FOXL2 Is an Essential Activator of SF-1-Induced Transcriptional Regulation of Anti-Müllerian Hormone in Human Granulosa Cells

Hanyong Jin1☯, Miae Won2☯, Si Eun Park1, Seunghwa Lee3, Mira Park1, Jeehyeon Bae1*

1 School of Pharmacy, Chung-Ang University, Seoul, Korea, 2 Department of Pharmacy, CHA University, Seongnam, Korea, 3 Department of Life Science, Chung-Ang University, Seoul, Korea

☯ These authors contributed equally to this work.
* jeehyeon@cau.ac.kr

Abstract

Anti-Müllerian hormone (AMH) is required for proper sexual differentiation by regulating the regression of the Müllerian ducts in males. Recent studies indicate that AMH could be an important factor for maintaining the ovarian reserve. However, the mechanisms of AMH regulation in the ovary are largely unknown. Here, we provide evidence that AMH is an ovarian target gene of steroidogenic factor-1 (SF-1), an orphan nuclear receptor required for proper follicle development. FOXL2 is an evolutionally conserved transcription factor, and its mutations cause blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES), wherein affected females display eyelid defects and premature ovarian failure (POF). Notably, we found that functional FOXL2 is essential for SF-1-induced AMH regulation, via protein–protein interactions between FOXL2 and SF-1. A BPES-inducing mutant of FOXL2 (290–291delCA) was unable to interact with SF-1 and failed to mediate the association between SF-1 and the AMH promoter. Therefore, this study identified a novel regulatory circuit for ovarian AMH production; specifically, through the coordinated interplay between FOXL2 and SF-1 that could control ovarian follicle development.

Introduction

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a member of the transforming growth factor β (TGF-β) family. This is known to be an essential hormone for proper sexual differentiation as it regulates the regression of the Müllerian ducts in males [1]. In recent years, a new functional role for AMH in ovarian follicle development has been reported based on studies using Amh knockout mice; these animals display infertility associated with the early depletion of the follicle pool [2]. AMH is highly expressed in the granulosa cells of small, growing follicles of the ovary [3]. In women, serum AMH levels decrease with age, leading to undetectable levels during menopause [4]. In addition, serum AMH levels in patients with primary ovarian insufficiency (POI) or premature ovarian failure (POF) are extremely low or undetectable [5, 6]. Together, these findings imply that AMH might be an
important hormone for maintaining the ovarian reserve. However, regulatory mechanisms of ovarian AMH production remain largely unknown.

Steroidogenic factor-1 (SF-1), also known as NR5A1, is an orphan nuclear receptor that has multiple functions in reproduction, steroidogenesis, and sexual differentiation [7]. SF-1 consists of two zinc finger DNA-binding domains, a ligand-binding domain, and a hinge region in between [8]. Sf-1-deficient mice exhibit adrenal and gonadal development abnormalities [9, 10]. In addition, female mice with a conditional Sf-1 knockout in granulosa cells display infertility associated with decreased follicle numbers [11]. SF-1 regulates the transcription of genes crucial for reproduction, including AMH, DAX-1, steroidogenic acute regulatory protein (STAR), cholesterol side chain cleavage enzyme (SCC), CYP17, and CYP19 (aromatase) [12–16]. The effect of interplay between SF-1 and SOX8, SOX9, Wilms’ tumor 1 (WT1), DAX-1, GATA4, and NF-κB on the transcription of AMH in Sertoli cells during testis development is relatively well defined [17–22]. However, to the best of our knowledge, the role of SF-1 in AMH regulation in the ovary remains unknown.

FOXL2 is an evolutionally conserved transcription factor that belongs to the forkhead family [23]. This family of genes encodes key molecules that regulate fundamental functions, including apoptosis, differentiation, proliferation, metabolism, and developmental processes [24]. In humans, FOXL2 mutations cause blepharophimosis-ptosis-epicanthus inversus syndrome (BPES; OMIM #110100), which is an autosomal dominant disease, and affected individuals display eyelid defects with or without POF [25]. Female, FoxL2-deleted, homozygous mice are infertile due to the premature exhaustion of ovarian follicles [26, 27]. FOXL2 ablation in the ovary of adult mice induces somatic sex reprogramming of ovaries to testes [28]. These findings underscore the crucial role of FOXL2 in the ovary. Recently, we identified that AMH is a target gene of FOXL2, and that depletion of AMH accelerates follicle growth, which could be effectively overcome by FOXL2 overexpression in mouse ovaries [29]. We also reported that FOXL2 interacts with SF-1 in granulosa cells [30].

