Transcriptional Regulation of the Y-Linked Mammalian Testis-Determining Gene *SRY*

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Disorders of sexual development · Epigenetic modifier · Gene expression · Gonadal differentiation · *SRY* · Testis development · Transcription factor

Abstract

Mammalian male sex differentiation is triggered during embryogenesis by the activation of the Y-linked testis-determining gene *SRY*. Since insufficient or delayed expression of *SRY* results in XY gonadal sex reversal, accurate regulation of *SRY* is critical for male development in XY animals. In humans, dysregulation of *SRY* may cause disorders of sex development. Mouse *Sry* is the most intensively studied mammalian model of sex determination. *Sry* expression is controlled in a spatially and temporally stringent manner. Several transcription factors play a key role in sex determination as trans-acting factors for *Sry* expression. In addition, recent studies have shown that several epigenetic modifications of *Sry* are involved in sex determination as cis-acting factors for *Sry* expression. Herein, we review the current understanding of transcription factor- and epigenetic modifier-mediated regulation of *SRY/Sry* expression.

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Introduction

Sex differentiation plays an indispensable role not only in the inheritance of genomic information but also in the generation of genomic diversity in sexually reproducing organisms. The first step of the sex differentiation process is sex determination, which fixes the fate of sexually undifferentiated gonads to develop into either testes or ovaries. Mammalian sex is determined genetically; the presence of the sex-determining gene, *SRY*, leads to testis development, whereas its absence leads to ovary development. *SRY/Sry* was first discovered in humans and mice in 1990 [Gubbay et al., 1990; Sinclair et al., 1990]. XX mice carrying a transgene of a genomic fragment containing only the *Sry* gene developed testes, revealing that *Sry* alone is sufficient and that other Y chromosomal genes are not required for male sex determination [Koopman et al., 1991].

SRY is a member of the SOX (*SRY*-like box) gene family. *SRY* protein can bind DNA with its high-mobility group (HMG) box and acts as a transcription factor. *SRY* directly binds to the enhancer of *Sox9* and activates its transcription [Sekido and Lovell-Badge, 2008; Gonen et

Fig. 1. Transcription factor-mediated regulation of *Sry* expression. The mouse *Sry* locus encodes 2 SRY isoforms: single-exon-type SRY (SRY-S), which carries a C-terminal degron, and 2-exon-type SRY (SRY-T), which is degron-free. Nuclear receptor transcription factor NR5A1, zinc finger transcription factor GATA4/FOG2 complex, and zinc finger transcription factor WT1 play fundamental roles in *Sry* activation. Several studies have indicated that these 3 transcription factors directly bind to the *Sry* promoter. CBX2 contributes to testis development in 2 ways: it upregulates *Nr5a1* and *Gata4* expression and suppresses ovary-promoting genes. SIX1 and SIX4 positively regulate *Nr5a1* and *Gata4* expression. WT1 acts cooperatively with a transcription factor, CITED2. GADD45G contributes to *Sry* expression by promoting GATA4 phosphorylation.

al., 2017, 2018]. It has been shown that the human SRY protein binds to the (A/T)ACAA(T/A) sequence in the minor groove of DNA through its HMG box and induces DNA bending at 60–80°. As most human XY females carry mutations in the HMG box, the DNA-binding activity of SRY seems to be essential for sex determination [Harley and Goodfellow, 1994].

SOX3 is a member of the SOX gene family and encodes a protein that is most similar to SRY [Bowles et al., 2000]. SOX3 is a single-exon gene and is considered the ancestor of SRY [Stevanovic et al., 1993; Sutton et al., 2011]. Accordingly, all previously characterized eutherian SRY genes have been single exons. However, in mice, the second exon of *Sry* was discovered in 2020 [Miyawaki et al., 2020]. The 2-exon-type *Sry* transcript encodes a previously unidentified SRY protein (SRY-T). Mice lacking SRY-T but still expressing canonical single-exon-type SRY (SRY-S) exhibit male-to-female sex reversal. This result indicates that SRY-T is essential for male sex determination whereas SRY-S alone is insufficient for male sex determination. SRY-S and SRY-T have similar HMG boxes and poly-glutamine sequences but different C-terminal sequences, owing to the presence and absence of splicing, respectively. The C-terminal sequence of SRY-S

was found to be a protein degradation motif, whereas that of SRY-T was degron-free (Fig. 1). The second exon of *Sry* seems to have evolved recently in mice, resulting in the emergence of a sex-determining factor resistant to protein degradation.

