

# Gonadal Dysgenesis-with Special Emphasis on the Molecular Mechanisms of SRY Mutations in Disorders of Sex Development (DSD) Resulting in Female Sex Reversal in 46XY Males

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## Abbreviations

SRY: Sex determining Region in the Y chromosome; Sox: SRY related high mobility box transcription factor; HMG: High Mobility Box; CGD: Complete Gonadal Dysgenesis; NLS: Nuclear Localization Signals; NPC: Nuclear Pore Complex; CaM: Calmodulin; AMH: Antimullerian Hormone; MIS: Mullerian Inhibiting Substance; T: Testosterone; HCG: Human Chorionic Gonadotrophin; PGD: Partial Gonadal Dysgenesis; WT1: Wilms Tumor-related gene; SF1/NR5A1: Steroidogenic Factor1/ Nuclear Receptor subfamily5group Amember1; ARX: Aristaless Related Homeobox; ATRX:  $\alpha$ -Thalassemia, mental Retardation on the X; DMRT1: Double sex and Mab-3 Related Transcription factor 1; WNT4: Wingless type mouse mammalian Tumour viral integration site; RSPO1: R-spondin 1; DAX1/NROB1: Dosage Sensitive Sex Reversal Adrenal hypoplasia acute Regulatory Protein; DHH: Desert Hedgehog; HHAT: O-Acetyl Methyl Transferase Hedgehog Acyl Trasferase; LoF: Loss of Functional variants; AA: Amino Acids; lncRNA: Long noncoding RNA; Xist: X Inactive Specific Transcript; TES: Testis Enhancer Sequence; TESCO: Testis Enhancer Sequence Core element; TCON0025195&6: lnc RNA in SOX9 regulation; MHM: lnc RNA-2.2 kb sequence on chicken Z chromosome only in birds; FGF: Fibroblast Growth Factor; FGFR2: Fibroblast Growth Factor Receptor 2; PGD2: Prostaglandin D2; PgdS: Prostaglandin Synthase

## Introduction

SRY related high mobility group box (Sox) transcription factors have emerged in the animal kingdom to help cells maintain stemness, commit to a specific lineage, proliferate or die. Encoded by 20 genes in humans and mice they show a highly conserved high-mobility group box domain, which was originally identified in SRY, the sex determining region on the Y chromosome. This has derived from a high mobility group domain characterized of chromatin associated proteins. HMG (high mobility group) non histone chromosomal proteins include the AT hook, HMGN, and HMG domain families [1].

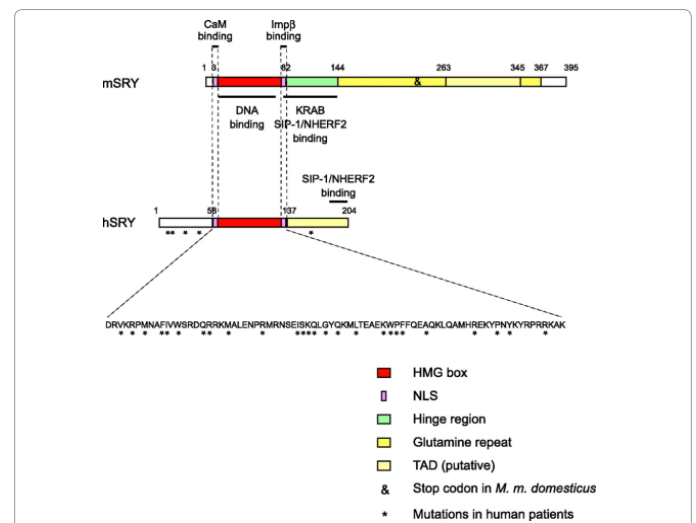
Members of the Sox SRY (Sex determining region on the Y chromosome) related HMG box family of chromatin remodelling factors play important development roles. SRY plays a key role in mammalian sex determination as determined by the fact that 15% of all XY sex reversal individuals carry mutations in SRY [2-5]. It is located on the end of short arm of Y chromosome and encodes a protein of 204 amino acids. It is not a typical eukaryotic transcript unit but a single exon-containing gene without any intron. It is not a conservative gene as well in mammals SRY is expressed 7 weeks post fertilization in humans with a specific role in the nucleus, activating/coordinating the expression of genes such as related proteins Sox9 which also results in differentiation of presertoli cells to produce a testis and suppress genes favouring formation of female gonads [3-4,6,7]. In the case of XY sex reversal due to impaired action of SRY (Swyers syndrome), patients present with complete gonadal dysgenesis (CGD) or partial gonadal dysgenesis, which causes a defective phenotype, with female external genitalia and 1 in 2 presenting with a gonadal tumor. SRY is able to bind and bend specific DNA targets through its HMG box domains like other HMG proteins [3,8]. The majority of sex reversal mutations in SRY results in impaired DNA binding/ bending, but a