In the present study, we investigated the inter-regulatory network of three key molecules (AMH, FOXL2, and SF-1) crucial for controlling the ovarian reserve. Here, we present, for the first time, direct experimental evidence indicating that AMH is a target gene of FOXL2, and that depletion of AMH accelerates follicle growth, which could be effectively overcome by FOXL2 overexpression in mouse ovaries [29]. We also reported that FOXL2 interacts with SF-1 in granulosa cells [30].

Materials and Methods

Cell culture and transfection

Human, adult-type granulosa cell tumor (GCT)-derived KGN cells (Drs. Yoshihiro Nishi and Toshihiko Yanase, Kyushu University, Fukuoka, Japan) and juvenile-type GCT-derived COV434 cells (Sigma-Aldrich, St. Louis, MO, USA), and human kidney-derived 293T (American Type Culture Collection, Rockville, MD, USA) cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) or DMEM-F12 containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. All cells were transfected with Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

Plasmid construction

Generation of constructs, Myc-tagged FOXL2, FLAG-tagged SF-1, human AMH promoter, and its FOXL2-binding elements (FBE) mutants was described previously [29, 30]. Constructs driving the expression of Myc-tagged mutated FOXL2 (290–291delCA) were generated by recombinant PCR using the following primers: FOXL2 wild type (WT)-F (5’-CTAGAATTCAATGATGGCCAGCTACCCC-3’) with 290–291delCA-R (5’-CCGGTCTCGGGGCAAGCA
G-3'); 290–291delCA-F (5'-GAGACC CGTCGCACA-3') with FOXL2 WT-R (5'-CATT CGC GCCTCGATCT CTCGAGTAG-3'). The PCR products were digested with EcoRI and XhoI (Takara Bio, Otsu, Shiga, Japan) and ligated into the pCMV-Myc vector (Clontech, Mountain View, CA, USA).

**Luciferase reporter assay**

KGN and COV434 cells (2 x 10^5) were transfected with 600 ng of AMH-luciferase reporter, 100 ng of pCMV β-galactosidase (Clontech), in addition to the indicated plasmids (300 ng) as well as small interfering nucleotides (200 nM) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Cells were then incubated in 12-well plates containing medium for 24 h. Luciferase activity was assessed as previously described [30]. The absorbance was measured with the FlexStation3 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

**Small interfering RNAs**

Small interfering RNA (siRNA) target sequences specific to FOXL2 and SF-1 were 5'-GCUC CUGUCGCUCUCUUU-3' and 5'-GUCUGCCUCAAGUUCAUCA-3', respectively. The control siRNA sequence used was 5'-CCUACGCCACCAAUUUCGU-3'. The sense and anti-sense oligonucleotides were annealed in the presence of annealing buffer (Bioneer, Daejeon, South Korea).

**Immunoblotting and immunoprecipitation**

KGN cells were transfected with the indicated plasmids and specific siRNAs. Twenty-four hours after transfection, cell lysates were prepared for immunoprecipitation with antibody-linked protein G Dynabeads® (Invitrogen) according to the manufacturer’s instructions. After incubation, the samples were boiled and subjected to SDS-PAGE and immunoblotting with appropriate antibodies. The membranes were imaged using a ChemiDoc™ XRS+ System Imager (Bio-Rad Laboratories Inc., Hercules, CA, USA). The following antibodies were purchased and used in this study: anti-AMH (AP9940c; Abgent, San Diego, CA, USA), anti-SF-1 (sc-28740; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-GAPDH (sc-25778; Santa Cruz Biotechnology), anti-PARP (sc-74469; Santa Cruz Biotechnology), and control rabbit IgG (sc-2027; Santa Cruz Biotechnology), anti-α-tubulin (LF-PA0146; AbFrontier Seoul, Korea), anti-c-Myc (631206; Clontech), anti-FLAG (2368) (Cell Signaling, Danvers, MA, USA), and anti-FLAG (F1804; Sigma-Aldrich). The polyclonal rabbit FOXL2 antibody was described previously [30].

