living A^y mice are invariably heterozygotes [9]. Thus, we can simultaneously utilize two different F_1 genetic backgrounds (i.e., F_1 A^y and F_1 non-A^y backgrounds). The A^y allele is known to signifi-

cantly reduce testis weight [34], and as such, this mating scheme allows us to examine this further by including the A^y allele effect into the analyses as a potential autosomal effect.

The A^y allele at the agouti locus causes obesity in mice [9]. In normal mice, the agouti gene is expressed only in the skin [4, 27] where it functions as an inverse agonist of the melanocortin 1 receptor to regulate pigmentation [23, 31]. However, in A^y mice, the A^y allele is associated with a large deletion, thus causing agouti gene expression to be aberrantly controlled by the unrelated *Raly* gene promoter [10, 25–27]. This leads to the ectopic overexpression; and therefore, A^y mice have a yellow coat and develop maturity-onset obesity. The molecular bases underlying these phenotypes are well understood; however, those leading to the reduction in testis weight are still unknown [6, 18, 29].

The B6.Cg-A^y strain was maintained at the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Japan) along with the following Y-consomic strains: DH-Chr Y^A(Y chromosome from A/J strain), DH-Chr Y^{AKR}(AKR/J), DH-Chr Y^{B6}(C57BL/6J), DH-Chr Y^{BALB}(BALB/cA), DH-Chr Y^{C3H}(C3H/HeJ), DH-Chr Y^{CAS}(CAST/EiJ), DH-Chr Y^{CBA}(CBA/N), DH-Chr Y^{CF1}(CF1/Sgn), DH-Chr Y^{DBA}(DBA/2J), DH-Chr Y^{DDD}(DDD/Sgn), DH-Chr Y^{DH}(DH/Sgn), DH-Chr Y^{KK}(KK/Ta), DH-Chr Y^{RF}(RF/J), DH-Chr Y^{RR}(RR/Sgn), DH-Chr Y^{SJL}(SJL/J), DH-Chr Y^{SS}(SS/Sgn) and DH-Chr Y^{SWR}(SWR/J).

Males of each Y-consomic strain were crossed with B6.Cg-A^y females to produce (♀B6.Cg-A^y × ♂DH-Chr $Y^@$) F_1 strains (@ is an arbitrary inbred strain name). Thus, F_1 mice comprised F_1 A^y and F_1 non-A^y mice. Hereafter, we designated F_1 A^y mice as F_1-Y and F_1 non-A^y mice as F_1-A. Occasionally, the origin of the Y chromosome was specifically indicated for the F_1 mice. For example, the ♀B6.Cg-A^y × ♂DH-Chr Y^{B6} F_1-Y strain was designated as F_1-Y (Y^{B6}). F_1-Y mice were visually distinct from F_1-A mice, because the former had a yellow coat [9]. Three to five male mice of the same strain, irrespective of F_1-A or F_1-Y mice, were housed in a single cage. The number of mice used in this

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study is summarized in Table 1. All mice were maintained in a specific-pathogen-free facility with a regular light cycle (12 hr light and 12 hr dark) and controlled temperature and humidity. Food (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water were freely available throughout the experimental period. All animal procedures were approved by the Institutional Animal Care and Use Committee of NIAS, and experiments were conducted in accordance with the committee-approved guidelines.

At 16 weeks of age, the mice were weighed on an electric balance to the nearest 0.01 g. They were euthanized by ether over dose, and their testes were removed and placed in physiological saline. After being rinsed and wiped with wet chromatography paper, the paired testes were weighed on the electric balance to the nearest 1 mg.

As mentioned above, the A^y allele reduces testis weight in mice [34]. The degree of this reduction varied among our F_1-Y strains. Therefore, the effect of the A^y allele in reducing testis weight in each individual F_1-Y mouse was defined as the "Tw ratio." For example, the "Tw ratio" in the F_1-Y (Y^A) strain was calculated as follows:

Tw ratio=(Testis weight in each individual F_1-Y (Y^A) mouse −Average testis weight in F_1-A (Y^A) mice)/[Average testis weight in F_1-Y (Y^{DH}) mice −Average testis weight in F_1-A (Y^{DH}) mice]