number of which do not affect DNA binding map to one of SRY's two independently functioning nuclear localization signals [NLS], which flank the HMGbox domain [3,4,9-11] of these C terminal  $\beta$ -NLS mediates nuclear import conventionally through the molecule importin  $\beta$  (Imp- $\beta$ ) (Figure 1) courtesy by Wilhelm D [12].

This facilitates transport through the nuclear pore complex (NPC) found embedded in nuclear envelope and release the nucleus on interaction with G protein monomeric binding proteins Ran activated G protein bound form [13,14]. The 2<sup>nd</sup> N terminal NLS, Calmodulin (CaM)-NLS binds the Ca<sup>2+</sup> binding protein CaM [15] Kaur, et al., showed a dual nuclear import and calmodulin dependent nuclear import importance in role of SRY in sex reversal after examining missense mutations in SRY CaM NLS from human XY sex reversal females [16,17].

## Clinical features

Patients with pure or Complete Gonadal Dysgenesis (CGD) also



**Figure 1:** A model of the most relevant molecular pathways in epithelial and stromal ectopic endometrial cells involved in the pathophysiology of endometriosis. \*Major proinflammatory cytokines are produced in both peritoneal macrophages and endometriotic cells. \*\*Altered peritoneal cell mediated immunity seen in endometriosis is related to inflammatory cytokine expression profile [1-9,12].

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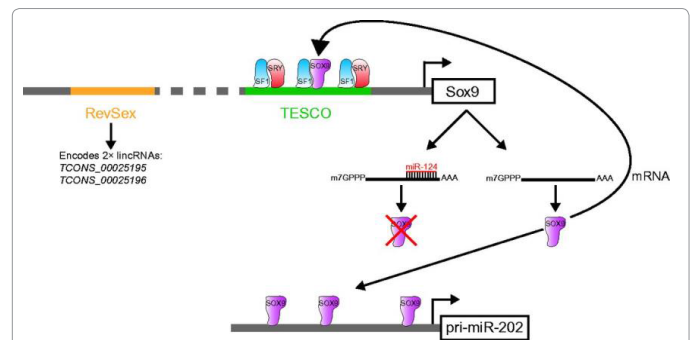
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known as Swyer's Syndrome have a normal female phenotype, including uterus and fallopian tubes but they have streak gonads, müllerian structures due to insufficient AMH/MIS secretion and a complex absence of androgenisations. AMH/MIS is low and testosterone (T) response to human chorionic gonadotropin (HCG) stimulation is impaired. These patients are free of turners like malformations and attain normal height. Patients with partial gonadal dysgenesis (PGD/dysgenetic gonads) may provide enough MIS to regress the uterus and sometimes sufficient for partial androgenisation. GD can result from mutations or deletions of testis promoting genes WT1 (Wilms tumor-related gene), SF1 (steroidogenic factor 1), SRY, SOX9 (SRY related HMG box gene 9), DHH (desert hedgehog), ATRX ( $\alpha$ -thalassaemia, mental retardation on the X), ARX (Aristaless related homeobox, X linked), DMRT (double sex and mab3 related transcription factor 1). Also duplication of chromosomal loci containing antitestis genes e.g. WNT4 (wingless type mouse mammalian tumor virus integration site 4), RSPO1 (R-spondin 1), DAX 1 also called NROB1 account for ~1% of the resolved cases [18] (dosage sensitive sex reversal adrenal hypoplasia acute regulatory protein) as reviewed by Wilhelm D and Mendonca BB [12,19]. Among these deletions or mutations of SF1 (NR5A1) appear to be the most common but still collectively account for <25% of cases. Associated clinical features may be present reflecting additional functional roles for these genes. For example renal dysfunction occurs in patients with specific WT1 mutations (Denys Drash and Fraser's syndrome). Primary adrenal failure occurs in some patients with SF1 mutations, DHH mutations cause GD associated with peripheral neuropathy and severe cartilage abnormalities (Campomelic dysplasia-a familial dwarf) are the predominant clinical features of SOX 9 mutations. Similarly recently 46XY DSD with CGD and chondrodysplasia has been found with a homozygous mutation (G287V) within coding sequence of O-acetyl transferase HHAT gene. HHAT gene codes for attachment of palmitoyl residues that are critical for multimerization and long term signaling of hedgehog secreted proteins [19]. Similarly recently 46XY DSD with CGD and chondrodysplasia has been found with a homozygous mutation (G287V) within coding sequence of O-acetyl transferase HHAT gene. HHAT gene codes for attachment of palmitoyl residues that are critical for multimerization and long term signaling of hedgehog secreted proteins [19]. A family history of DSD or premature ovarian insufficiency is important (eg. SF1/NR5A1). Intra-abdominal dysgenetic testis should be removed or prevent malignancy and oestrogens can be used to induce secondary sex reversal in 46XY individuals raised as females with absent (vanishing testes syndrome-(bilateral anorchia)-reflect regression of the testis during development. The etiology is unknown but the absence of müllerian structures indicates adequate secretion of AMH in utero and in most cases androgenisation of the external genitalia is either normal or slightly impaired e.g. small penis, hypospadias). These individuals can be of feredprosthesis and should receive androgen replacement in adolescence.

