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Polymorphism in MRL and AKR mice *Sry*: a candidate gene for the appearance of testicular oocyte

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Abstract

Although mammals produce either sperm or eggs depending on their sex, newborn MRL/MpJ male mice contain oocytes within their testes. In our previous study, the testicular oocyte appears as early as day 0 afterbirth and has morphological characteristics as an oocyte such as zona pellucida and follicular epithelial cells. Based on the observation of F1 between MRL/MpJ and C57BL/6, one of the genes causing the appearance of testicular oocyte exists on the Y chromosome. In the present study, we found testicular oocytes within newborn AKR mice. We have also analyzed the *Sry* genes from several inbred mouse strains and identified a shortened glutamine repeat near the C-terminal region that is unique to MRL and AKR. These results suggest that polymorphism of glutamine repeat within *SRY* correlates with the appearance of testicular oocyte and this phenotype is derived from AKR, one of the original strains of MRL mice.

Key words: AKR, glutamine repeat, MRL/MpJ, *SRY*, testicular oocyte

Introduction

It has been generally believed that in mammals, males produce only sperm, and oocytes are only produced in females. The sex of a mammal is determined when an ovum containing a haploid genome with an X chromosome is fertilized by a sperm possessing a haploid genome with either an

X or Y chromosome. However, the anatomical determination of sex occurs later in development as gonads first arise as bipotential primordia with plasticity to develop into ovaries or testes. Determination of the bipotential primordia into male gonads requires expression of *Sry* (sex-determining region on Y), which initiates the differentiation of Sertoli cells, as well as their structural organiza-

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tion into a testis cord¹⁶⁾. If the primordial gonad proceeds to develop along the ovarian cascade despite being in an XY animal, this could result in sexual reversal of the animal, or the development of ovotestes. This has been observed in animals with translocations or deletions of the *Sry* gene, or animals with delayed *Sry* expression^{2,7,16)}. As another factor contributing to the plasticity of sexual determination, it is reported that the differentiation of germ cells into oogonia or prospermatogonia is directed by signals from somatic cells in the gonads, not by the germ cells themselves²¹⁾. In mice, *SRY* contains the HMG box at its N-terminus (5-73 amino acids) and a glutamine (CAG in nucleotide sequence) repeat region interspersed by a sequence FHDHHH^{a)} or similar sequences at the caudal half region (144-367 amino acids). The HMG box region has been known as DNA-binding domain suggesting that it is predominantly responsible for the role of *SRY* protein as a transcription factor^{12,26)}. On the other hand, the glutamine repeat region has been thought to mediate interaction with other proteins and to have an essential role in sex determination^{3,8,18,41)}.

Although some genetic abnormalities of sexual differentiation in mammals, including experimental chimeras, can cause the appearance of oocytes in the testes or the development of ovotestis, it has never been reported that fertile male animals can produce oocytes during spermatogenesis except newborn MRL/MpJ (MRL) mice²⁷⁾. The MRL inbred mouse strains, MRL/MpJ-+/+(M+) and MRL/MpJ-Fas^{lpr}/Fas^{lpr}(*lpr*), originate from a series of crosses with strains C57BL/6 (B6) (0.3%), C3H/He (C3H) (12.1%), AKR (12.6%) and LG/J (75%) and then followed by inbreeding initiated²⁴⁾. These MRL strains are known to develop several autoimmune diseases, such as systemic lupus erythematosus, polyarteritis nodosa, rheumatoid arthritis, and systemic sclerosis caused by the MRL genetic background, but not by the *lpr* allele³⁵⁾. The testicular oocyte in MRL mice appears

as early as day 0 afterbirth and has morphological characteristics as an oocyte such as zona pellucida and follicular epithelial cells²⁷⁾. Furthermore, based on the observation of MRL strains and F1 between MRL and B6, this phenotype is due to the MRL genetic background, and one of the genes causing the appearance of testicular oocyte exists on the Y chromosome.

In the present study, we re-examined the existence of testicular oocyte in inbred strains including B6, C3H, and AKR, which are contained in the MRL background and F1 generations between MRL and B6. Additionally, we focused on the *Sry* to reveal its relevance to the appearance of testicular oocytes by comparison of its nucleotide sequences among MRL and other inbred strains.

Materials and Methods

Mice: Several inbred mouse strains and F1, AKR/N (AKR), BALB/c (BALB), C57BL/6 (B6), C3H/He (C3H), DBA/2 (DBA), A/J (AJ), MRL/MpJ-+/+(M+), MRL/MpJ-*lpr*/*lpr* (*lpr*), B6MRLF1 (F1 between female B6 and male MRL), and MRLB6F1 (F1 between female MRL and male B6) were used in the present study. Eight-week-old male and female mice purchased from Japan SLC (Shizuoka, Japan) were maintained with free access to food and water in our facility. In the handling of experimental animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, Hokkaido University, Graduate School of Veterinary Medicine." In this study, 10 to 49 newly born mice from each strain were obtained by free-breeding, and were sacrificed at 14 days after birth by cervical dislocation.