We identified the following thirty single nucleotide polymorphisms (SNPs) in the 16 Y-consomic strains (DH-Chr Y^{DH} was not genotyped) [36]: rs47359684, rs47900677, rs46080695, rs52139814, rs45850354, rs48064925, rs51995337, rs51133250, rs50647790, rs51277152, rs48685451, rs48834187, rs46947134, rs51756947, rs48554025, rs47574660, rs51766109, rs49468864, rs49623242, rs51230091, rs49614307, rs48926479, rs51025923, rs48512209, rs47293184, rs51685350, rs47616691, rs51560704, rs46643293 and rs51529727. We also identified the following nine polymorphisms in *Sry* (nt numbers are based on the GenBank entry X67204): nt 8491, nt 8701, nt 8711, nt 8731, number of first CAG repeats starting at nt 8733, number of second CAG repeats starting at nt 8811, nt 8930, nt 8934 and nt 9006 [11]. Based on the *Sry* sequences, the DH-Chr Y^A, DH-Chr Y^{B6}, DH-Chr Y^{BALB}, DH-Chr Y^{C3H}, DH-Chr Y^{CBA}, DH-Chr Y^{CF1}, DH-Chr Y^{DH}, DH-Chr Y^{KK}, DH-Chr Y^{RR} and DH-Chr Y^{SS} strains were defined as possessing the *Mus musculus musculus*-type Y chromosome (Y^{Mus}), whereas the DH-Chr Y^{AKR}, DH-Chr Y^{DDD}, DH-Chr Y^{RF}, DH-Chr Y^{SJL} and DH-Chr Y^{SWR} strains were defined as possessing the *M. m. domesticus*-type Y chromosome (Y^{Dom}). The strains, Y^{Mus} vs. Y^{Dom}, were classified on the basis of the following criteria: (1) a C-to-T transitional substitution at nt 8491 in the high mobility group (HMG) box of *Sry* (Y^{Mus}=T and Y^{Dom}=C) [20] and (2) the presence of a C-to-T change that created a TAG termination codon at nt 9006 in the third major CAG repeat starting at nt 8985 in Y^{Dom} [8] but not Y^{Mus} (the mouse *Sry* gene has four major sites comprising approximately 10 CAG repeats). The partitioning of the strains into either Y^{Mus} or Y^{Dom} was compatible with the descriptions set forth in previous studies [2, 28].

Table 1. Number of F_1 mice and their average body weight

Y chromosome donor strain (abbreviation)	Number of F_1-A mice (mean ± SE body weight, g)	Number of F_1-Y mice (mean ± SE body weight, g)
A/J (A)	18 (33.32 ± 0.91)	12 (47.04 ± 0.98)
AKR/J (AKR)	5 (31.91 ± 1.05)	13 (44.18 ± 0.56)
C57BL/6J (B6)	17 (33.96 ± 0.66)	18 (46.49 ± 0.78)
BALB/cA (BALB)	7 (35.77 ± 1.93)	13 (48.43 ± 0.76)
C3H/HeJ (C3H)	8 (35.51 ± 2.05)	15 (47.11 ± 0.64)
CAST/EiJ (CAS)	12 (34.47 ± 0.37)	12 (47.97 ± 0.58)
CBA/N (CBA)	12 (36.06 ± 0.54)	10 (49.09 ± 0.94)
CF#1/Sgn (CF1)	12 (35.86 ± 0.83)	10 (49.10 ± 0.97)
DBA/2J (DBA)	11 (32.91 ± 0.36)	13 (46.28 ± 0.66)
DDD/Sgn (DDD)	15 (36.32 ± 0.59)	13 (47.72 ± 0.57)
DH/Sgn (DH)	9 (34.20 ± 0.99)	10 (46.93 ± 0.84)
KK/Ta (KK)	16 (29.69 ± 0.47)[a]	12 (41.46 ± 0.95)[a]
RF/J (RF)	8 (33.29 ± 1.29)	10 (48.44 ± 0.62)
RR/Sgn (RR)	13 (32.27 ± 0.41)	11 (45.99 ± 0.50)
SJL/J (SJL)	16 (35.60 ± 1.01)	17 (47.62 ± 0.58)
SS/Sgn (SS)	18 (32.09 ± 0.70)	17 (45.44 ± 0.64)
SWR/J (SWR)	10 (33.60 ± 0.50)	12 (45.69 ± 0.48)

a) According to the Dnnett's multiple comparison test using DH as a reference, strain carrying Y^{KK} was significantly associated with reduced body weight in both F_1-A and F_1-Y mice [37].