### Role of noncoding RNA'S in male differentiation

In mammals SRY is expressed for a short period in presertoli cells, which in this small time organizes for all other cell types where key roles for SRY in up regulation of Sox 9 which encodes a transcription factor belonging to the same SRY like HMG domain family [20]. Before SRY expression is up regulated in XY genital ridges Sox 9 is expressed at low levels both in developing testis and ovary [21] due to the binding and activation of SF1 to the testis enhancer sequence (TES)1. 4 Kb upstream of SOX 9 Transcription start site [22]. Subsequently SRY binds together to SF1 to a 1.4 kb core element (TESCO) located within the TES resulting in up regulation of Sox 9 transcription within the testis whereas Sox9 becomes undetectable in ovary. After that Sox binds along with SF1 to TESCO to maintain its own expression.



**Figure 2:** Putative regulation of Sox 9 expression by ncRNAs in mice: Sox 9 transcription is up regulated by the binding of SF1 and SRY/SRY or SF1&SOX9 to the enhancer region TESCO which is located 13 kb upstream of the Sox 9 transcription start site. The TESCO sequence is also present in humans but its relevance for testis specific Sox 9 expression is not clear. Rather the analysis of human patients with 46XX and 46XYDSD identified a second control region called Revsex located upstream of the Sox 9 Transcription start site. This region harbours two linc RNAs TCON 00025195, and TCON 00025196 which might be involved in regulating Sox 9 expression. In ADDITION Sox9 expression can be regulated by miR124 by binding to the 3'UTR of Sox 9mRNA. However it is unclear if this regulation occurs during gonadal differentiation. Finally Sox 9 likely functions as a transcriptional activator of miRNAs such as miR 202 and miR140 [31].