Light microscopic analysis: To examine testicular oocytes, fresh whole-mount preparations were used, in which the testes of a 14-day-old of the inbred strains were removed, and immediately

^a Single-letter abbreviations were used for amino acid residues.

mounted on glass-slides with cover slips. They were then crushed to make whole-mounted single tubular sheets, and were observed under a differential interference microscope (BX50F4, Olympus, Tokyo, Japan). The number of oocytes per testis (number of oocyte/number of testis) was calculated as an oocyte score. After the observation and counting, oocyte-containing seminiferous tubules were removed and immediately fixed with Bouin's solution for 24 hr, cut into 5 μ m-thick serial paraffin sections, and stained with hematoxylin-eosin (HE).

Genomic DNA extraction and Sry PCR and nucleotide sequencing: To determine the relationship between *Sry* from the MRL Y chromosome and the appearance of testicular oocyte, we compared *Sry* nucleotide sequences of MRL and other inbred strains. Since *Sry* cDNA contains only one exon, genomic DNA was used for *Sry* nucleotide sequencing. Genomic DNA was prepared from the tail with length of approximately 5 mm for each of the inbred strains. These samples were incubated in lysis buffer containing 50 mM Tris-HCl, 100 mM NaCl, 20 mM EDTA, 1% sodium dodecyl sulfate, and 100 μ g/ml proteinase K, overnight at 56°C, and then treated with phenol extraction. Finally, genomic DNA was purified by ethanol precipitation and resolved in 500 μ l of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

PCR was carried out with ExTaq (Takara, Tokyo, Japan) under the following PCR conditions: 5

min at 95°C, 35 cycles of 40 sec at 95°C, 30 sec at 62°C and 1 min at 72°C followed by 5 min at 72°C. We used proper combinations of forward and reverse PCR primers listed in Table 1. After the amplified samples were electrophoresed with 2% agarose gel containing ethidium bromide, bands were cut and PCR products were purified with the GENECLAN II Kit (Funakoshi, Tokyo, Japan). Extracted PCR products were labeled with a cycle sequencing kit containing fluorescent terminators employing standard methods (Applied Biosystems, CA, USA) using the listed primer in Table 1, and finally analyzed with a model 310 automatic sequencer (Applied Biosystems).

Additionally, in order to compare *Sry* nucleotide sequence at the polymorphic CAG repeat, a two-step PCR method was created as the following protocol. The first PCR products were obtained and purified by procedures written above with primers mSry-834F and mSry1318R. Then extracted PCR products were diluted by TE to 1:20,000 and used as templates for the second PCR. We targeted on the sequence FHDHH which interspersed the glutamine repeat region and created a forward primer as 5'-TTCCATGACCACCACCAC-3'. For the reverse primer, we used mSry1255R as listed in Table 1. The second PCR was carried out with *Taq* DNA polymerase (Promega, WI, USA) under the following PCR conditions: 5 min at 95°C, 40 cycles of 20 sec at 95°C, 20 sec at 64°C and 30 sec at 72°C, followed by 5 min at 72°C. Finally, amplified samples were electrophoresed with 2%

Table 1. Primers used for *Sry* sequence

	Name	5' → 3'	Position
Forward	mSry-834F	TGCATGGTGATGCTGTAAGG	-834 ~ -815
	mSry-534F	TACTAATGATAGGTTCCATGC	-534 ~ -514
	mSry45F	TCTTAAACTCTGAAGAAGAGAC	45 ~ 66
	mSry153F	GTGAGAGGCACAAGTTGGC	153 ~ 171
	mSry370F	GCCTGCACTTGCTCAACAA	370 ~ 389
	mSry489F	GCTTTTATTGGCAGCCTGTTG	489 ~ 509
	mSry666F	AGCAGCAGTTTCATGACCAC	666 ~ 685
	mSry298R	GATTGAAGATCCTACACAGAG	298 ~ 277
Reverse	mSry1255R	ATACACCATAACCAGGAGCAC	1255 ~ 1235
	mSry1318R	TCTCCTACCTATGCCAACAC	1318 ~ 1299

NuSieve 3:1 agarose gel (Cambrex, ME, USA) containing ethidium bromide.

