Identifying mystery sex reversals in mice WORKSHEET Biol4490

LEARNING OBJECTIVES

- To apply critical thinking skills in analyzing data from multiple experimental methodologies to determine the identity of a disorder of sex development (DSD).
- To be able to evaluate the advantages and limitations of experimental methodologies presented in this exercise.

LEARNING GOALS

- To understand the molecular/genetic basis of mammalian sex determination.
- To understand and construct your own workflow containing molecular/genetic information pertaining to 1° and 2° sex determination in mammals.
- To gain an appreciation not only for the molecular/genetic complexity underlying human disorders of sex development but also the functional, sexual and psychological issues.

Case study 5.1 assignment. Due Nov. 4 at 11:59 pm (30 points total)

Instructions:

Group case study exercise: This will be carried out in class in your groups as usual. You will go through sequential experiments to identify the nature of the mystery sex reversal numbers assigned to your group below. We will fill out the results of these experiments on a class jamboard document.

Assigned in-class mystery sex reversals

4490 group	SR numbers
#1	#2
#2	#4
#3	#5

Follow-up group exercise for Case Study 5.1:

1) Solve mystery sex reversals #1 and #3.
2) Complete the guiding questions for sex reversals #1 and #3.

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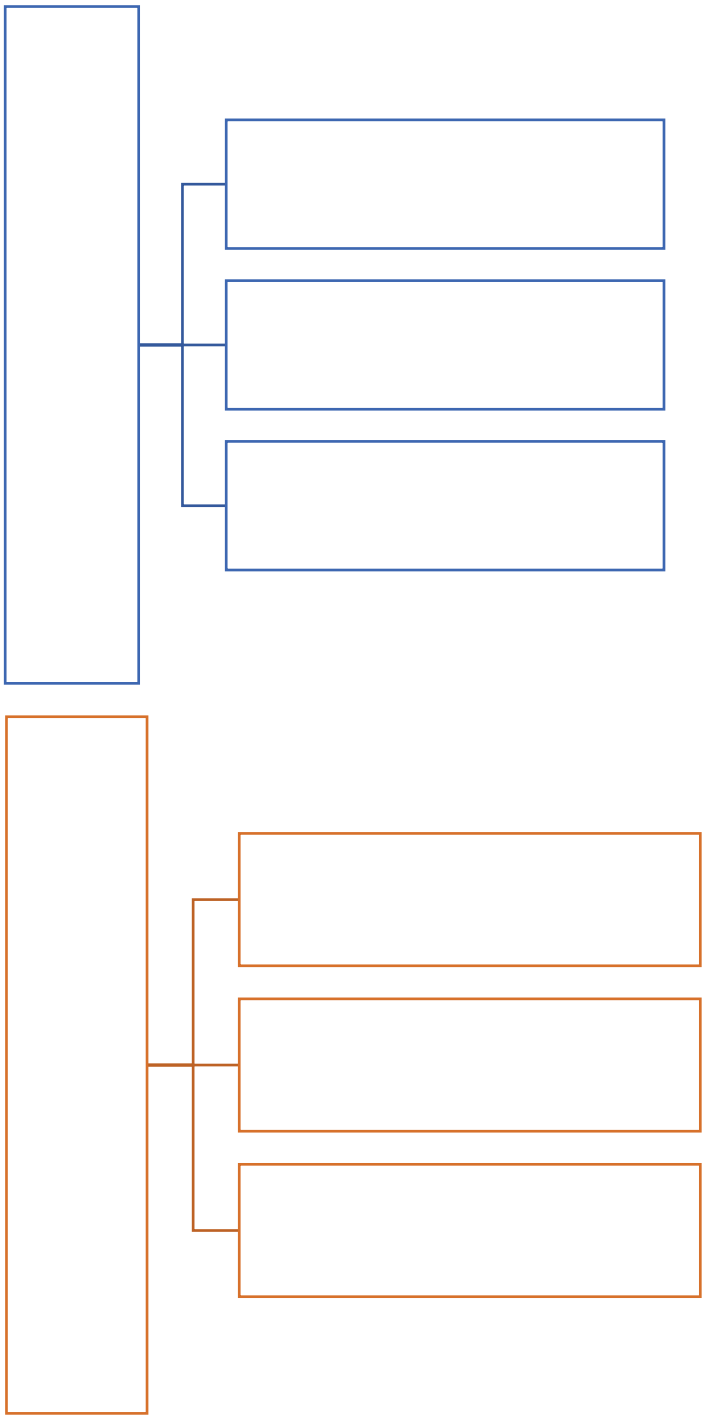
Complete your workflow diagrams for male and female mice. What steps would you take to determine the molecular genetic basis of the sex reversal in your mice? (1 point)



Part A: You will complete experiments #1 to #3 and fill out the columns in Table 1 below.