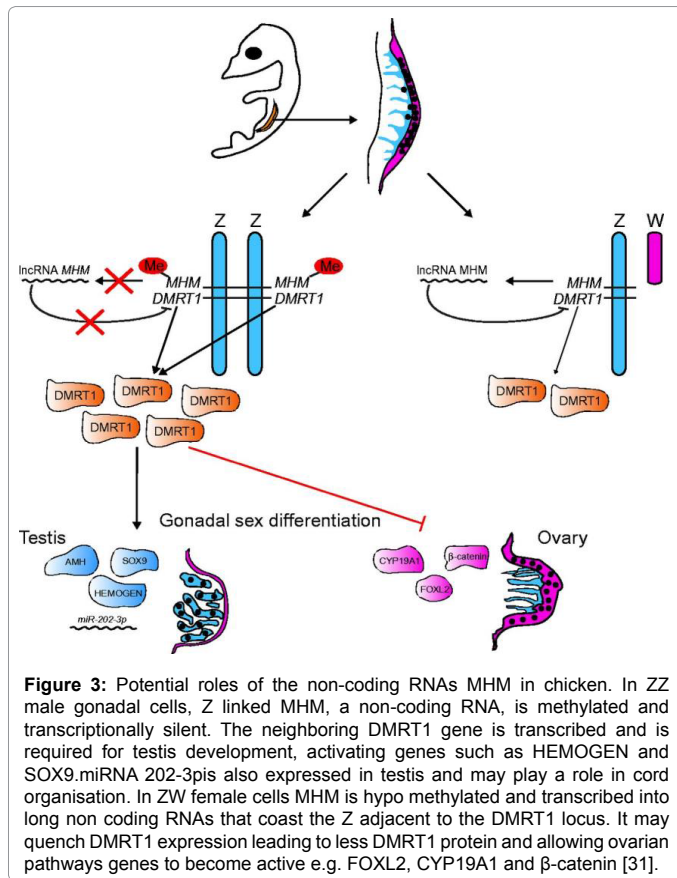
- Sox 9 directly or indirectly up regulates FGF9, which then activates FGF signaling via FGFR2 resulting in Sox 9 up regulation [23,24].
- Sox 9 directly stimulates expression of the prostaglandin D synthase (ptgds) gene leading to the production of prostaglandinD2 (PGD2) which leads to the translocation of SOX 9 protein from the cytoplasm into the nucleus and up regulation of its expression [25-27].

Although TESCO has been located in human genome ~13 kb upstream of the SOX 9 TSS [22], it is not known that if TESCO also mediates testis specific expression of SOX 9 in humans. Mapping of copy number variants in human patients with 46 XX and 46 XY female development identified a long distance regulatory region upstream of Sox 9 called Revsex which is likely to harbour an enhancer driving testis specific expression [28]. This region encodes two linc RNAs (TCONS00025195 and TCONS00025196 [29], which might be involved in up regulation of SOX9 expression in human testis (Figure 2).

In ovary miR 124 has been implicated in the down regulation of Sox9 [30] as reviewed by Rastetter, et al. [31]).

### The Role of long non-coding RNA (lncRNA) in dosage compensation

In species with genetic sex determination such as XX female, XY male system in mammals and in the ZW female; ZZ male system in birds, males and females have a difference in sex chromosome-linked gene dosage which has resulted in the evolution of dosage compensation mechanism in mammals which is realized by the inactivation of one of the X chromosomes through coating by an lncRNA called Xist (Xinactive specific transcript). The 19 kilobase long transcript Xist is only transcribed from inactive X chromosome and coats hundreds of genes. Prior to inactivation an lncRNA that is antisense to Xist called Tsix is down regulated from one of the X chromosomes resulting in the Xist and inactivation of the X chromosome. On the active X, the maintenance expression of Tsix prevents the full length Xist expression and X linked gene expression is unaffected reviewed by Moran [32]. This phenomenon on dosage compensation in mammals is clearly regulated by lncRNAs. But in other groups such as birds there is no inactivation of one sex chromosome of the homogametic sex. However



there is potential involvement of lncRNAs in dosage compensation in chicken as well. Chickens and other birds have a ZZ male, ZW female sex chromosome system. The Z linked transcription factor gene DMRT1, is thought to play a central role in avian sex determination by directing testis development in Z embryos. Overexpression of DMRT1 induces the male specific genes HEMGN, SOX9 and AMH [33] (Figure 3).

MHM is a 2.2 kb sequence absent in other birds and MHM is located within a region of Z chromosome which corresponds to hyper acetylation of histone H4 which is associated with increased gene expression or second hypothesis MHM may regulate by in male cells ZZ MHM is hyper methylated and transcriptionally silent, whereas in female cells ZW it is hypo methylated and transcribed. Being near DMRT1, it is suggested it may influence to dampen DMRT1 in female cells, by MHM lncRNA coating the chromosome adjacent to DMRT1 locus, inducing local chromatin conformational changes which may interfere by TF binding [34] -further reviewed by Rastetter, et al. [31].

### Role of SRY mutations

Till now 80 mutations have been reported from a gene which encodes 204 aa's, and although all cause either CGD/PGD, very little is understood how the mutations damage SRY function at the molecular level. Fan, et al. [35] reported a novel mutation in a case of Swyer syndrome which is a denovo mutation at nucleotide 224 of SRY coding region with guanine replaced by thymine and at protein level arginine is replaced at position 75 in aa of entire SRY region with methionine R75M in a 46 XY Karyotype with phenotypic female and associated dysgerminoma. They cloned a wild and mutated type SRY and showed mutated SRY greatly accumulated in cytoplasm compared to wild type SRY which only localizes in nucleus [35].