Results

Number of testicular oocytes in various mouse strains

The number of testicular oocytes per testis

was counted in whole-mount preparations. We found testicular oocytes in M+, *lpr*, AKR, and B6MRLF1, but not in other inbred strains including MRLB6F1 mice (Table 2 and Fig. 1). The oocyte scores of M+ and *lpr* were almost the same (M+: 1.23, *lpr*: 1.27), however, those of AKR and B6MRLF1 were less than the MRL strains (AKR: 0.15, B6MRLF1: 0.29). Morphologically, the testicular oocytes in B6MRLF1 and AKR contained

Table 2. Comparison of oocyte scores

Strain	No. of testis	No. of oocyte	Oocyte score	No. of testis with oocyte
M+	98	121	1.23	35
<i>lpr</i>	30	38	1.27	15
AKR	78	12	0.15	9
AJ	24	0	0	0
BALB	20	0	0	0
B6	50	0	0	0
C3H	46	0	0	0
DBA	20	0	0	0
B6MRLF1	68	20	0.29	13
MRLB6F1	68	0	0	0

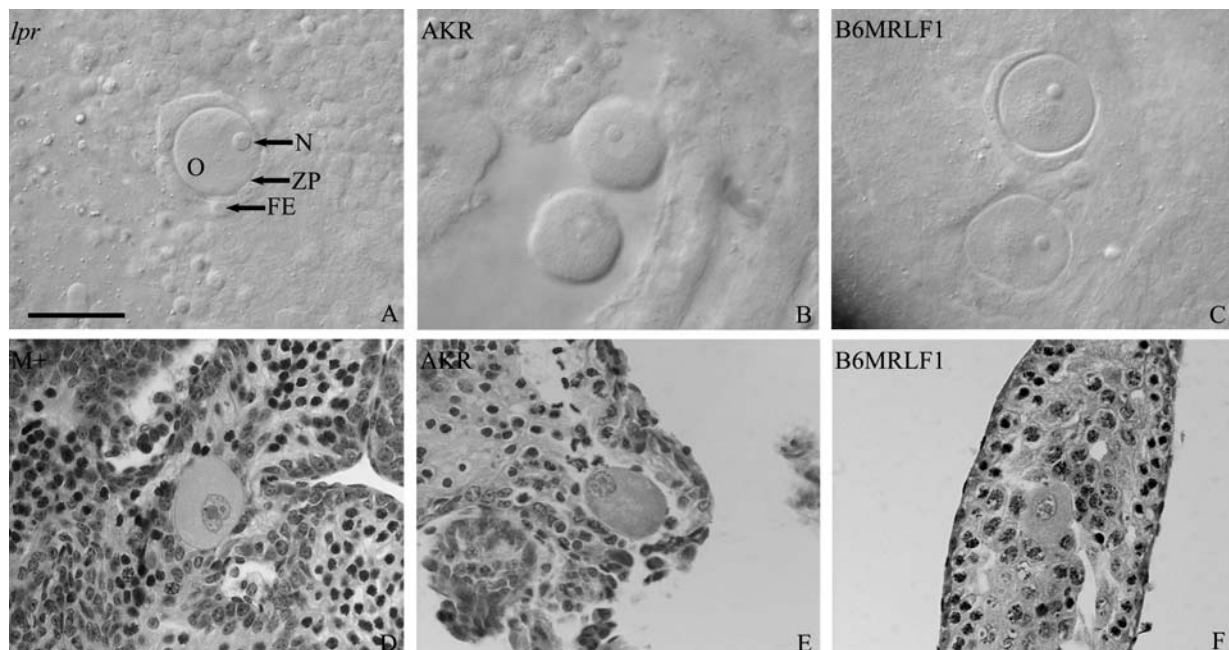


Fig. 1. Testicular oocytes *lpr*, M+, AKR, and B6MRLF1 aged 14 days.

Testicular oocytes in whole mount testis of *lpr* (A), AKR (B), and B6MRLF1 (C) and in HE staining of M+ (D), AKR (E), and B6MRLF1 (F). Scale bar = 50 μ m. All images are the same magnification. FE, follicular epithelial cell; N, nucleus; O, oocyte; ZP, zona pellucida.