**Subcellular fractionation**

KGN cells were transfected with the indicated plasmids as well as siRNAs for 24 h and fractionation of nuclear and cytosolic compartments was performed as previously described [30].

**Electrophoretic mobility shift assay (EMSA)**

EMSA was performed as previously reported [30]. The probe was designed based on known SF-1 binding sequences (SBEs) at -92 to -84 and -218 to -210 [21]. Double-stranded oligonucleotides were generated based on the following human AMH sequences: 5'-TGTTCCCCCA AAGGTCGCGCAG-3' or 5' - CTGCCGCCACCGTGGGGACA-3'T oligonucleotides were annealed before use.
Chromatin immunoprecipitation–quantitative polymerase chain reaction (ChIP–qPCR) analysis

ChIP assays were performed as previously described [30]. DNA was amplified using primer sets flanking the putative SF-1 binding element 1 (SBE1; -134/-6) and SF-1 binding element 2 (SBE2; -293/-133) of the AMH promoter as follows: forward (5'-GAAGGCCACTCTGCTG GAGT-3') with reverse (5'-GGGCTGGGCTGCCTGCTTAA-3'); forward (5'-CAGCG CTGTCCTAGTTGGTT-3') with reverse (5'-TCTCAAGAGCCCTTCTGT-3'). The primers of the FOXL2-binding motifs (FBE1 and FBE2) in the AMH promoter were described in our previously study [29]. Products were analyzed by quantitative real-time PCR.

Recombinant protein purification

SF-1 as well as WT and mutated (MT; 290–291delCA) FOXL2 were purified from mammalian cells after transfection of 293T cells with pcDNA3-FLAG-NR5A1 (FLAG-tagged SF-1) as well as Myc-tagged WT and MT FOXL2 plasmids. Twenty four hours after transfection, the cells were lysed in RIPA buffer containing 1 mM Na3VO4, 10 mM NaF, and a protease inhibitor cocktail, and were frozen at −80°C for 2 h. Following incubation, the supernatant was separated from 293T cell lysates and incubated with BS3-crosslinked anti-FLAG or anti-Myc-Dynabeads protein G (Invitrogen) overnight, at 4°C. The antibody–protein G crosslinked Dynabeads were washed with precooled PBS-T three times and protein G Dynabead-linked proteins were eluted with 0.1 M glycine buffer (pH 2.0) for 1–2 min, and immediately neutralized with 1 M Tris buffer (pH 10.0).

Immunofluorescence analysis

KGN cells (2 × 10^4) were seeded onto round coverslips in 24-well plates. At 24 h post-transfection, cells were fixed with 4% paraformaldehyde for 15 min at room temperature. The fixed cells were permeabilized with 0.2% Triton-X 100 in TBS for 15 min and then incubated with 2% BSA in TBS containing 0.1% Tween 20 (TBS-T) for 2 h. Fixed cells were incubated for 12 h with primary antibodies in TBS-T. Anti-Myc (1:100) and anti-SF-1 (1:100) antibodies were used to detect the localization of FOXL2 and SF-1, respectively. After washing three times with TBS-T, the cells were stained with Alexa Fluor® 488 goat anti-mouse IgG (1:1000) (Invitrogen) and Alexa Fluor® 546 goat anti-rabbit IgG (1:1000) (Invitrogen) for 1 h. After additional washing with TBS-T, the coverslips were mounted on slides using mounting solution with DAPI. Fluorescence was detected using a Zeiss LSM 510 META confocal microscope (Carl Zeiss, Göttingen, Deutschland).

Statistical analysis

Data analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC, USA). Statistically significant differences were identified using either the Student–Newman–Keuls multiple range test or the Fisher’s least significant difference test, at the 5% level of significance.