To rule out other gene involvement trio base whole exomic sequencing studies using the DNA samples from proband and parents revealed no mutations especially in Desert Hedge Hog (DHH), NROB1 (DAX1), NR5A1/SF1, SOX9 which implicated denovo mutations in SRY is a single defect for sex reversal. On an estimation human genome, contains >100 genuine loss of functional (LoF) variants with >20 genes completely inactivated and on an approximation 5 common compete knockout genes in autosomes which may be associated with disorders or may be completely harmless [36,37]. They further used bioinformatics simulation analysis to predict about impact of analysis of R75 on SRY function and found R75 in wild type SRY can form a Hydrogen Bond with serine at 91 (S91) which makes the SRY protein fit well into the minor groove of DNA, while M75 in mutated SRY can't do so. The authors reviewed the SRY mutations based on available references and analysed distribution pattern accordingly in density and continuity which may be useful for further study of structure and function and its relatedness with disorders of sex development.

They found a total of 80 mutations reported in 204 aa's in full length of SRY from the gene bank available regarding human mutations [38]. 80 mutations occur/59 Codons, that show 1, 39599 mutations at an average. Among these 80 mutations, 66 concentrated in HMB domain (57-136) which take 82.59% (66/80) of total mutations. Of these HMG domain occurs in 46 codons, including 54 missense mutations (81.82%; 54/66), and 12 nonsense mutations (18.18%; 12/66), 18 double mutations (39.13%; 18/46), and 1 triple mutation (2.1%; 1/46). Hence based on the continuity and density of mutations in HMG group -they divided it into 4 sub regions separated by 3 or more consecutive codons without mutations) region carries 22 codons (from 57-78) which include 7 as mutation free, 14 carrying 23 mutations because of 1 triple and 7 double mutations and a cluster of 3 codons from 74-76 that all show mutation without interruption. Their case of denovo R75 Moccurs in the middle of it. Second sub region spans 20 codons from 82-101 which contain a cluster of codon 10 codons from 87-96 with 14 mutations because of 4 double. Third sub region covering 13 codons 107-119 with 11 mutations including 5 mutations in a continuous 4 codons 107-110 because of 1 double mutation and 4th covers 12 codons-125-136 with 12 mutations. It is said 7 codons from 130-136 encode C terminus NLS, but observed mutation pattern suggested C terminal NLS may cover codons from 125-136.

Based on these mutation distribution and the experiments by authors they hypothesized that the N terminal NLS covers 22 amino acids from 57-78, while the C terminal NLS covers 12 aa's from 125-136. The epitope that determines the SRY-DNA interaction maybe strongly associated with 10 codons from 87-96 and 4 codons from 107-110 in the HMG. The final confirmation they are still waiting the results of experiments in their lab oratory right now. Further they commented on the rare mutations occurring in first 56 codons-only 9 mutations of which all scattered in first 43 codons and nearest missense mutation at 2nd and 4th codon's being understandable but the nearest missense mutation occurs in 3rd codon which causes a complete XY female sex reversal [39]. 5 mutations downstream to 137-163 with no mutations in last 40 codons from 164-204 (41/204), the observation could suggest some aa's proximal downstream HMG still play a crucial role to keep SRY functional, with aa at distal downstream HMG being less functional. This gets further confusing as at least 40 aa's contribute little to SRY function, why does the truncated SRY by nonsense mutations at codon 163 cause XY female sex reversal as reported [40]. The similar situation can be applied to the frame shift mutations at codon 158 and 159. It becomes interesting to know that the missense mutations of serine at codon 145 can diminish SRY-DNA interaction [41], means the aa's outside the HMG domain can exert certain influences to SRY



function by some way. HMG and occurrence of mutations and Swyer syndrome still suggests some crucial role in amino acids distal to HMG, but puzzle that can't be solved is if last 40 aa's contribute little to SRY function why truncated SRY with nonsense mutation in 163 Codon causes sex reversal and certain unanswered questions which they are still trying to solve in their laboratory.

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