B6	ATG GAG GGC CAT GTC AAG CGC CCC ATG AAT GCA TTT ATG GTG TGG TCC CGT GGT GAG AGG CAC AAG TTG GCC CAG CAG AAT CCC AGC ATG	90
MRL	...	
B6	M E G H V K R P M N A F M V W S R G E R H K L A Q Q N P S M	30
MRL	* *	
	CAA AAT ACA GAG ATC AGC AAG CAG CTG GGA TGC AGG TGG AAA AGC CTT ACA GAA GCC GAA AAA AGG CCC TTT TTC CAG GAG GCA CAG AGA	180
	Q N T E I S K Q L G C R W K S L T E A E K R P F F Q E A Q R	60
	* *	
	TTG AAG ATC CTA CAC AGA GAG AAA TAC CCA AAC TAT AAA TAT CAG CCT CAT CGG AGG GCT AAA GTG TCA CAG AGG AGT GGC ATT TTA CAG	270
	L K I L H R E K Y P N Y K Y Q P H R R A K V S Q R S G I L Q	90
	* *	
	CCT GCA GTT GCC TCA ACA AAA CTG TAC AAC CTT CTG CAG TGG GAC AGG AAC CCA CAT GCC ATC ACA TAC AGG CAA GAC TGG AGT AGA GCT	360
	P A V A S T K L Y N L L Q W D R N P H A I T Y R Q D Q S R A	120
	* *	
	GCA CAC CTG TAC TCC AAA AAC CAG CAA AGC TTT TAT TGG CAG CCT GTT GAT ATC CCC ACT GGG CAC CTG CAG CAG CAG CAG CAG CAG CAG	450
	A H L Y S K N Q Q S F Y W Q P V D I P T G H L Q Q Q Q Q Q Q Q	150
	* *	
	CAG CAG CAG CAG TTC CAT AAC CAC CAC CAG CAG CAA CAG CAG TTC TAT GAC CAC CAC CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG	540
	Q Q Q Q F H N H H Q Q Q Q Q F Y D H H Q Q Q Q Q Q Q Q Q Q	180
	* * - - *	
	CAG TTC CAT GAC CAC CAC CAG CAG AAG CAG CAG TTT CAT GAC CAC CAC CAG CAG CAA CAG CAG TTC CAT GAC CAC CAC CAC CAC CAC CAG	630
	Q F H D H H Q Q K Q Q F H D H H Q Q Q Q Q F H D H H H H H Q	210
	* *	
	GAG CAG CAG TTC CAT GAC CAC CAC CAG CAG CAA CAG CAG TTC CAT GAC CAC CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG	720
	E Q Q F H D H H Q Q Q Q Q F H D H Q Q Q Q Q Q Q Q Q Q F H	240
	* *	
	GAC CAC CAC CAG CAG AAG CAG CAG TTC CAT GAC CAC CAC CAC CAC CAA CAG CAG CAG CAG TTC CAT GAC CAC CAG CAG CAG CAG CAG CAG	810
	D H H Q Q K Q Q F H D H H H H Q Q Q Q Q F H D H Q Q Q Q Q Q	270
	* *	
	TTC CAT GAC CAC — CAG CAG CAG CAG CAG CAG TTC CAT GAC CAC CCC CAG CAG AAG CAG CAG TTC CAT GAC CAC CCC — CAG CAG CAA	900
	F H D H — Q Q Q Q Q Q F H D H P Q Q Q K Q Q F H D H P — Q Q Q	300
	* * * * * H *	
	CAG CAG TTC CAT GAC CAC CAC CAG CAG CAG CAG AAG CAG CAG TTC CAT GAC CAC CAC CAG CAG AAG CAG CAG TTC CAT GAC CAC CAC	990
	Q Q F H D H H H Q Q Q Q K Q Q F H D H H Q Q K Q Q F H D H H	330
	* * * * * Q *	
	CAG CAG AAG CAG CAG TTC CAT GAC CAC CAC CAG CAG CAA CAG CAG TTC CAT GAC CAC CAC CAG CAG CAG CAG CAG CAG CAG CAG CAG	1080
	Q Q K Q Q F H D H H Q Q Q Q Q F H D H H Q Q Q Q Q Q Q Q Q Q	360
	* *	
	CAG CAG CAG TTC CAC GAC CAG CAG CTT ACC TAC TTA CTA ACA GCT GAC ATC ACT GGT GAG CAT ACA CCA TAC CAG GAG CAC CTC AGC ACA	1170
	Q Q Q F H D Q Q L T Y L L T A D I T G E H T P Y Q E H L S T	390
	* *	
	GCC CTG TGG TTG GCA GTC TCA TGA	
	...	
	A L W L A V S /	
	* * * * * * *	

Fig. 2. Alignment of B6 and MRL *Sry* sequences.

First line and second lines show DNA sequences and third and fourth lines show amino acid sequences, respectively. Dot (.), identical DNA sequences; hyphen(-), deletion; asterisk (*), identical amino acids; slash (/), stop codon.

large nuclei with nucleoli, zona pellucida, and follicular epithelial cells that were similar to those of MRL. However, the follicular epithelial cells in AKR mice were less developed than those of MRL and B6MRLF1.