Results

SF-1 transactivates ovarian AMH in a FOXL2-dependent manner

To understand the regulatory function of SF-1 on AMH in the ovary, the effect of SF-1 on transcriptional regulation of the AMH promoter was determined after ectopic expression of SF-1 in the human, GCT-derived KGN cell line. As shown in Fig 1A, SF-1 clearly activated the AMH promoter in granulosa cells. Overexpression of WT FOXL2 also transactivated AMH (Fig 1B),
as we previously reported [29]. In contrast, a FOXL2 mutant harboring a dinucleotide deletion (290–291delCA), detected in a patient with BPES type I who exhibited POF [31], failed to elicit transactivation of AMH (Fig 1B). Co-expression of WT FOXL2 further potentiated SF-1-induced AMH activation, but the FOXL2 mutant did not (Fig 1B). Furthermore, SF-1-induced AMH transcriptional activation was completely abolished in FOXL2-silenced KGN cells (Fig 1C). A similar effect was observed in COV434 cells, another type of human granulosa cell line (Fig 1D). Regulation of AMH by SF-1 and FOXL2 was further confirmed by immunoblot analysis; herein, the level of AMH protein increased after SF-1 overexpression, but this increase was prevented when FOXL2 was silenced (Fig 1E). In contrast, SF-1 depletion did not significantly affect the capacity of FOXL2 to induce AMH transcriptional activation (Fig 1F).

FOXL2 is required for the binding of SF-1 to the AMH promoter

To investigate how FOXL2 regulates SF-1-mediated AMH transcription, we determined if FOXL2 affected the binding of SF-1 to the AMH promoter. The human AMH promoter contains two SBEs at -92 to -84 (SBE1) and -218 to -210 (SBE2) (Fig 2A) [21]. Oligonucleotide probes specific for SBE1 were generated, and EMSA experiments on nuclear extracts from KGN cells overexpressing SF-1 and/or FOXL2 were performed. The results indicated the formation of a specific complex between SF-1 and the probe corresponding to SBE1, which was abolished or supershifted by the addition of the cold probe or antibody, respectively (Fig 2A). The formation of this complex, between SF-1 and AMH, was increased by FOXL2 overexpression (Fig 2A). In addition, the SF-1–AMH complex was greatly reduced in FOXO2-knockdown cells (Fig 2B). To further confirm the EMSA results, ChIP-qPCR experiments were performed. The promoter regions corresponding to SBE1 (Fig 2C) and SBE2 (Fig 2D) of endogenous AMH were significantly enriched in anti-SF-1 immunoprecipitates (Fig 2C and 2D). However, these enrichments were abolished in FOXL2-depleted KGN cells (Fig 2C and 2D), indicating an essential role for FOXL2 in the association between SF-1 and the AMH promoter. Similar results were observed in COV434 cells (S1A Fig).

Because endogenous or overexpressed FOXL2 can also bind to the AMH promoter, at two forkhead-binding elements (FBEs) (S1B Fig) [29] located in the proximal vicinity of the SBEs (Fig 3), we determined whether FOXL2-association to AMH is prerequisite for SF-1-binding to AMH. Using AMH promoters with mutated FBEs to which FOXL2 is unable to bind [29], SF-1-induced reporter activities were measured. As shown in Fig 3, SF-1 was still able to activate these FBE mutated AMH promoters. Thus, this result indicates that the association of FOXL2 with AMH promoter is not necessary for SF-1-binding to AMH.
Fig 2. FOXL2 is essential for SF-1 association with AMH. (A) Two putative SF-1-binding elements (SBEs), SBE1 (-92/-84) and SBE2 (-218/-210), in the human AMH promoter are shown. (A–B) EMSA was performed using nuclear extracts (5 μg) from KGN cells transfected with the indicated plasmids or siRNAs. For the cold probe, a 200-fold excess of unlabeled oligonucleotides was used. The supershift of the band was determined by incubation of the nuclear extract with an anti-FLAG antibody. Radiolabeled-probes corresponding to the SBE1 sequences in the AMH promoter were used. Expression of SF-1 and FOXL2 proteins were determined by immunoblotting using the indicated antibodies (A; bottom panel). Efficient FOXL2 silencing and nuclear
The 290–291delCA-FOXL2 mutant fails to regulate SF-1-AMH binding due to defects in intracellular localization and interaction with SF-1.