The mouse is a model animal in which mammalian sex determination has been most extensively studied. In mice, *Sry* expression is strictly controlled in a cell type- and temporal-specific manner. *Sry* is expressed exclusively in the precursors of Sertoli cells (pre-Sertoli cells). Other types of gonadal somatic cells and germ cells do not express *Sry*. *Sry* expression starts at embryonic day 10.5 (E10.5) [Koopman et al., 1990; Hacker et al., 1995; Jeske et al., 1996]. *Sry* is detected only in the central region of the genital ridges in the early stages of expression [Bullesjos and Koopman, 2001; Wilhelm et al., 2005]. *Sry* expression extends to the whole region of the genital ridges at about E11.5 and is extinguished by E12.5. When the Y chromosome of *Mus poschiavinus* (Ypos) was introduced into the C57BL/6J (B6) autosomal background, the corresponding XY mice developed ovotestes, gonads containing both ovarian and testicular tissue [Eicher et al., 1982]. The expression peak of *Sry* in XY B6 (Ypos) gonads is delayed by up to 10 h compared with that of control XY

B6 gonads [Bullejos and Koopman, 2005], indicating the importance of the time window of *Sry* expression in inducing Sertoli cell differentiation. Interestingly, a recent whole-genome sequence analysis of a certain colony of XY B6 (Ypos) has revealed that a variant region on chromosome 13, that is not derived from B6, protects against XY gonadal sex reversal [Livermore et al., 2020]. A transgenic mouse study further confirmed that a narrow time window of *Sry* expression is critical for inducing Sertoli cell differentiation. Hiramatsu et al. [2009] have established mouse lines carrying *Sry* transgenes whose expression is driven by the promoter of *heat shock protein 70.3* (*Hsp70.3-Sry*). In this system, *Sry* expression was induced experimentally at various time points, revealing that only a 6 h delay in *Sry* induction results in a failure to initiate testis development [Hiramatsu et al., 2009]. In contrast to mouse *Sry*, *SRY* in other mammals such as human [Clepet et al., 1993], bovine [Daneau et al., 1995], goat [Montazer-Torbati et al., 2010], rats [Turner et al., 2007], and marsupials [Harry et al., 1995] are expressed more broadly.

It is likely that timely and spatially accurate induction of *SRY* is critical for mammalian male development other than that of mice. Differences of disorders of sex development (DSDs) in humans are accompanied by atypical conditions of external or internal sexual organs [Bashamboo and McElreavey, 2016; Audi et al., 2018]. Genetic screening of human patients exhibiting XY gonadal dysgenesis has led to the identification of several types of transcription factors that potentially regulate *SRY* expression. Indeed, genetic and biochemical approaches using knockout/transgenic mice have demonstrated that multiple transcription factors are involved in *Sry* regulation. Current evidence shows that *Sry* expression is also controlled in an epigenetic manner, at least in mice. Herein, we discuss transcription factor- and epigenetic modifier-mediated regulation of *Sry* expression, which were mainly obtained from mouse genetic studies.

Transcription Factor-Mediated Regulation of *Sry* Expression

Transcription factors are proteins that bind to specific DNA sequences and, in turn, upregulate or downregulate the expression of the adjacent genes. Transcription factors are grouped into classes based on the structure of their DNA-binding domains. As *Sry* expression is prolonged in the XY gonads lacking SOX9 [Chaboissier et al., 2004; Barrionuevo et al., 2006], SOX9 may play a role in the downregulation of *Sry*. Herein, we describe the fun-

damental roles of a nuclear receptor family transcription factor and 2 zinc finger transcription factors in the upregulation of *Sry* (Fig. 1).