Comparison of Sry nucleotide sequences and corresponding amino acids between M+ and B6

We initially compared the whole nucleotide sequence of Sry between M+ and B6. Only open reading frames of Sry and corresponding amino acids sequences are shown in Fig. 2. The open reading frame of B6 Sry encodes a protein of 395 amino acids, while MRL Sry contains 389 amino acids, and both of the nucleotide sequences and amino acids homology of these two strains were approximately 97%. There was no substitution at the upstream of the open reading frame within genomic Sry. There were only three single-base changes, which caused amino acid alternations within upper half of Sry sequences between B6 and MRL, I63T in HMG box and W133T and L143P in the junction site. However, there were many substitutions within the glutamine repeat region such as deletion of glutamine at codon 153 and 154, H169Q, insertion of histidine at codon 275 and 297, P286Q, K289Q, P296H, and H307Q. Strikingly, Q351H and the following deletion of six CAG repeat causing shortened glutamine repeat was observed in MRL SRY. There was no difference between B6 and MRL at the downstream of the glutamine repeat region at the C-terminus.

Comparison of SRY glutamine repeats among representative inbred strains

Since polymorphism between MRL and B6 was concentrated in the glutamine repeat region, we compared the glutamine regions between testicular oocyte-producing strains (MRL and AKR) and other non-producing inbred strains (BALB, B6, C3H, DBA and AJ). Only residues amino acid 282-381 are shown in Fig. 3. As a result of the comparison, polymorphism common in MRL and AKR but not in other strains was only found in two regions. One was at amino acid 294-302 in which DHHHQ QQQQ was in MRL and AKR, while DHQ-QQQQQ, DHP-QQQQQ, and DHH-QQQQQ were in AJ, B6, and other strains, respectively. The other was the deletion of seven glutamine repeat at 351-357 in MRL and AKR.

In order to prove the shortened glutamine repeat, we compared the polymorphic glutamine repeat with a two-step PCR method using the forward primer corresponding to the region FHDHH H. From a deduced sequence (Fig. 3), SRY of the inbred strains except MRL and AKR contained three FHDHHH regions at 202-207, 279-284, and 333-338 amino acids which should result as PCR product of 552, 411, and 255 bp sizes respectively. On the other hand, four amplified products should be observed in MRL because of the substitution or insertion of histidines at codons 296, 297, and 307 resulting in the existence of FHDHHH regions at 202-207, 279-284, 292-297, and 376-381 with products of 537, 396, 270, and 108 bp. In AKR mice, since there was no substitution of histidine at codon 307, five PCR products should be obtained at

	282	292	302	312	322	332	342	352	362	372
AKR	FHDHPQQQQ	FHDHHHQQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	———	QQQQ QGFHDQQLTY LLTADITGEH
MRL	FHDHQQQQQ	FHDHHHQQQ	QFHDHGHQQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	———	QQQQ QGFHDQQLTY LLTADITGEH
AJ	FHDHHHQQQ	FHDH—QQQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QQQQQQQQQ	QGFHDQQLTY LLTADITGEH
B6	FHDHPQQKQ	FHDHP—QQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QQQQQQQQQ	QGFHDQQLTY LLTADITGEH
BALB	FHDHPQQQQ	FHDHH—QQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QQQQQQQQQ	QGFHDQQLTY LLTADITGEH
C3H	FHDHQQKQ	FHDHH—QQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QQQQQQQQQ	QGFHDQQLTY LLTADITGEH
DBA	FHDHQQKQ	FHDHH—QQQ	QFHDHHHQ	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QKQQFHDHH	QQQQQQQQQ	QGFHDQQLTY LLTADITGEH

Fig. 3. Comparison of glutamine repeat region (282-381) of SRY among inbred strains. Hyphens indicate deletions.

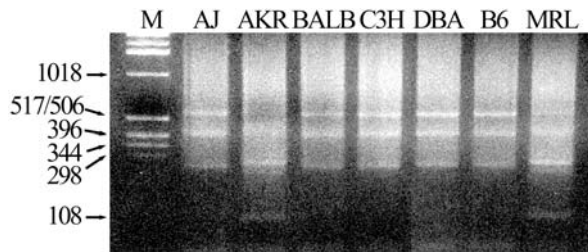


Fig. 4. Comparison of *Sry* electrophoresis patterns.

537, 396, 270, 237, and 108 bp. The results of PCR shown in Fig. 4 correspond to the expectation, especially the presence of the 108-bp band, which provides evidence of the shortened glutamine repeat in MRL and AKR.

Discussion

The existence of testicular oocytes has been reported in several cases such as chimeric mice, XXsxr sex-reversed mice, and the fetal testis transplanted under an adult female kidney capsule^{14, 20, 25, 28, 29}. Thus, to date, testicular oocyte in MRL mice was the only report of the appearance of testicular oocytes in XY fertile males. In this study, the testicular oocytes were also found in newborn AKR mice. As AKR is one of the MRL ancestor strains, it is suggested that the appearance of testicular oocyte was genetically derived in part from AKR. However, AKR including B6MRLF1 showed much lower appearance rates than MRL, indicating that multiple genes, probably on the autosomes, are also required for the development of this phenotype.