We further investigated the possible mechanism for the defective transcriptional regulatory activity of mutant (290–291delCA) FOXL2 (observed in Fig 1B). Immunoprecipitation experiments to analyze protein–protein interactions revealed that the mutant could not bind to SF-1,
whereas WT FOXL2 interacted with SF-1 (Fig 4A). In addition, mutant FOXL2 showed abnormal intracellular localization, as it was widely detected in the cytoplasm rather than in the nucleus; WT FOXL2 was mainly localized in the nucleus of KGN cells (Fig 4B). Moreover, EMSA using purified, recombinant proteins of WT FOXL2, mutant FOXL2, and SF-1 (S2 Fig), revealed that mutant FOXL2 failed to enhance SF-1 binding to the AMH promoter sequences (Fig 4C).

**Discussion**

The development and growth of ovarian follicles, to generate competent oocytes, are complex processes that require highly ordered crosstalk with critical molecules and remains largely unspecified. Accumulating evidence implies that FOXL2, SF-1, and AMH could be important factors governing the ovarian follicle development. Herein, we provide, for the first time, experimental evidence that supports the presence of a coordinated regulatory network of FOXL2 and SF-1 on AMH production, which could be potentially important for controlling the ovarian folliculogenesis.
The ovarian reserve is comprised of a pool of resting primordial follicles and a pool of activated growing follicles. Approximately 680,000 non-growing follicles are endowed at birth, and follicles decline to 460,000 around puberty [32–35]. Monthly, cyclic recruitment of follicles continuously depletes the ovarian reserve until menopause [32, 35, 36]. Recently, serum AMH levels have gained attention as the most promising biomarker to accurately predict the ovarian reserve in clinical settings [37]. In addition, AMH measurement is accepted as a good method to predict the response to ovarian stimulation in assisted reproductive technology [38–40]. Although a decade has passed since the discovery of the inhibitory function of AMH on initial and cyclic ovarian follicle recruitments [2, 41, 42], the underlying regulatory mechanism of ovarian AMH production is still unclear.

Nuclear receptors are transcription factors that mostly function as homodimers or heterodimers [43]. SF-1 is a nuclear receptor acting as a monomer, and its function is regulated by binding to its interacting proteins [44]. Regarding AMH gene regulation, WT-1, DAX-1, SOX8, SOX9, GATA4, and NF-κB interact with SF-1 and positively or negatively regulate SF-1-induced AMH transcription during male sexual differentiation [17, 18]. Here, we first demonstrated that SF-1 is a transcriptional activator of ovarian AMH and further identified FOXL2 as an indispensable factor for SF-1-induced AMH regulation, based on the observation that SF-1 was unable to bind and transactivate the AMH promoter in the absence of a functional FOXL2 protein (Figs 1 and 2 and S1A Fig). The mechanism underlying this regulation by FOXL2 is likely to be through its interaction with SF-1 that subsequently allows association between SF-1 and SBEs in the human AMH promoter. Since the protein–protein interaction of SF-1 and FOXL2 involves their DNA-binding domains [30], it is plausible that the FOXL2 interaction at the DNA-binding domain of SF-1 may induce a conformational change in SF-1, particularly in the DNA-binding region, allowing SF-1 to bind to the AMH promoter. Meanwhile, as FOXL2 itself can bind to the AMH promoter, at two FBEs [29] located in the proximal vicinity of the SBEs (Fig 3), it is also possible that FOXL2 binding to AMH may alter the chromatin architecture, facilitating SF-1 access to SBEs on AMH. However, this is unlikely because SF-1 could still activate FBE-mutated AMH promoters (Fig 3), to which FOXL2 is unable to bind [29]. Therefore, the regulatory role of FOXL2 identified in this study is unique and critical because FOXL2 is an essential factor required for SF-1-induced AMH transcription in contrast to other SF-1-interacting proteins, such as WT-1, DAX-1, SOX8, SOX9, GATA4, and NF-κB, that merely function as modulators of SF-1-mediated transcriptional activation of AMH.

