NOTE Laboratory Animal Science

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-consomic mouse strain) F1 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′ 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′ allele in mediating this phenomenon.

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

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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 F1 mice using the Y-consomic strains as sires to alter their genetic backgrounds. Therefore, we produced and analyzed F1 mice between B6.Cg-A′ females and Y-consomic strain males. Because A′ is an autosomal dominant allele and homozygous A′/A′ mice are lethal in utero [9], the living A′ mice are invariably heterozygotes [9]. Thus, we can simultaneously utilize two different F1 genetic backgrounds (i.e., F1 A′ and F1 non-A′ backgrounds). The A′ allele is known to significantly reduce testis weight [34], and as such, this mating scheme allows us to examine this further by including the A′ allele effect into the analyses as a potential autosomal effect.

The A′ 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′ mice, the A′ 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′ 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′ strain was maintained at the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Japan) along with the following Y-consomic strains: DH-Chr Y(DH/Sgn), DH-Chr YAKR(AKR/J), DH-Chr YBALB(BALB/cA), DH-Chr YCBA(CBA/N), DH-Chr YCF1(CF1/Sgn), DH-Chr YDBA(1DBA/2J), DH-Chr YYDD(DDD/Sgn), DH-Chr YDDH(D/H/Sgn), DH-Chr YKK(KK/Ta), DH-Chr YRF(RF/J), DH-Chr YRR(RR/Sgn), DH-Chr YSSL(SJL/J), DH-Chr YSS(S/Sgn) and DH-Chr YSWR(SWR/J).

Males of each Y-consomic strain were crossed with B6.Cg-A′ females to produce (♀B6.Cg-A′ × ♂DH-Chr Y@) F1 strains (@ is an arbitrary inbred strain name). Thus, F1 mice comprised F1 A′ and F1 non-A′ mice. Hereafter, we designated F1 A′ mice as F1-Y and F1 non-A′ mice as F1-A. Occasionally, the origin of the Y chromosome was specifically indicated for the F1 mice. For example, the ♀B6.Cg-A′ × ♂DH-Chr YB6 F1-Y strain was designated as F1-Y (YB6). F1-Y mice were visually distinct from F1-A mice, because the former had a yellow coat [9]. Three to five male mice of the same strain, irrespective of F1-A or F1-Y mice, were housed in a single cage. The number of mice used in this
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’ allele reduces testis weight in mice [34]. The degree of this reduction varied among our F1-Y strains. Therefore, the effect of the A’ allele in reducing testis weight in each individual F1-Y mouse was defined as the “Tw ratio.” For example, the “Tw ratio” in the F1-Y (YA) strain was calculated as follows:

\[
Tw \text{ ratio}=\frac{\text{Testis weight in each individual } F_1-Y \text{ (YA) mouse} - \text{Average testis weight in } F_1-Y \text{ (YA) mice}} {\text{Average testis weight in } F_1-Y \text{ (YDH) mice}}
\]

We identified the following thirty single nucleotide polymorphisms (SNPs) in the 16 Y-consomic strains (DH-Chr YDH was not genotyped) [36]: rs47359684, rs47900677, rs460080695, rs52139814, rs48580354, rs48064925, rs51995337, rs51133250, rs50647790, rs51277152, rs48685451, rs48834187, rs46947134, rs51756947, rs48554025, rs47574660, rs51766109, rs49468864, rs49623242, rs51230091, rs49614307, rs48926479, rs51029923, rs48512209, rs47293184, rs51685350, rs47616691, rs51560704, rs46632939 and rs5129727. 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 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 YCA, DH-Chr YDB, DH-Chr YEB, DH-Chr YCB, DH-Chr YCF, DH-Chr YDF, DH-Chr YKG, DH-Chr YRF and DH-Chr YSS strains were defined as possessing the Mus musculus musculus-type Y chromosome (YMus), whereas the DH-Chr YYAKR, DH-Chr YDDP, DH-Chr YDP, DH-Chr YSSL and DH-Chr YSSR strains were defined as possessing the M. m. domesticus-type Y chromosome (YDom). The strains, YMus vs. YDom, 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 (YMUS=5 and YDOM=6) [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 YDOM [8] but not YMus (the mouse Sry gene has four major sites comprising approximately 10 CAG repeats). The partitioning of the strains into either YMus or YDom was compatible with the descriptions set forth in previous studies [2, 28].

| Y chromosome donor strain (abbreviation) | Number of F1-A mice (mean ± SE body weight, g) | Number of F1-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/HaeIII (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)                             |
| CF1/Sgn (CF1)                            | 12 (38.56 ± 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 (46.72 ± 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 Dunnett’s multiple comparison test using DH as a reference, strain carrying YSS was significantly associated with reduced body weight in both F1-A and F1-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 strain DH 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 F1-A and F1-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 DDD strains to have significantly heavier testis than the YDH strain in F1-Y mice (Fig. 1). The mean testis weight ± SE in F1-A mice was 247.0 ± 1.4 mg (n=208), and that in F1-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,
heavier testis than those with the A 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@ F1 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 F1 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 YMus and YDom. In this case, YCAS was classified as YDom. In the case of rs48685451 in Kdm5d and others, YCAS was classified in YMus, and according to the literature [1], YCAS was classified as YMus.

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 heavier testis than mice with 9 or 12 repeats. In contrast, starting at 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 YDom. 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-

Fig. 1. Distribution of mean testis weight with SE in F1-A and F1-Y strains. Each strain symbol on the y-axis represents the kind of Y-chromosome donor strain; for example, “C3H” represents F1-A and F1-Y mice carrying the Y chromosome of C3H strain. Strain symbols were sorted in descending order according to the mean testis weight of F1-A strain. Statistical comparisons were made with Dunnett’s multiple comparison tests using YDom as a reference. F1-A and F1-Y strains were analyzed separately.
significantly 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 \textit{Sry} was significantly associated with testis weight in our previous study \cite{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 $A^r$ 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 \textit{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 \textit{Sry} modified the effect of the $A^r$ allele in reducing testis weight.

\textit{Sry} is expressed in the gonad and plays roles in testis determination. On the basis of the findings on B6.Y\text{Dom} sex reversal \cite{12}, Y\text{Dom} can be classified into several functional classes. That is, Y\text{Dom} associated with either of (1) complete

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

\text{a)} In addition to rs48685451 in \textit{Kdm5d}, many SNPs showed similar polymorphic pattern \cite{28}. \text{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 | Tw ratio (mg, mean ± SE)$^{c)}$ | $P$ value$^{c)}$ |
|------------------------|------|-------------------------------|----------------|
| rs48685451$^{a)}$       |      |                               |                |
| \textit{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             |      |                               |                |
| \textit{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             |      |                               |                |
| \textit{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 |      |                               |                |
| \textit{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 |      |                               |                |
| \textit{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 |      |                               |                |
| \textit{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) |

\text{a)} Combined data are expressed as standardized values. \text{b)} NS, not significant.
sex reversal, (2) partial (fetal) sex reversal/hermaphroditism 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 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). F1 mice were partitioned into five groups (Table 5). There was a significant difference in testis weight among the five groups in F1-A (P=4.57 × 10−11) and F1-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 YCAS, had the heaviest testis among five groups in both F1 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-Ay) F1Ay and (B6 × DDD.Cg-Ay) F1Ay males as well as their F1 non-Ay littermate males are fully fertile.

Table 4. Effects of gene polymorphisms on Tw ratio

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

a) NS, not significant.
Therefore, we expect that there are no substantial defects in the spermatogenic ability in DH-Chr Y®, F1-A and F1-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 Ay 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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