Y-linked genetic variations controlling testis weight were identified using the following three-step approach [36]: (1) The effects of genes on autosomes and the X chromosome were eliminated by the use of Y-consomic strains, and the net phenotypic effects of Y-linked genes were assessed. (2) Either Dunnett's multiple comparison test (with the background DH strain as a reference) or the Tukey–Kramer honest significant difference (HSD) test was used to determine if a trait was Y-linked. (3) The data from all strains were assembled on the basis of SNP genotypes, and the statistical significance of the differences was assessed. Two groups partitioned by genotype were compared using the Student's or Welch's *t*-test, and three groups were compared with one-way analysis of variance (ANOVA). Based on the number of SNP loci (n) genotyped, the significant threshold P value was determined as 0.05/n with the Bonferroni correction test. Unless otherwise noted, $P<0.05$ was considered statistically significant.

Testis weight showed a bell-shaped distribution curve for both F_1-A and F_1-Y mice, but did not show a normal distribution on the basis of the Shapiro-Wilk W test (JMP8, SAS Institute Japan Inc., Tokyo, Japan). Therefore, the Box-Cox method was used to normalize the data prior to subsequent statistical analyses. According to Dunnett's multiple comparison tests, a Y chromosome substitution caused Y^{SJL} and Y^{CAS} strains to have significantly heavier testis than the Y^{DH} strain in F_1-A mice, and the Y^{CAS} strain to have significantly heavier testis than the Y^{DH} strain in F_1-Y mice (Fig. 1).

The mean testis weight ± SE in F_1-A mice was 247.0 ± 1.4 mg (n=208), and that in F_1-Y mice was 214.5 ± 1.0 mg (n=218), the difference of which was highly significant (P=1.09 × 10^{-60}). Thus, consistent with our previous finding,
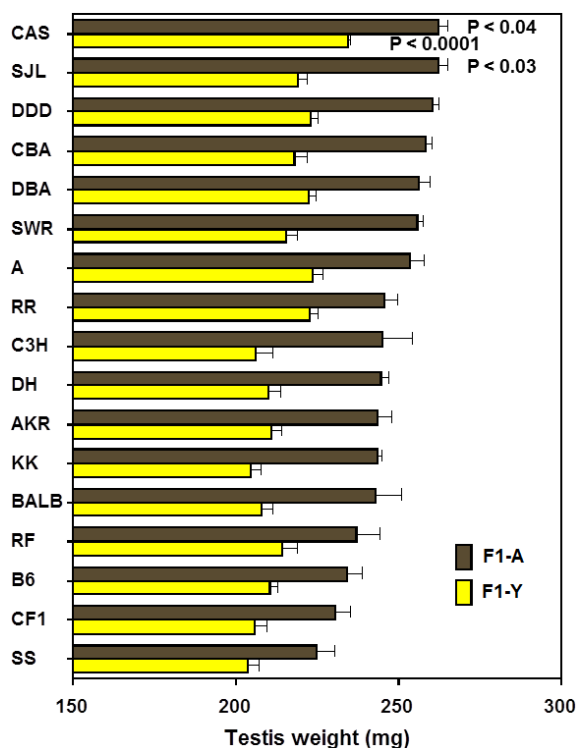
Fig. 1. Distribution of mean testis weight with SE in F_1-A and F_1-Y strains. Each strain symbol on the y-axis represents the kind of Y-chromosome donor strain; for example, "C3H" represents F_1-A and F_1-Y mice carrying the Y chromosome of C3H strain. Strain symbols were sorted in descending order according to the mean testis weight of F_1-A strain. Statistical comparisons were made with Dunnett's multiple comparison tests using Y^{DH} as a reference. F_1-A and F_1-Y strains were analyzed separately.

the A^y allele reduced testis weight.