POI occurs by premature exhaustion of the ovarian reserve, leading to infertility, but its etiology is not well-defined because of the complex and heterogeneous nature of this condition [45, 46]. However, elucidation of ovarian AMH regulation at the molecular level can aid in understanding POI and normal ovarian folliculogenesis, based on the facts of the inhibitory role of AMH being demonstrated in follicle growth in mice [2, 41, 47] and extremely low or undetectable AMH levels in patients with POI [37]. A familial deletion mutant of FOXL2 (290–291delCA) that results in the production of a truncated FOXL2 protein lacking the forkhead DNA-binding domain was previously identified [31]. This mutant was employed in this study and can serve as a good model system, allowing us to gain molecular insights into POI, as the FOXL2 mutant was unable to interact with SF-1, failing to mediate the association between SF-1 and the AMH promoter. The inhibitory function of AMH on follicle recruitment [2, 41, 42] led us to speculate that the aberrant regulation of AMH results from loss-of-function mutations in FOXL2. This would result in insufficient production of AMH that would in turn lead to accelerated follicle growth, followed by premature exhaustion of the ovarian reserve. In addition to the activating role of FOXL2 in SF-1-induced AMH transcription identified in this study, FOXL2 itself transactivates AMH and the released AMH is further positively upregulated by FOXL2 [29]. Coinciding spatiotemporal expression of AMH, FOXL2, and SF-1 in...
granulosa cells of small follicles, and similar spectrums of ovarian phenotypes in mice with knockouts of these genes [27, 47, 48], also support the presence of this physiologically relevant regulatory network. Although more studies are needed for a complete understanding of AMH regulation and its molecular function in the ovary, this study demonstrates the presence of a critical regulatory network that could control ovarian folliculogenesis.

Supporting Information

S1 Fig. Transcriptional activation of AMH by SF-1 and FOXL2. Quantitative ChIP assays in COV434 (A) and KGN (B) cells were performed using SF-1-specific primers that target SBE1 or the FOXL2-specific primers for FOXL2 binding elements (FBEs) in the AMH promoter. (A) COV434 cells were transfected with control or SF-1 plasmids together with control siRNA or specific FOXL2 siRNA. (B) Enrichment of endogenous AMH and FOXL2 was determined by ChIP assay without transfection of plasmid in KGN cells. AMH luciferase activity was analyzed 24 h after transfection. Quantitative real-time PCR results are shown as fold enrichment. Control IgG was used for immunoprecipitation as a negative control. Asterisks indicate significant values compared with control values. The results are from three independent experiments performed in duplicate ($p < 0.05$).

(TIF)

S2 Fig. Production and purification of recombinant proteins. The purity of (A) recombinant FLAG-tagged SF-1, (B) Myc-tagged WT FOXL2, and (C) Myc-tagged mutant FOXL2 (290–291delCA) proteins are demonstrated by coomassie blue staining. Arrows indicate the expected position of the proteins.

(TIF)

Author Contributions

Conceived and designed the experiments: JB. Performed the experiments: HJ MW SP SL MP. Analyzed the data: JB HJ. Contributed reagents/materials/analysis tools: JB. Wrote the paper: JB HJ MW.

References

1. Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, et al. Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell. 1986; 45(5):685–98. PMID:3754790.

2. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology. 1999; 140(12):5789–96. PMID:10579345.

3. Bezard J, Vigier B, Tran D, Mauleon P, Josso N. Anti-mullerian hormone in sheep follicles. Reprod Nutr Dev. 1988; 28(4B):1105–12. PMID:3244904.

4. Dollemans M, Depman M, Eijkemans MJ, Heimensem J, Broer SL, van der Stroom EM, et al. Anti-Mullerian hormone is a more accurate predictor of individual time to menopause than mother’s age at menopause. Hum Reprod. 2014; 29(3):584–91. PMID: 24435779. doi: 10.1093/humrep/det446

5. Broekmans FJ, Visser JA, Laven JS, Broer SL, Themmen AP, Fauser BC. Anti-Mullerian hormone and ovarian dysfunction. Trends Endocrinol Metab. 2008; 19(9):340–7. PMID: 18805020. doi: 10.1016/j.tempo.2008.08.002

6. Meduri G, Massin N, Guibourdenche J, Bachelot A, Fiori O, Kuttern F, et al. Serum anti-Mullerian hormone expression in women with premature ovarian failure. Hum Reprod. 2007; 22(1):117–23. PMID: 16954410.

7. Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet. 1999; 22(2):125–6. PMID: 10369247.
8. Lin L, Philibert P, Ferraz-de-Souza B, Kelberman D, Homfray T, Albanese A, et al. Heterozygous missense mutations in steroidogenic factor 1 (SF1/Ad4BP, NR5A1) are associated with 46,XY disorders of sex development with normal adrenal function. J Clin Endocrinol Metab. 2007; 92(3):991–9. PMID: 17200175.

9. Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev. 1997; 18(3):361–77. PMID: 9183568.

10. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell. 1994; 77(4):481–90. PMID: 8187173.

11. Pelusi C, Ikeda Y, Zubair M, Parker KL. Impaired follicle development and infertility in female mice lacking steroidogenic factor 1 in ovarian granulosa cells. Biol Reprod. 2008; 79(6):1074–83. PMID: 18703422. doi: 10.1095/biolreprod.108.069435

12. Yu RN, Ito M, Jameson JL. The murine Dax-1 promoter is stimulated by SF-1 (steroidogenic factor-1) and inhibited by COUP-TF (chicken ovalbumin upstream promoter-transcription factor) via a composite nuclear receptor-regulatory element. Mol Endocrinol. 1998; 12(7):1010–22. PMID: 9658405.

13. Sugawara T, Holt JA, Kiriakidou M, Strauss JF 3rd. Steroidogenic factor 1-dependent promoter activity of the human steroidogenic acute regulatory protein (StAR) gene. Biochemistry. 1996; 35(28):9052–9. PMID: 8703908.

14. Hatano O, Takayama K, Imai T, Waterman MR, Takakusu A, Omura T, et al. Sex-dependent expression of a transcription factor, Ad4BP, regulating steroidogenic P-450 genes in the gonads during prenatal and postnatal rat development. Development. 1994; 120(10):2787–97. PMID: 7607070.

15. Lynch JP, Lala DS, Peluso JJ, Luo W, Parker KL, White BA. Steroidogenic factor 1, an orphan nuclear receptor, regulates the expression of the rat aromatase gene in gonadal tissues. Mol Endocrinol. 1993; 7(6):776–86. PMID: 8395654.

16. Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates the mullerian inhibiting substance gene: a link to the sex determination cascade. Cell. 1994; 77(5):651–61. Epub 1994/06/03. doi: 0092-8674(94)90050-7 [pii]. PMID: 8205615.

17. Ito M, Yu R, Jameson JL. DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. Mol Cell Biol. 1997; 17(3):1476–83. PMID: 9032275.

18. Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA. Wilms’ tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell. 1998; 93(3):445–54. PMID: 9500178.

19. Schepers G, Wilson M, Wilhelm D, Koopman P. SOX8 is expressed during testis differentiation in mice and synergizes with SF1 to activate the Amh promoter in vitro. J Biol Chem. 2003; 278(30):28101–8. PMID: 12732652.

20. Tremblay JJ, Viger RS. Transcription factor GATA-4 enhances Mullerian inhibiting substance gene transcription through a direct interaction with the nuclear receptor SF-1. Mol Endocrinol. 1999; 13(8):1388–401. PMID: 10446911.

21. De Santa Barbara P, Bonneaud N, Boizet B, Desclozeaux M, Moniot B, Sudbeck P, et al. Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Mullerian hormone gene. Mol Cell Biol. 1998; 18(11):6653–65. PMID: 9774680.

22. Hong CY, Park JH, Seo KH, Kim JM, Im SY, Lee JW, et al. Expression of MIS in the testis is downregulated by tumor necrosis factor alpha through the negative regulation of SF-1 transactivation by NF-kappa B. Mol Cell Biol. 2003; 23(17):6000–12. PMID: 12917325.

23. Uhlenhaut NH, Treier M. Foxl2 function in ovarian development. Mol Genet Metab. 2006; 88(3):225–34. PMID: 16647286.

24. Thackray VG. Fox tales: regulation of gonadotropin gene expression by forkhead transcription factors. Mol Cell Endocrinol. 2014; 385(1–2):62–70. PMID: 24099863. doi: 10.1016/j.mce.2013.09.034

25. Crispioni L, Deiana M, Lai O, Chieppe F, Uda M, Amati P, et al. The putative forkhead transcription factor FOXL2 is mutated in male pseudohermaphroditism/ptosis/epicanthus inversus syndrome. Nat Genet. 2001; 27(2):159–66. Epub 2001/02/15. doi: 10.1038/84781 PMID: 11175783.