Nuclear Receptor Transcription Factor, NR5A1

NR5A1, previously known as SF-1 or AD4BP, contains several domains typically conserved in other nuclear receptor family proteins, such as the N-terminal DNA-binding domain and C-terminal ligand-binding domain. NR5A1 is considered an orphan nuclear receptor protein. Historically, NR5A1 was identified by screening for proteins with the capacity to activate transcription from P450 steroidogenic enzyme promoters [Lala et al., 1992; Morohashi et al., 1992]. NR5A1 has multiple functions in sex development, such as steroid synthesis [Morohashi et al., 1992], gonadal development [Luo et al., 1994], and activation of *Sox9* [Sekido and Lovell-Badge, 2008]. In humans, mutations in *NR5A1* have been found to cause XY sex reversal [Achermann et al., 1999]. NR5A1 binding sites have been identified in *SRY* promoters in pigs [Pilon et al., 2003]. Furthermore, reporter expression analysis has demonstrated that these NR5A1-binding sites in the pig *SRY* locus are necessary for *SRY* activation. NR5A1-binding sites in the mouse *Sry* locus have not been identified.

The expression of *Nr5a1* in gonadal somatic cells is regulated by multiple molecular pathways. Mutation of CBX2 (also called M33), a component of polycomb-group repressive complex 1 (PRC1), causes XY sex reversal in humans and mice [Katoh-Fukui et al., 1998; Biason-Lauber et al., 2009]. Embryonic gonads lacking CBX2 exhibit reduced expression of *Nr5a1* [Katoh-Fukui et al., 2012]. This result indicates that CBX2 positively regulates *Nr5a1* expression. An alternative function of CBX2 in sex development is the epigenetic suppression of ovary-determining genes, thereby blocking testis development [Garcia-Moreno et al., 2019]. PRC1-mediated epigenetic regulation of sex development is discussed below. Mice lacking the homeobox transcription factors SIX1 and SIX4 exhibit perturbed *Sry* expression and male-to-female sex reversal [Fujimoto et al., 2013]. SIX1 and SIX4 are involved in the upregulation of *Nr5a1* expression in developing gonads.

Zinc Finger Transcription Factors GATA4/FOG2 and WT1

GATA4/FOG2

GATA4 is a transcription factor with 2 zinc finger domains. Mouse genetic studies have shown that conditional depletion of *Gata4* at E10.5, but not at later stages, induces XY sex reversal [Manuylov et al., 2011]. In humans,

Fig. 2. Epigenetic modifier-mediated regulation of *Sry* expression. H3K9me2 is a representative epigenetic mark of transcriptionally suppressed chromatin. The G9a/GLP complex is responsible for depositing H3K9me2 at the *Sry* promoter. CpG sequences at the *Sry* promoter are also methylated when *Sry* is silent. JMJD1A upregulates *Sry* expression by removing H3K9me2 at the *Sry* promoter against G9a/GLP complex-mediated H3K9 methylation. TET2 actively demethylates CpG sequences at the *Sry* promoter by hydroxylating methylated cytosine. JMJD1A and TET2 utilize Fe^{2+} and $\alpha\text{-KG}$ for the demethylation reaction. Histone acetyltransferases CBP and p300 synergistically upregulate *Sry* expression by acetylating H3K27 at the *Sry* promoter. CBP and p300 utilize acetyl-CoA as a donor of the acetyl group.

a heterozygous missense mutation in the first zinc finger domain was found in patients with XY DSD [Lourenco et al., 2011]. Chromatin immunoprecipitation analysis has confirmed that GATA4 directly binds to the proximal promoter of *Sry*, which contains the consensus binding sites of GATA4 in mouse gonads [Gierl et al., 2012]. FOG2, also known as ZFPM2, is a zinc finger protein that forms a heterodimer with GATA family proteins. Interaction between GATA4 and FOG2 is required for *Sry* expression in mice [Tevesian et al., 2002]. *Gata4* expression in developing gonads is positively regulated by CBX2 [Katoh-Fukui et al., 2012]. Furthermore, SIX1 and SIX4 contribute to *Fog2* expression during the sex-determination process [Fujimoto et al., 2013].