As a result of comparison, we found three amino acid alternations within the upper half of SRY sequences between B6 and MRL, I63T in HMG box and W133T and L143P in the junction site. These alternations were also observed in AKR (data not shown). Unlike the junction site between HMG box and glutamine repeat region, the importance of HMG box in SRY function is well known as the mutations in this DNA binding domain cause sex reversal in humans¹³. However,

since many of the *Mus musculus domesticus* contain threonine at positions 63 and 133 and proline at position 143 like MRL and AKR^{1,37}, these alternations do not seem to be important for the appearance of testicular oocyte. At the glutamine repeat region, there were two regions of polymorphism common in MRL and AKR, but not in other strains, codons 294-302 and deletion of seven glutamines at 351-357. The amino acid residues 294-302 contained DHHHQQQQQ in MRL and AKR, and DHQ-QQQQQ, DHP-QQQQQ, or DHH-QQQQQ in other strains. However, this substitution and insertion of histidine in MRL and AKR seemed less likely to have a relationship with the appearance of testicular oocyte as substitutions varied among strains. On the other hand, the deletion of seven glutamines in amino acid residues 351-357 was only found in MRL and AKR, and is suggested as one of the candidate factors for the appearance of testicular oocyte.

Polymorphism of the glutamine repeat has been known to cause many diseases in humans. Many of these diseases, such as Huntington disease, spinal bulbar muscular atrophy, several spinocerebellar ataxias, and dentatorubral-pallidoluysian atrophy are results of the extension of glutamine repeat^{32, 34, 36}. Conversely, there are only few reports about the effect of shortening glutamine, mostly about the relationship between androgen receptor gene and risk for cancer^{11, 40}. Several mechanisms causing these diseases have been identified such as protein aggregation, mitochondrial dysfunction, oxidative stress, transcriptional dysregulation, aberrant apoptosis, altered proteosomal function, and excitotoxicity^{15, 31, 32, 24}.

The importance of glutamine repeat has also been reported in mice (*Mus musculus*) SRY. When the Y chromosome of a *Mus musculus domesticus* mouse strain is placed onto the C57BL/6J genetic background, the XY (B6.Y^{DOM}) progeny show sex reversal which correlates with polymorphism of a glutamine repeat within SRY⁸. Additionally, SRY constructs without the glutamine repeat region could not induce testis formation in XX embryos³. Although the exact roles of this structure within

mouse SRY protein functions have not been identified yet, it is suggested that they contribute to the biological functions of mouse SRY through protein-protein interactive roles^{18,41}. Hence the glutamine repeat region of mouse SRY is thought to have an essential role in sex determination.

Sex differentiation of mouse primordial germ cell occurs at E13.5 when female germ cells enter meiosis while male germ cells are arrested in G0/G1 of mitosis. However, fetal germ cells are intrinsically programmed to enter meiosis and initiate oogenesis unless specifically prevented by a meiosis-inhibiting mechanism possessed in a fetal testis²². This meiosis-inhibiting mechanism can be mainly divided into two events, formation of testis cords and prevention of retinoic acid known as a meiosis-inducing factor. The formation of testis cords occurs at E12.0-12.5 as a result of interaction of many genes, which are thought as downstream targets of *Sry* such as *Nr0b1* (nuclear receptor subfamily 0, group B, member 1), *Dhh* (desert hedgehog), *Pd/gra* (platelet-derived growth factor receptor α), and *Ntf3* (neurotrophin 3)^{5,6,9,10,19,23,30,33,39}. Additionally, testicular somatic cells express *Cyp26b1* (cytochrome P450, family 26, subfamily b, polypeptide 1), an enzyme involved in retinoid metabolism, under the regulation of *Sry*^{4,17}. Thus, by being sequestered within testis cords, germ cells are prevented to enter meiosis in the XY gonad with normal *Sry* expression. In spite of the existence of the meiosis-inhibiting mechanism, it was noted that meiosis prematurely progresses in MRL fetal testes starting as early as E13.5²⁷. Taking all of the above into consideration, it is indicated that MRL fetal testes possess a unique meiotic progression mechanism, which might be the result of the shortened glutamine repeats in SRY and involved in the appearance of testicular oocytes. Additionally, the two-step PCR method that we used to compare CAG repeats can be an easy way to find mouse strains with the possibility to contain testicular oocytes. However, B6.Y^{DOM} with shortened SRY and mice with delayed or lower expression of *Sry* shows only sex reversal or ovotestis, but never testicular oocyte^{7,12}. This indi-

cated again the existence of other genes concerning the appearance of testicular oocytes as discussed above.

Since the glutamine repeat in SRY is unique in mice³⁸, it is suggested that the testicular oocyte can appear only in mice. Although it is necessary to identify the complicity genes, clarification of the relationship between SRY with shortened glutamine repeat and testicular oocyte can provide more clues concerning the development of the reproductive system in mice, such as the mechanisms of sex differentiation and the mechanism that prevents entry into meiosis in male embryos.