26. Uda M, Ottolenghi C, Crispioni L, Garcia JE, Deiana M, Kimber W, et al. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum Mol Genet. 2004; 13(11):1171–81. PMID: 15056605.

27. Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredested L, Treier AC, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development. 2004; 131(4):933–42. PMID: 14736745.

28. Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, et al. Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell. 2009; 139(6):1130–42. PMID: 20005806. doi: 10.1016/j.cell.2009.11.021
29. Park M, Suh DS, Lee K, Bae J. Positive cross talk between FOXL2 and antimullerian hormone regulates ovarian reserve. Fertil Steril. 2014; 102(3):847–55 e1. PMID: 24973035. doi: 10.1016/j.fertnstert.2014.05.031

30. Park M, Shin E, Won M, Kim JH, Go H, Kim HL, et al. FOXL2 interacts with steroidogenic factor-1 (SF-1) and represses SF-1-induced CYP17 transcription in granulosa cells. Mol Endocrinol. 2010; 24(5):1024–36. PMID: 20207836. doi: 10.1210/me.2009-0375

31. De Baere E, Dixon MJ, Small KW, Jabs EW, Leroy BP, Devriendt K, et al. Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype—phenotype correlation. Hum Mol Genet. 2001; 10(15):1591–600. PMID: 11468277.

32. Block E. Quantitative morphological investigations of the follicular system in women; variations at different ages. Acta Anat (Basel). 1952; 14(1–2):108–23. PMID: 14932631.

33. Block E. [Follicle variations during the genital cycle of woman]. Arch Gynakol. 1953; 183:294–6. PMID: 13125448.

34. Forabosco A, Sforza C. Establishment of ovarian reserve: a quantitative morphometric study of the developing human ovary. Fertil Steril. 2007; 88(3):675–83. PMID: 17434504.

35. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod. 2008; 23(3):699–708. PMID: 18192670. doi: 10.1093/humrep/dem048

36. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab. 1987; 65(6):1231–7. PMID: 3119654.

37. Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update. 2014; 20(5):688–701. PMID: 24821925. doi: 10.1093/humupd/dmu020

38. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril. 2002; 77(3):675–71. PMID: 11872196.

39. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update. 2010; 16(2):113–30. PMID: 19793843. doi: 10.1093/humupd/dmp038

40. Peluso C, Fonseca FL, Rodart IF, Cavalcanti V, Gastaldo G, Christofolini DM, et al. AMH: An ovarian reserve biomarker in assisted reproduction. Clin Chim Acta. 2014; 437:175–82. PMID: 25086280. doi: 10.1016/j.cca.2014.07.029

41. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001; 142(1):4891–9. PMID: 11606457.

42. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004; 10(2):77–83. PMID: 14742691.

43. Jagannathan V, Robinson-Rechavi M. The challenge of modeling nuclear receptor regulatory networks in mammalian cells. Mol Cell Endocrinol. 2011; 334(1–2):91–7. PMID: 20600584. doi: 10.1016/j.mce.2010.06.012

44. Schimmer BP, White PC. Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. Mol Endocrinol. 2010; 24(7):1322–37. PMID: 20203099. doi: 10.1210/me.2009-0519

45. Hoek A, Schoemaker J, Drexhage HA. Premature ovarian failure and ovarian autoimmunity. Endocr Rev. 1997; 18(1):107–34. PMID: 9034788.

46. Conway GS. Premature ovarian failure. Br Med Bull. 2000; 56(3):643–9. PMID: 11255551.

47. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Antimullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002; 143(3):1076–84. PMID: 11861555.

48. Falender AE, Lanz R, Malenfant D, Belanger L, Richards JS. Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary. Endocrinology. 2003; 144(8):3598–610. PMID: 12865342ler AE, Lanz R, Malenfant D, Belanger L, Richards JS. Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary. Endocrinology. 2003; 144(8):3598–610. PMID: 12865342.

A Regulatory Network for AMH Production

PLOS ONE | DOI:10.1371/journal.pone.0159112 July 14, 2016 13 / 13