The MAPK signaling pathway is involved in *Sry* activation. A forward genetic screen in mice indicated that a mutation in the gene encoding MAP3K4 (also known as MEKK4) induces a reduction in *Sry* expression and male-to-female reversal in mice [Bogani et al., 2009]. In humans, mutations of MAP3K4-related protein MAP3K1 lead to XY gonadal dysgenesis [Pearlman et al., 2010]. A stress-response protein, GADD45G, binds to and activates MAP3K4, thereby promoting the phosphorylation of p38 MAPK [Takekawa and Saito, 1998; Lu

et al., 2001]. Mice deficient in GADD45G also display male-to-female sex reversal [Gierl et al., 2012; Warr et al., 2012]. The loss of GADD45G, as well as the loss of MAP3K4, results in reduced phosphorylation levels of GATA4 [Warr et al., 2012]. These results suggest that MAPK signaling upregulates *Sry* expression through the phosphorylation of GATA4. The combined loss of insulin receptor family proteins induces male-to-female sex reversal with reduced expression of *Sry* [Nef et al., 2003]. As the expression levels of *Gadd45g* and *p38 β* are perturbed in gonads lacking insulin receptor and insulin-like growth factor 1, a cross-talk between insulin signaling and MAPK signaling in *Sry* regulation might exist [Pitetti et al., 2013].

WT1

WT1 is a transcription factor that carries 4 zinc finger domains. Frasier syndrome patients with male pseudohermaphroditism exhibit reduced expression of WT1(+KTS), an isoform of WT1 [Barboux et al., 1997]. Loss of WT1(+KTS) in mice also results in the failure of testis development accompanied by reduced expression of *Sry* [Hammes et al., 2001]. WT1 can bind to the promoter region of *SRY* and upregulate its expression in hu-

mans [Hossain and Saunders, 2001]. Reporter expression analysis using HeLa cells has shown that WT1 and GATA4 synergistically enhance the transcription of mouse, pig, and human *Sry*/*SRY* promoter constructs [Miyamoto et al., 2008]. It has been proposed that WT1 cooperates with the transcription cofactor CITED2 in sex determination [Buaas et al., 2009]. There are 2 regions in the proximal 2 kb of the mouse *Sry* 5' region (−132 to +14 and −1992 to −1897 from TSS of mouse *Sry*) that show moderate homology with human *SRY* 5' region [Hacker et al., 1995]. A DNA-binding assay has demonstrated that the WT1-binding site is located within the former region [Miyamoto et al., 2008].

Epigenetic Modifier-Mediated Regulation of *Sry* Expression

Epigenetics is defined as the study of gene regulation with no change in the DNA sequence. Accurate gene regulation generally depends not only on the function of transcription factors but also on chromatin structure. DNA and histones undergo covalent modifications, thereby changing the chromatin structure. Players in epigenetic modifications are categorized as writers (enzymes depositing modifications), readers (proteins that bind and decode modifications), and erasers (enzymes removing modifications) [Biswas and Rao, 2018]. The levels of epigenetic modification can fluctuate because of the opposing activities of writers and erasers. This mechanism allows the tuning of gene activity in response to environmental stimuli. Accordingly, numerous studies have demonstrated that the levels of epigenetic modifications change dynamically during mammalian development. These dynamic changes contribute to cell fate decisions and cellular differentiation. Recently, a compelling body of evidence regarding the indispensable role of epigenetic regulation in sex determination/differentiation has been accumulated. In this section, we provide an overview of the current knowledge about the role of epigenetic regulation in *Sry* expression (Fig. 2).