Y-linked SNPs and *Sry* polymorphisms could be summarized into six polymorphic patterns (Table 2). Although we performed statistical analyses on these data, the significance threshold at $\alpha=0.05$ was 0.0013 (0.05/39), because we actually genotyped 39 polymorphisms.

rs48685451 in *Kdm5d* and additional 25 SNPs had the same polymorphic pattern, which was consistent with the distinction between Y^{Mus} and Y^{Dom}. When the F_1-A and F_1-Y mice were analyzed separately, only F_1-A mice showed a significant association with testis weight (Table 3). However, because two-way ANOVA showed no statistically significant interactions between the agouti locus genotype (A or Y) and the rs48685451 genotype (A or G), data on F_1-A and F_1-Y mice were combined and analyzed after standardization within each F_1 type. As a result, rs48685451 was significantly associated with testis weight. Mice with the A allele (Y^{Dom}) had significantly heavier testis than mice with the G allele (Y^{Mus}), which is in accordance with our previous findings [36]. Subsequent analyses were performed in a similar manner.

rs51766109 in *Usp9y* was significantly associated with testis weight, and mice with the T allele had significantly

heavier testis than those with the C allele. The result differed with our previous study in which rs51766109 had no significant effect on absolute testis weight [36]. rs46947134 in *Uty* was not significantly associated with testis weight. The result also differed with our previous study in which rs46947134 had significant effect on absolute testis weight [36]. A major difference between the two studies is the strain of mice used (i.e., our previous study used DH-Chr $Y^@$ strains, whereas the present study was conducted with B6.Cg-A^y × DH-Chr $Y^@$ F_1 strains). Thus, this suggests that the effect of the Y chromosome on testis weight was considerably influenced by genetic backgrounds. This result is not surprising, because genes on the X chromosome, in mitochondria, and half of all autosomes were thoroughly replaced by the B6 genome in F_1 male mice. Indeed, there is evidence that the Y chromosome interacts with the X-linked locus in controlling testis weight [36]. In addition, testis weight measurements were taken on 16-week-old mice in the present study and 80-day-old mice in our previous study, suggesting that the discrepancies between the two studies were partly due to the difference in the ages of the mice investigated. QTLs for testis weight have been shown to vary between pigs of different ages [30]. Alternatively, it is possible that the discrepancies between two studies are caused by the differences in epigenomes.

Uty and *Usp9y* are histocompatibility Y antigen-coding genes. Both genes are expressed extensively in testis; however, physiological significance of them remains unknown. For example, *Usp9y* has been implicated in human infertility associated with oligospermia and azoospermia [19, 33]; however, a more recent study demonstrated that the *Usp9y* is not essential for normal spermatogenesis [24]. Several lines of evidence suggest that *Uty* and *Usp9y* have roles in cell division, proliferation and/or differentiation [13, 39]; therefore, it is possible that they control testis mass through these physiological processes.

Nucleotides at positions 8491 and 8711 in *Sry* were significantly associated with testis weight. Mice with the C allele had significantly heavier testis than mice with the T allele at both sites. As with rs48685451 in *Kdm5d*, *Sry* polymorphisms at these positions were mostly consistent with the distinction between Y^{Mus} and Y^{Dom}. In this case, Y^{CAS} was classified in Y^{Dom}. In the case of rs48685451 in *Kdm5d* and others, Y^{CAS} was classified in Y^{Mus}, and according to the literature [1], Y^{CAS} was classified as Y^{Mus}.

Starting at nt 8733, the number of the first major CAG repeats in *Sry* was significantly associated with testis weight. Mice with 11 repeats had significantly lighter testis than mice with 9 or 12 repeats. In contrast, starting at nt 8811, the number of the second major CAG repeats was not significantly associated with testis weight. In mice, *Sry* contains four large CAG trinucleotide repeat regions, resulting in four major polyglutamine stretches [8]. The C-to-T transitional substitution at nt 9006 creates a premature TAG termination codon in the third major CAG repeat region of Y^{Dom}. Therefore, polymorphisms in the first and second CAG repeats are potentially important. Consistent with our previous findings [36], the present study demonstrated that variation in the number of CAG repeats starting at nt 8733 of *Sry* was sig-