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1. SR#	2. External phenotype *(Expt#1)* (M/F)	3. Karyotype *(Expt#2) (XX or XY)*	4. Diagnosis of sex reversal (XX or XY)	5. Predicted sex-specific genes	6. Sex-specific genes (PCR, *Expt#3*)	7. ID#	8. Unexpected genes
#1							
#2							
#3							
#4							
#5							

Experiment #1: Characterization of external genitalia

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Fill in the external genitalia phenotype of your mice in Table 1 column 2. (2 points)

Questions

Q1a. What does the examination of the external genitalia tell you about your mice? **(1 point)**

Q1b. What experiment would you carry out next to determine if your mouse has a sex reversal?

(1 point)

Experiment 2: Karyotyping your mice

Analyze the karyotypes of your assigned mice and complete column 3 in Table 1 above.

Questions

Q2a. Based on the karyotype analysis, what is the chromosomal sex of your mouse? (1 point)

Q2b. Fill in the diagnosis of your sex reversal mouse in column 4 of Table 1. (2 points)

Q2c. Which SRs appear to be the same and how? Compare all SRs for the assignment. (2 points)

Q2d. Given your knowledge of the molecular genetics of 1° sex determination, what is your next question for each SR scenario? (1 point)

Q2e. Use the gene glossary to predict which sex-specific genes you would expect to see by PCR to confirm the chromosome identities of your SR mice? Fill in column 5 of Table 1. (1 point)

Experiment #3: PCR Genotyping

After analyzing experiment 3, fill out columns #6-#8 in Table 1.

Questions:

Q3a. Now describe any changes in SR similarities/differences compared to those in question 2c. Outline the consistencies/inconsistencies with respect to the presence/absence of sex-linked genes and external adult genitalia phenotypes. **(5 points)**

DSD#1-

DSD#2-

DSD#3-

DSD#4-

DSD#5-

Q3b. Consult the preliminary information sheets and gene glossary to pose some hypotheses about what happened to your mice. **(1 point)**

DSD#1-

DSD#2-

DSD#3-

DSD#4-

DSD#5-

Part B: Secondary sex determination

Table 2- Analysis of sex-specific mRNA expression in the genital ridge (Experiment #4). Embryonic expression of sertoli AMH and newborn gonad morphology (Experiment #5). It may help to enter your original karyotypes again.

1. SR # and karyotype	2.Wnt4/ Follistatin+ genital ridge (yes/no)	3.Sox9/ Dhh+ genital ridge (yes/no)	4.Meiotic genital ridge cells (yes/no)	5.AMH+ Sertoli cells (yes/no)	6. Internal genitalia (ovaries/testes/ both/neither)	7.Possible genetic scenarios
#1						
#2						
#3						
#4						
#5						

Experiment #4: Embryonic expression of sex-specific genes.

1. Transfer the data from Table 2 of the class jamboard to Table 2 above.
2. Fill out columns 2 to 4 of Table 2 above for SRs #1 and #3.
3. Summarize the *in situ* hybridization results for SRs #1 and #3 below.

Summary: (2 points)

DSD#1-

DSD#2-

DSD#3-

DSD#4-

DSD#5-

Questions:

Q4a. Explain why a combination of *Sox9* and *Dhh* mRNA expression was analysed in the developing genital ridge of your SR mice. **(2 points)**

Q4b. Likewise, why was a combination of Wnt4, Follistatin and Dmc1 expression analysed in the developing genital ridge of your SR mice? **(2 points)**

Experiment #5: Analysis of 2° sex determination

Analyze the results of your experiments and fill out columns 5 and 6 of Table 2 (above).

Questions:

Q5a. What does the presence of AMH in the developing genital ridge indicate? **(1 point)**

Q5b. Is the expression of AMH in the developing gonads of your SR mice consistent with your previous data and with your analysis of the internal and external genitalia? Provide details. **(5 points)**

DSD#1-

DSD#2-

DSD#3-

DSD#4-

DSD#5-

Q5c. Did your analysis of the newborn morphologies of your newborn SR mice help in the diagnosis of your sex reversal?

DSD#1-

DSD#2-

DSD#3-

DSD#4-

DSD#5-

Final question #6.

Consider the following statement: "The Y chromosome is an accurate indicator of a person's external sex organs."