H3K9 Demethylation

Histone 3 on lysine 9 (H3K9) methylation is generally enriched in transcriptionally inactive “closed” chromatin, which is called heterochromatin. JmjC domain-containing histone demethylase JMJD1A (also called KDM3A/JHDM2A/TSGA) removes the methyl group from di-methylated H3K9 (H3K9me2) [Yamane et al., 2006]. Mice lacking JMJD1A exhibit male-to-female sex reversal

[Kuroki et al., 2013]. JMJD1A protein is predominantly detected in gonadal somatic cells but not in mesonephric cells in embryos at E11.5. A recent single-cell RNA sequencing analysis demonstrated that *Jmjd1a* and *Sry* exhibit similar expression patterns during gonadal differentiation; their mRNAs are expressed specifically in pre-Sertoli cells [Stevant et al., 2018]. JMJD1A deficiency did not change the number of NR5A1-expressing cells per gonad but reduced the number of SRY-expressing cells per gonad to approximately 25% of that in the control. The subpopulation of JMJD1A-deficient mice showed a partial sex reversal phenotype with a testis and an ovary. These results indicate that the number of SRY-expressing cells in JMJD1A-deficient gonads at E11.5 may be near the threshold of the cell number required for inducing testis development. ChIP analysis demonstrated that JMJD1A accumulates at the *Sry* locus in pre-Sertoli cells. Furthermore, loss of JMJD1A results in an increase in the H3K9me2 modification at the *Sry* locus. These results indicate that JMJD1A catalyzes H3K9 demethylation at the *Sry* locus [Kuroki et al., 2013].

There are multiple H3K9-methylating enzymes in mammalian cells [Hyun et al., 2017]. There should be an H3K9-methylating enzyme that acts in opposition to JMJD1A-mediated H3K9 demethylation at the *Sry* locus. G9a (also called EHMT2) and its related molecule GLP (also known as EHMT1) exhibit an intrinsic methyltransferase activity toward H3K9 [Tachibana et al., 2002, 2005]. GLP and G9a exist as heterodimers in vivo; this heterodimer formation is essential for the H3K9-methylating activity [Tachibana et al., 2008]. Heterozygous mutation of *GLP* reduced H3K9 hypermethylation at the *Sry* locus in JMJD1A-deficient gonads, indicating that the GLP/G9a complex is responsible for hypermethylation of H3K9me2 in JMJD1A-deficient gonads [Kuroki et al., 2017]. Furthermore, perturbed *Sry* expression in JMJD1A-deficient embryos and the sex-reversal phenotype in JMJD1A-deficient mice were rescued by *GLP* heterozygous mutation. These results show that GLP/G9a suppresses *Sry* by methylating H3K9 at the *Sry* locus.

Histone Acetylation

Histone acetylation is enriched in transcriptionally active “open” chromatin. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play critical roles in controlling histone acetylation levels. The cAMP-response-element-binding protein (CREB)-binding protein p300 (EP300/KAT3B) and CBP (CREBBP/KAT3A) are 2 members of the KAT3 subfamily of HATs [Dancy and Cole, 2015]. There is a pos-

itive correlation between H3K9/K27 acetylation and *Sry* expression in developing gonads [Gierl et al., 2012; Carre et al., 2018]. XY mice deficient in CBP or p300 show a partial male-to-female sex reversal phenotype, whereas those deficient in both enzymes show a complete sex reversal phenotype [Carre et al., 2018]. mRNA expression analysis revealed an approximately 6-fold reduction in *Sry* expression in gonads lacking CBP/p300. ChIP analysis demonstrated that the level of H3K27ac was reduced at the *Sry* promoter in E11.5 gonads lacking CBP/p300, suggesting that H3K27ac might play a pivotal role in *Sry* activation. In contrast, the levels of pan-H3ac and H3K9ac were unaffected in E11.5 gonads lacking CBP/p300, indicating that other unidentified HATs are responsible for histone H3 acetylation other than H3K27ac at the *Sry* locus.