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References

- 1) Albrecht, K. H. and Eicher, E. M. 1997. DNA sequence analysis of *Sry* alleles (subgenus *Mus*) implicates misregulation as the cause of C57BL/6J-Y (POS) sex reversal and defines the SRY functional unit. *Genetics*, **147**: 1267-1277.
- 2) Berta, P., Hawkins, J. R., Sinclair, A. H., Taylor, A., Griffiths B. L., Goodfellow, P. N. and Fellous, M. 1990. Genetic evidence equating SRY and the testis-determining factor. *Nature*, **348**: 448-450.
- 3) Bowles, J., Cooper, L., Berkman, J. and Koopman, P. 1999. *Sry* requires a CAG repeat domain for male sex determination in *Mus musculus*. *Nat. Genet.*, **22**: 405-408.
- 4) Bowles, J., Knight, D., Smith, C., Wilhelm, D., Richman, J., Mamiya, S., Yashiro, K., Chawengsaksophak, K., Wilson, M. J., Rossant, J., Hamada, H. and Koopman, P. 2006. Retinoid signaling determines germ cell fate in

- mice. *Science*, **312**: 596-600.
- 5) Brennan, J., Tilmann, C. and Capel, B. 2003. Pdgfr- α mediates testis cord organization and fetal Leydig cell development in the XY gonad. *Genes Dev.*, **17**: 800-810.
 - 6) Brennan, J. and Capel, B. 2004. One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat. Rev. Genet.*, **5**: 509-521.
 - 7) Bullejos, M. and Koopman, P. 2005. Delayed *Sry* and *Sox9* expression in developing mouse gonads underlies B6-Y^{DOM} sex reversal. *Dev. Biol.*, **278**: 473-481.
 - 8) Coward, P., Nagai, K., Chen, D., Thomas, H. D., Nagamine, C. M. and Lau, Y. F. 1994. Polymorphism of a CAG trinucleotide repeat within *Sry* correlates with B6. Y^{Dom} sex reversal. *Nat. Genet.*, **6**: 245-250.
 - 9) Cupp, A. S., Tessarollo, L. and Skinner, M. K. 2002. Testis developmental phenotypes in neurotrophin receptor *trkA* and *trkC* null mutations: role in formation of seminiferous cords and germ cell survival. *Biol. Reprod.*, **66**: 1838-1845.
 - 10) Cupp, A. S., Uzumcu, M. and Skinner, M. K. 2003. Chemotactic role of neurotrophin 3 in the embryonic testis that facilitates male sex determination. *Biol. Reprod.*, **68**: 2033-2037.
 - 11) De Abreu, F. B., Pirolo, L. J., Canevari Rde, A., Rosa, F. E., Moraes Neto, F. A., Caldeira, J. R., Rainho, C. A. and Rogatto, S.R. 2007. Shorter CAG repeat in the *AR* gene is associated with atypical hyperplasia and breast carcinoma. *Anticancer Res.*, **27**: 1199-1205.
 - 12) Dubin, R. A. and Ostrer, H. 1994. *Sry* is a transcriptional activator. *Mol. Endocrinol.*, **8**: 1182-1192.
 - 13) Harley, V. R., Clarkson, M. J. and Argentaro, A. 2003. The molecular action and regulation of the testis-determining factors, *SRY* (sex-determining region on the Y chromosome) and *SOX9*[*SRY*-related high-mobility group (HMG) box 9]. *Endocr. Rev.*, **24**: 466-487.
 - 14) Isotani, A., Nakanishi, T., Kobayashi, S., Lee, J., Chuma, S., Nakatsuji, N., Ishino, F. and Okabe, M. 2005. Genomic imprinting of XX spermatogonia and XX oocytes recovered from XX $\leftrightarrow$ XY chimeric testes. *Proc. Natl. Acad. Sci. U.S.A.*, **102**: 4039-4044.
 - 15) Katsuno, M., Banno, H., Suzuki, K., Takeuchi, Y., Kawashima, M., Tanaka, F., Adachi, H. and Sobue, G. 2008. Molecular genetics and biomarkers of polyglutamine diseases. *Curr. Mol. Med.*, **8**: 221-234.
 - 16) Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. and Lovell-Badge, R. 1991. Male development of chromosomally female mice transgenic for *Sry*. *Nature*, **351**: 117-121.
 - 17) Koubova, J., Menke, D. B., Zhou, Q., Capel, B., Griswold, M. D. and Page, D.C. 2006. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc. Natl. Acad. Sci. U.S.A.*, **103**: 2474-2479.
 - 18) Lau, Y. F. and Zhang, J. 1998. *Sry* interactive proteins: implication for the mechanisms of sex determination. *Cytogenet. Cell Genet.*, **80**: 128-132.
 - 19) Levine, E., Cupp, A. S. and Skinner, M. K. 2000. Role of neurotrophins in rat embryonic testis morphogenesis (cord formation). *Biol. Reprod.*, **62**: 132-142.
 - 20) McLaren, A. 1980. Oocytes in the testis. *Nature*, **283**: 688-689.
 - 21) McLaren, A. 1995. Germ cells and germ cell sex. *Philos. Trans. R. Soc. Lond., B.*, **350**: 229-233.
 - 22) McLaren, A. and Southee, D. 1997. Entry of mouse embryonic germ cells into meiosis. *Dev. Biol.*, **187**: 107-113.
 - 23) Meeks, J. J., Crawford, S. E., Russell, T. A., Morohashi, K., Weiss, J. and Jameson, J. L. 2003. *Dax1* regulates testis cord organization during gonadal differentiation. *Development*, **130**: 1029-1036.
 - 24) Murphy, E. D. 1981. Lymphoproliferation (*lpr*) and other single-locus models for murine pus. In: *Immunologic defects in laboratory animals*, pp.143-173. Plenum Press, New York.
 - 25) Mystkowska, E. T. and Tarkowski, A. K. 1968. Observations on CBA/-p/CBA-T6T6 mouse chi-