Table 2. Patterns of SNPs and *Sry* polymorphisms in Y-consomic strains

Gene	SNP/polymorphism	A	B6	BALB	C3H	CBA	CF1	DBA	KK	RR	SS	CAS	AKR	DDD	RF	SJL	SWR
Kdm5d	rs48685451[a]	G	G	G	G	G	G	G	G	G	G	G	A	A	A	A	A
Usp9y	rs51766109	C	C	C	T	T	C	T	C	T	C	T	T	T	T	T	T
Uty	rs46947134	C	C	C	G	G	C	G	C	G	C	C	C	C	C	C	C
Sry	Nts at 8491 and 8711[b]	T	T	T	T	T	T	T	T	T	T	C	C	C	C	C	C
Sry	No. of CAG repeats starting at nt 8733	11	11	11	12	12	11	12	11	12	11	11	9	9	9	9	9
Sry	No. of CAG repeats starting at nt 8811	12	12	12	10	10	12	10	12	10	12	12	13	12	13	12	12

a) In addition to rs48685451 in *Kdm5d*, many SNPs showed similar polymorphic pattern [28]. b) Nt position is based on the numbering of GenBank entry X67204.

Table 3. Effects of gene polymorphisms on testis weight in Y-consomic strains

SNP/Polymorphism (Gene)	Mice	Paired testis weight (mg, mean ± SE)[a]			*P* value[b]
rs48685451		A	G		
(*Kdm5d*)	F_1-A	254.9 ± 2.1 (n=54)	244.2 ± 1.7 (n=144)		0.00023
	F_1-Y	216.8 ± 1.6 (n=65)	213.8 ± 1.3 (n=143)		NS (0.24)
	Combined F_1	0.268 ± 0.076	−0.096 ± 0.063		0.00090
rs51766109		C	T		
(*Usp9y*)	F_1-A	238.1 ± 2.3 (n=88)	254.3 ± 1.5 (n=110)		2.52×10^{-9}
	F_1-Y	209.1 ± 1.5 (n=82)	218.4 ± 1.3 (n=126)		1.63×10^{-6}
	Combined F_1	−0.415 ± 0.078	0.317 ± 0.058		1.09×10^{-13}
rs46947134		C	G		
(*Uty*)	F_1-A	245.9 ± 1.7 (n=154)	251.5 ± 2.5 (n=44)		NS (0.12)
	F_1-Y	214.2 ± 1.2 (n=159)	216.7 ± 2.2 (n=49)		NS (0.26)
	Combined F_1	−0.041 ± 0.058	0.184 ± 0.099		NS (0.058)
Nts at nt 8491 and 8711		C	T		
(*Sry*)	F_1-A	256.3 ± 1.8 (n=66)	242.6 ± 1.8 (n=132)		4.01×10^{-7}
	F_1-Y	219.6 ± 1.5 (n=77)	211.9 ± 1.3 (n=131)		0.00016
	Combined F_1	0.403 ± 0.070	−0.203 ± 0.064		4.07×10^{-9}
No. of CAG repeats starting at nt 8733		9	11	12	
(*Sry*)	F_1-A	254.9 ± 2.1 (n=54)	241.0 ± 2.2 (n=100)	251.5 ± 2.5 (n=44)	4.36×10^{-5}
	F_1-Y	216.8 ± 1.6 (n=65)	212.3 ± 1.6 (n=94)	216.7 ± 2.2 (n=49)	NS (0.12)
	Combined F_1	0.268 ± 0.090	−0.231 ± 0.071	0.184 ± 0.102	1.65×10^{-5}
No. of CAG repeats starting at nt 8811		10	12	13	
(*Sry*)	F_1-A	251.5 ± 2.5 (n=44)	246.5 ± 1.8 (n=141)	239.4 ± 4.9 (n=13)	NS (0.11)
	F_1-Y	216.7 ± 2.2 (n=49)	214.5 ± 1.3 (n=136)	212.4 ± 2.7 (n=23)	NS (0.39)
	Combined F_1	0.184 ± 0.104	−0.016 ± 0.060	−0.234 ± 0.168	NS (0.080)

a) Combined data are expressed as standardized values. b) NS, not significant.

nificantly associated with testis weight. However, although testis weight was inversely correlated with the numbers of CAG repeats in our previous study, here we show that the testis was significantly lighter only in mice with 11 CAG repeats. Furthermore, although variation in the numbers of CAG repeats starting at nt 8811 of *Sry* was significantly associated with testis weight in our previous study [36], no significant association was observed in this study. We cannot presently explain these discrepancies between the two studies, but we speculate that they are caused by differences in genetic backgrounds, ages and/or epigenomes.