The K136 residue of the human SRY protein can be acetylated by p300 [Thevenet et al., 2004]. Acetylation of SRY enhances its nuclear localization by increasing its interaction with importin-beta. On the other hand, deacetylation of K136 by HDAC3 stimulates cytoplasmic localization of SRY. Exome sequencing analysis revealed mutations in human *CBP* and *p300* in DSD patients with 46,XY gonadal dysgenesis [Bagheri-Fam et al., 2015; Eggers et al., 2016], suggesting that the role of CBP/p300 in male sex development might be conserved in mice and humans.

Polycomb Group-Mediated Gene Silencing

Polycomb group proteins are a family of protein complexes, first discovered in fruit flies, that silence developmental genes, including *Hox* cluster genes. Polycomb group proteins are classified into 1 of 2 multiprotein complexes, PRC1 and PRC2 [Aloia et al., 2013]. PRC1 and PRC2 catalyze the ubiquitination of H2AK119 and methylation of H3K27, respectively. CBX2 is a core component of PRC1. CBX2 is thought to be responsible for targeting PRC1 to chromatin through specific binding to H3K9me3 and H3K27me3 [Kaustov et al., 2011]. Transcriptomic and immunohistochemical analyses demonstrated that *Sry* and *Sox9* expression is downregulated in CBX2-deficient gonads. Furthermore, the expression levels of genes essential for gonadal development, such as *Lhx9*, *Nr5a1*, *Dax1/Nr0b1*, *Gata4*, *Arx*, and *Dmrt1*, were also perturbed in *Cbx2*-deficient gonads [Katoh-Fukui et al., 2012]. These results suggest that CBX2 is an activator of testis-promoting genes.

Among a cohort of human DSD patients, a 46,XY girl with normal female genitalia, uterus, and ovarian-like tissue carried double heterozygous alleles for *CBX2*

[Biaison-Lauber et al., 2009]. Each of the alleles corresponded to the missense mutations CBX2:P98L and R443P. In vitro transfection experiments demonstrated that these mutations influence the ability of CBX2 to bind to the NR5A1 promoter region and activate *NR5A1* expression. Accordingly, mouse embryonic gonads lacking CBX2 failed to activate *Nr5a1* [Katoh-Fukui et al., 2012].

Another possible function of CBX2 in sex determination has recently been proposed. Testis- and ovary-promoting genes are initially co-expressed in XX and XY gonads prior to sex determination and are bivalently marked by H3K4me3 and H3K27me3 [Garcia-Moreno et al., 2019]. XY sex-reversal caused by *Cbx2* mutation was repaired by an additional mutation of *Wnt4*, an ovary-promoting gene. Furthermore, *Sry* expression was restored in XY *Cbx2/Wnt4* double-mutant mice. These findings suggest that CBX2 stabilizes testis fate by suppressing ovary-promoting genes, which accords well with the general function of PRC1 as the negative regulator of transcription.

DNA Demethylation

DNA methylation occurs predominantly on cytosine residues, followed by guanine residues (CpG). DNA methylation is catalyzed by the de novo DNA methyltransferases DNMT3A/DNMT3B and is maintained by DNA methyltransferase DNMT1 during DNA replication.

It has been reported that the CpG sequences of the *Sry* promoter are demethylated in gonadal somatic cells during the sex-determination period. These observations show that DNA demethylation in the *Sry* promoter precedes the onset of *Sry* expression and that DNA demethylation is more prominent in the *Sry* promoter region than in other regions of the *Sry* locus [Nishino et al., 2004; Gierl et al., 2012]. In vitro promoter activity analysis demonstrated that methylation of the 5'-flanking region suppresses the activity of a *Sry* reporter plasmid [Nishino et al., 2004]. The non-CpG (CCTGG) sequence in the *Sry* locus is also demethylated during the sex-determination period [Nishino et al., 2011].