- Do you agree or disagree with this statement? State a CLAIM that addresses the question and support that claim with EVIDENCE and REASONING from the case. (3 points)

Gene Glossary

Gene	Chromosome (cytogenetic) location	Function	Sex reversal	References
Anti-Mullerian hormone (AMH)	*19p13.3*	Causes regression of mullerian ducts. Indicator	XY	https://omim.org/entry/600957?search=AMH&highlight=amh

		of Sertoli cell differentiation.		
Androgen receptor (AR)	Xq12	Nuclear steroid receptor that promotes internal (Wolffian ducts) & external male sex differentiation & testes descent.	XY	McPhaul et al., 1993 https://omim.org/entry/313700?search=androgen%20receptor&highlight=androgen%20receptor Mutated in androgen insensitivity syndrome rendering tissues insensitive to androgens. Affected males have female external genitalia, female breast development, blind vagina, absent uterus and female adnexa, and abdominal or inguinal testes. https://omim.org/entry/300068
ß-Catenin	3p22.1	Essential part of the Wnt signaling pathway. Indicates activation of female gonadal sex determination.	46XX sex reversal	https://ghr.nlm.nih.gov/gene/CTNNB1
Dhh (desert hedgehog)	12q13.1	Member of the hedgehog signalling family. Involved in male gonadal differentiation. Specifies fetal Leydig cell fate after testis has been specified.	46XY https://omim.org/entry/233420	Yao et al., 2001 Defects in this protein have been associated with partial gonadal dysgenesis. https://omim.org/entry/605423?search=desert%20hedgehog&highlight=desert%20hedgehog
Dmc1 (DNA meiotic recombinase 1)	22q13.1	Marker for meiotic germ cells; indicator of ovarian development.		https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:2927
Follistatin	5q11.2	Regulator of activin and other members of the TGF-beta superfamily. Stimulated by β-catenin to promote pre-		https://omim.org/entry/136470?search=follistatin&highlight=follistatin https://pubmed.ncbi.nlm.nih.gov/15162500/

		granulosa cell formation.		
FOXL2 forkhead box L2	*3q22.3*	Transcription factor with DNA-binding motif called forkhead box. Ovarian development and fertility.	XX sex reversal	https://omim.org/entry/605597?search=Foxl2&highlight=foxl2 Promotes female and represses male gonad fates by repressing sox9 (Uhlenhaut et al. (2009); Ottolenghi et al. (2007)).
RSPO1	1p34.3	R-SPONDIN 1	XX sex reversal	https://omim.org/entry/609595 Promotes wnt1 signaling by inhibiting ZNRF3.
SF1	9q33.3	Steroidogenic factor 1 (Sf1) is an orphan nuclear receptor		https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:7983
SOX9 SRY-box transcription factor 9.	17q24.3	Promotes Sertoli cell differentiation. Upstream indicator of testes specification.	LOF=XY reversal GOF=XX reversal	https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:11204 Positively regulates itself, testes genes and AMH. Negatively regulates ß-catenin transcription.
SRY sex determining region Y.	Yp11.2	HMG box transcription factor. Triggers fetal genital ridge to form a testis by activating sox9	LOF=XY reversal GOF=XX reversal	Koopman et al., 1991; Wang et al., 2013a, b The SRY gene is located in the pseudoautosomal region and can aberrantly cross over from the Y chromosome to the X chromosome.
Testosterone		Promotes internal (Wolffian ducts) & external male sex differentiation & testes descent.		
Wnt4	1p36.12	Wnt family member 4 is an indicator of ovarian fate specification.	XX sex reversal	https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/HGNC:12783
WT1	11p13	Wilms tumor-1 is a zinc finger transcription factor that activates *Sry*.	Frasier syndrome. Heterozygous WT1+/-	https://ghr.nlm.nih.gov/gene/WT1 Hossain & Saunders, 2001 https://omim.org/entry/607102#29

Xist (X inactive specific transcript)	Xq13.2	Paternal X inactivation	Paternal X inactivation	
Zfy	Y	Zinc finger TF-the completion of the second meiotic division and sperm morphogenesis/function		Vernet et al., 2014; 2016

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http://www.jbc.org/content/276/20/16817.long In 3 patients with Frasier syndrome, Barbaux et al. (1997) identified mutations in the donor splice site of intron 9 of the WT1 gene (607102.0018; 607102.0019) with a predicted loss of the so-called +KTS isoform. Normally, an alternative splice site in intron 9 allows the addition of 3 amino acids (KTS) between the third and fourth zinc fingers of the WT1 protein. All 3 patients had male pseudohermaphroditism.

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