When the F_1-A strains were sorted in descending order with regard to their testis weight, the testis weight of the F_1-Y strains was not necessarily sorted in descending order (Fig. 1). That is, the effect of the A^y allele in reducing testis weight differed depending on Y chromosome type. Because there was variation in the Tw ratio among Y-consomic strains based on the Tukey–Kramer HSD test (Fig. 2), we performed association studies on the Tw ratio. The number of first major *Sry* CAG repeats (starting at nt 8733) was significantly associated with Tw ratio (Table 4). Mice with 11 repeats had a significantly lower Tw ratio than mice with 9 or 12 repeats. Thus, CAG repeat number polymorphism of *Sry* modified the effect of the A^y allele in reducing testis weight.

Sry is expressed in the gonad and plays roles in testis determination. On the basis of the findings on B6.Y^{Dom} sex reversal [12], Y^{Dom} can be classified into several functional classes. That is, Y^{Dom} associated with either of (1) complete
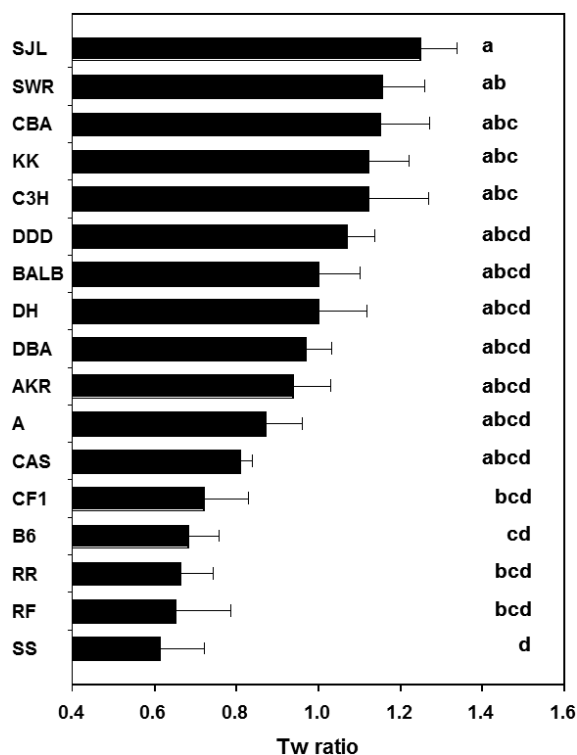
Fig. 2. Distribution of mean Tw ratio with SE in F_1-Y strains. Each strain symbol on the y-axis represents the kind of Y-chromosome donor strain; for example, "C3H" represents F_1-Y mice carrying the Y chromosome of C3H strain. Strain symbols were sorted in descending order according to the mean Tw ratio. Statistical comparisons were made with the Tukey-Kramer HSD tests. Values with different superscripts differ significantly.

disorders are associated with polyglutamine stretch expansion (OMIM, Online inheritance in man, http://www.ncbi.nlm.nih.gov/omim). Although the hypothesis by Coward *et al.* was denied later [5], it was important that they indicated a genetic link between CAG repeat number polymorphism and gonadal phenotypes of mice. According to Bowles *et al.* [3], the presence of CAG repeats in the mouse *Sry* is essential for testis determination. They consider that the absolute sequence and length of the CAG repeat domain are not necessarily critical to its function; however, it has not yet been proved enough. We would like to propose that the CAG repeat polymorphism has roles in other physiological processes including the control of testis weight.

The above-mentioned analyses were based on the alleles at SNPs/*Sry*. We further performed analyses based on the strains partitioned by Y-linked haplotypes on the basis of the six SNPs and *Sry* polymorphisms (Table 2). F_1 mice were partitioned into five groups (Table 5). There was a significant difference in testis weight among the five groups in F_1-A (P=4.57 × 10^{-11}) and F_1-Y (P=3.44 × 10^{-11}) mice. Tukey–Kramer HSD tests were used to compare testis weight among the five haplotype-based groups. A significant difference was observed between several pairs of groups. Group 4, which comprised only mice with Y^{CAS}, had the heaviest testis among five groups in both F_1 mice. For Tw ratio, there were significant differences among the five groups (P=0.000036). Tukey–Kramer HSD tests were used to compare testis weight among the five haplotype-based groups. Although there was no significant difference between Groups 1 and 5, Group 1 had the largest Tw ratio among the five groups and had a significantly larger Tw ratio than Groups 2, 3 and 4. Thus, the different aspects related to the effect of the Y chromosome on testis weight were elucidated only after the Y-consomic strains were analyzed by partitioning on the basis of Y-linked haplotypes.