CpG methylation marks can be removed by replication-dependent and -independent mechanisms. The former is regulated by inhibition of DNA methyltransferase activity during de novo DNA synthesis, whereas the latter (also known as active demethylation) is induced by the oxidation of 5-methylcytosine (5mC) by 10–11 translocation proteins (TET1/TET2/TET3), which produce 5-hydroxymethylcytosine (5hmC). 5hmC is further oxidized

to 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) by TET enzymes, both of which can be repaired by the base excision repair pathway to produce unmodified cytosine [Wu and Zhang, 2010]. 5hmC is highly enriched in male gonadal somatic cells during the sex-determination period [Okashita et al., 2019]. The 5hmC level increases in the *Sry* promoter concomitantly with *Sry* expression in gonadal somatic cells. Deficiency in TET2, but not TET1 and TET3, induces an increase in DNA methylation and the disappearance of 5hmC in the *Sry* promoter. Furthermore, TET2 deficiency enhances the sex reversal phenotype of *Jmjd1a*-deficient mice [Okashita et al., 2019]. These results reveal that TET2-mediated active DNA demethylation is involved in the regulation of *Sry* expression and male sex determination. Cloned canines generated by somatic cell nuclear transfer (SCNT) occasionally show male-to-female sex reversal. Reduced *Sry* expression and hypermethylation of the *Sry* locus have been observed in SCNT-derived XY gonads showing the DSD phenotype [Jeong et al., 2016]. These data suggest that incomplete demethylation of *Sry* can cause XY DSD in dogs.

In a case of human XY gonadal dysgenesis, the chromosome Y inversion juxtaposed *SRY*-containing euchromatin to the Yq12 heterochromatin, resulting in the silencing of *SRY* [Gimelli et al., 2006]. The CpG sequence located 3 kb 5' of *SRY* in this patient was methylated twice as much as that of her father, who showed mosaicism of a normal Y chromosome and several different rearrangements. These results suggest that the level of DNA methylation may be important for *SRY* expression in humans.

Perspective

As described, recent mouse genetic studies have revealed the fundamental role of epigenetic regulation in *Sry* expression. In addition, evidence that the cellular metabolic state can alter the cellular epigenetic state has been accumulated [Reid et al., 2017]. In this scenario, alteration of metabolism can change gene expression epigenetically by modulating the substrates or cofactors of chromatin-modifying enzymes. In this section, we discuss the possible involvement of metabolic pathways in epigenetic gene regulation by referring to previous studies.

Intermediate metabolites utilized in epigenetic gene regulation are summarized in Figure 2. DNA and histone methyltransferases utilize S-adenosyl methionine

(SAM) as a methyl donor. The reaction of histone demethylation by JmjC domain-containing enzymes and DNA demethylation by TET family enzyme DNA hydroxylase are both dependent on Fe^{2+} and α -ketoglutarate (α -KG). Acetyl coenzyme A (acetyl-CoA) is a universal donor for acetylation reactions in eukaryotes. It was presented that a variety of metabolites and the corresponding metabolic enzymes can modulate epigenetic modification levels and influence gene expression [Wolff et al., 1998; Wellen et al., 2009; Sutendra et al., 2014; Carey et al., 2015; Mews et al., 2017; Wang et al., 2020]. However, the relationship between metabolism and sex determination/differentiation remains elusive. Interestingly, 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (HMGCS2), encoding a metabolic enzyme for energy production from fatty acids, shows a very similar pattern as *Sry* in developing gonads [Bagheri-Fam et al., 2020]. Further, *Sry* activation initiates glycogenesis in pre-Sertoli cells, suggesting the importance of glucose metabolism for *SRY*/SOX9-downstream events in gonadal sex determination [Matoba et al., 2005, 2008]. More than half of human DSDs cannot be explained by alterations in the genes that are known to be required for sex determination or gonadal development [Munger and Capel, 2012]. It is possible that both genetic and epigenetic mutations during embryogenesis are responsible for the onset of DSDs.

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Conflict of Interest Statement

The authors declare no competing financial interests.

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Author Contributions

M.T. designed the content of the manuscript. N.O. and M.T. wrote the manuscript.

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