- meras. *J. Embryol. Exp. Morphol.*, **20**: 33-52.
- 26) Nasrin, N., Buggs, C., Kong, X. F., Carnazza, J., Goebel, M. and Alexander-Bridges, M. 1991. DNA-binding properties of the product of the testis-determining gene and a related protein. *Nature*, **354**: 317-320.
 - 27) Otsuka, S., Konno, A., Hashimoto, Y., Sasaki, N., Endoh, D. and Kon, Y. 2008. Oocytes in Newborn MRL Mouse Testes. *Biol. Reprod.*, **79**: 9-16.
 - 28) Ozdzenski, W. 1972. Differentiation of the genital ridges of mouse embryos in the kidney of adult mice. *Arch. Anat. Microsc. Morphol. Exp.*, **61**: 267-278.
 - 29) Ozdzenski, W. and Presz, M. 1984. Precocious initiation of meiosis by male germ cells of the mouse. *Arch. Anat. Microsc. Morphol. Exp.*, **73**: 1-7.
 - 30) Pierucci-Alves, F., Clark, A. M. and Russell, L. D. 2001. A developmental study of the Desert hedgehog-null mouse testis. *Biol. Reprod.*, **65**: 1392-1402.
 - 31) Rubinsztein, D. C. and Carmichael, J. 2003. Huntington's disease: molecular basis of neurodegeneration. *Expert Rev. Mol. Med.*, **5**: 1-21.
 - 32) Shao, J. and Diamond, M. I. 2007. Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Hum. Mol. Genet.*, **16**: R115-123.
 - 33) Smith, C. A., McClive, P. J., Hudson, Q. and Sinclair, A. H. 2005. Male-specific cell migration into the developing gonad is a conserved process involving PDGF signaling. *Dev. Biol.*, **284**: 337-350.
 - 34) Stack, E. C. and Ferrante, R. J. 2007. Huntington's disease: progress and potential in the field. *Expert Opin. Investig. Drugs*, **16**: 1933-1953.
 - 35) Theofilopoulos, A. N. and Dixon, F. J. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.*, **37**: 269-390.
 - 36) Walker, F. O. 2007. Huntington's disease. *Lancet*, **369**: 218-228.
 - 37) Washburn, L. L., Albrecht, K. H. and Eicher, E. M. 2001. C57BL/6J-T-associated sex reversal in mice is caused by reduced expression of a *Mus domesticus* Sry allele. *Genetics*, **158**: 1675-1681.
 - 38) Whitfield, L. S., Lovell-Badge, R., and Goodfellow, P. N. 1993. Rapid sequence evolution of the mammalian sex-determining gene SRY. *Nature*, **364**: 713-715.
 - 39) Yao, H. H. and Capel, B. 2002. Disruption of testis cords by cyclopamine or forskolin reveals independent cellular pathways in testis organogenesis. *Dev. Biol.*, **246**: 356-365.
 - 40) Yu, H., Bharaj, B., Vassilikos, E. J., Gai, M. and Diamandis, E. P. 2000. Shorter CAG repeat length in the androgen receptor gene is associated with more aggressive forms of breast cancer. *Breast Cancer Res. Treat.*, **59**: 153-161.
 - 41) Zhang, J., Coward, P., Xian, M. and Lau, Y. F. 1999. In vitro binding and expression studies demonstrate a role for the mouse Sry Q-rich domain in sex determination. *Int. J. Dev. Biol.*, **43**: 219-227.