Finally, we would like to mention the spermatogenic ability of mice used in this study. We have confirmed that all DH-Chr$^@$ strains breed well in their brother-sister mating. (B6 × KK.Cg-A^y) F_1 A^y and (B6 × DDD.Cg-A^y) F_1 A^y males as well as their F_1 non-A^y littermate males are fully fertile

sex reversal, (2) partial (fetal) sex reversal/hermaphrodism and (3) normal testis development [8, 12]. Coward *et al.* [8] once proposed a hypothesis that the degrees of sex reversal phenotype depend on polymorphism of CAG trinucleotide repeat of *Sry*. It has been known that many human genetic

Table 4. Effects of gene polymorphisms on Tw ratio

SNP/Polymorphism (Gene)	Tw ratio (mean ± SE)			P value[a]
rs48685451	A	G		
(*Kdm5d*)	1.04 ± 0.05 (n=65)	0.87 ± 0.03 (n=143)		NS (0.0049)
rs51766109	C	T		
(*Usp9y*)	0.81 ± 0.04 (n=82)	1.00 ± 0.04 (n=126)		NS (0.0015)
rs46947134	C	G		
(*Uty*)	0.91 ± 0.03 (n=159)	0.98 ± 0.06 (n=49)		NS (0.27)
Nts at nt 8491 and 8711	C	T		
(*Sry*)	1.01 ± 0.05 (n=77)	0.88 ± 0.04 (n=131)		NS (0.025)
No. of CAG repeats starting at nt 8733	9	11	12	
(*Sry*)	1.04 ± 0.05 (n=65)	0.81 ± 0.04 (n=94)	0.98 ± 0.06 (n=49)	0.0011
No. of CAG repeats starting at nt 8811	10	12	13	
(*Sry*)	0.98 ± 0.06 (n=49)	0.92 ± 0.03 (n=136)	0.81 ± 0.08 (n=23)	NS (0.25)

a) NS, not significant.

Table 5. Testis weight and Tw ratio in Y-consomic strains partitioned by haplotypes
based on Y-linked SNPs and *Sry* polymorphisms

Group	Strain	Testis weight (mg, mean ±SE)[1] (Sample size)		Tw ratio (mg, mean ±SE)[a] (Sample size)
		F$_1$-A mice	F$_1$-Y mice	
1	DDD	259.8 ± 1.6[A]	219.2 ± 1.8[B]	1.17 ± 0.06[A]
	SJL	(n=41)	(n=42)	(n=42)
	SWR			
2	AKR	239.4 ± 4.9[BC]	212.4 ± 2.7[BC]	0.81 ± 0.08[B]
	RF	(n=13)	(n=23)	(n=23)
3	A	238.1 ± 2.3[C]	209.1 ± 1.5[C]	0.81 ± 0.04[B]
	B6	(n=88)	(n=82)	(n=82)
	BALB			
	CF1			
	KK			
	SS			
4	CAS	262.4 ± 2.9[A]	234.4 ± 1.1[A]	0.81 ± 0.11[B]
		(n=12)	(n=12)	(n=12)
5	C3H	251.5 ± 2.5[AB]	216.7 ± 2.2[B]	0.98 ± 0.06[AB]
	CBA	(n=44)	(n=49)	(n=44)
	DBA			
	RR			

a) Values with different superscripts differ significantly.

[35, 38]. Therefore, we expect that there are no substantial defects in the spermatogenic ability in DH-Chr Y$^@$, F$_1$-A and F$_1$-Y strain males, despite variation in testis weight and Tw ratio among strains.

In conclusion, we confirmed our previous findings that the genes on the Y chromosome influenced testis weight in two genetic backgrounds. We also revealed that the effect of the A^y allele in reducing testis weight was modified by the genes on the Y chromosome. These data suggest that testis weight in mice is controlled by a cooperation of genes on autosomes and Y chromosome.

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