Regulation of Sex Determination

Activity
Educator Materials

Overview

This activity accompanies the HHMI BioInteractive module Sex Verification of Athletes (https://www.hhmi.org/biointeractive/testing-athletes). It provides students with exercises to explore the aspects of human anatomy and physiology associated with determination of biological sex as it pertains to sex verification in elite athletes. The specific focus of this activity deals with variation in testosterone production. Students are tasked with evaluating testosterone levels from elite athletes and individuals with differences in sex development (DSDs) and, using quantitative reasoning, evaluating the effectiveness of using testosterone levels to determine an individual's biological sex, especially in terms of sex verification in athletes.

Key Concepts

- Many gene products determine the development of biological sex characteristics in humans.
- Like many traits, biological sex, consist of a spectrum of phenotypes.
- Biological differences result in both males and females having wide ranges of testosterone levels, as can be seen in data collected from elite athletes.

Student learning Targets

- GOAL 1: Understand how sex determination is regulated.
  - LO1: Explain the roles of the SRY gene and testosterone in regulating sex determination.
  - LO2: Predict the effects of genetic variations on sex determination in humans.
- GOAL 2: Interpret biological data in relation to sex determination
  - LO3: Using the graph provided, determine the range of serum testosterone levels.
  - LO4: Use data to predict the impact of DNA mutations on testosterone levels in humans with DSDs.

Key terms

allosome, autosome, barr body, biological sex, chromosome, data analysis, deoxyribonucleic acid, differences in sex development (DSD), differentiation, endogenous, estrogen, external sex characteristics, gel electrophoresis, gender identity, genotype, gonads, hormone, mullerian duct, ovaries, phenotype, puberty, ribonucleic acid, secondary sex characteristics, sex determination , sex verification tests, somatic cell, sex-determining region Y (SRY) gene, testes, testosterone, transcription factor, wolffian duct, x-inactivation

Time Requirements

- Students should review the Sex Verification in Athletes BioInteractive Click and Learn prior to class = 20 minutes outside of class
- Sex Verification in Athletes Introduction Video and Review of Sex Verification in Athletes BioInteractive = 10 min (Optional, depending on class preparedness)
- Think-write-pair-share on video questions = 5 min
- Small group break out and activity instructions = 5 min
- Data analysis worksheet in small groups = 30 min
- Class debrief (student presentations) = 10 min

suggested audience

- High-School (upper level biology/anatomy & physiology, dual-enrollment); Lower Level College (Freshman/Sophomore, Introductory Biology for majors/non-majors, Introductory Human Anatomy and Physiology)

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Prior Knowledge

Students should be able to

- Explain the process of information transfer in cells and multicellular organisms.
    - Explain the Central Dogma of Molecular Biology
    - List the steps in information transfer
    - Identify the types of errors that can occur in information transfer, specifically in protein production
    - Differentiate between allosomes and autosomes.
- Explain what hormones are and their methods of action (in general terms).
- Explain the relationship between chromosomes and genes.
- Demonstrate a basic knowledge of meiosis.

Procedure

It is imperative that students review the HHMI BioInteractive Click and Learn "Sex Verification in Athletes"prior to attempting the in-class activity. This activity can be assigned prior to the in-class activity as homework. The web-link includes a supplemental handout that can be used as an assessment of preparedness for the in-class activity.

In the in-class activity, students will explore data in the form of a bar graph. Serum testosterone levels in female versus male elite athletes has been previously characterized (Healy, 2014). The data reveals a wide range of testosterone levels in both males and females and a great deal of overlap between the two biological sexes. After analyzing data representing typical serum testosterone levels in athletes, students are asked to predict testosterone levels in individuals with differences in sexual development (DSDs) and will compare their predictions to actual data. As such, students will build an understanding of biological sex determination, including how hormones regulate the development of sex characteristics, and thus phenotype. In so doing, students will also make the link between genetics and phenotype.

While students should have worked through HHMI BioInteractive's "Sex Verification in Athletes" prior to class, the instructor may want to play the Introduction video as an engagement piece. Questions at the end of the video may be discussed as a class or in think-pair-share. Students then work in groups of 2-4 students on one of four versions of the activity. The activity has been divided into four separate groups, each with a different DSD to explore. The students work together to analyze the graph and answer the questions in their Part 1. This part of the activity includes a prediction question. Once the students have made a prediction of testosterone levels they expect in individuals with their DSD, the instructor provides the group with Part 2 of the activity, the actual data to compare their prediction to. If the instructor feels that students are not prepared for making the predictions asked of them in the activity, the data sheets (Part 2) may be distributed with Part 1 allowing students to see the data while making their predictions. After completing the exercise, groups may present their DSDs to the class and/or a class discussion held.

Background (optional)

This student activity is based on the HHMI BioInteractive resource "Sex Verification in Athletes". The debate of how to categorize individuals as female versus male for the purpose of sport has long existed and was recently brought to light once again with the case of the elite female athlete, Dutee Chand, who has naturally occurring high testosterone levels. The question is: should females with high testosterone be allowed to compete as a female? The case of Dutee Chand is used as an engagement piece and the associated Click and Learn in the HHMI resource that accompanies this activity helps students to discover how genes and testosterone regulate sex determination in humans.

Materials:

- Four different group handouts accompany this activity. The group activity handouts will need to be downloaded and printed in advance - one of the four handouts per group.
- Each handout contains 3 pages:
    - Page 1 is the same across all handouts; it asks students to evaluate the bar graph data presented in the Healy et al (2014) paper.
        - Observing trends in data
        - Determining data ranges
        - Prediction/data evaluation related to biological sex determination is introduced
    - Page 2 introduces the differences in sex development (DSD) that students will be responsible for analyzing.
    - Page 3 provides unique data sets containing testosterone levels in individuals with various differences in sex development (DSD) and may be kept from students until after they complete the prediction questions on Page 2.

Suggested Student Grouping:

- Groups of 2-4 students work best for these activities.
- Each group will receive one of the four group handouts.
- End of class discussion about the DSDs and predictions to cover the Quantitative Reasoning aspect can be done by either letting each group report out, or by having groups with the same DSD have a large group discussion and then report out their successes (results) and concerns.

Scaffolding Suggestions to prepare for activity:

- The Pre-lesson Homework with Readiness Assessment Quiz are optional, but sets the stage for the Sex Verification of Athletes Click and Learn
- Sex Verification in Athletes Click and Learn
    - The Click and Learn contains a section on Human Development that covers most of the information students will need to be familiar with to complete the activity.
    - The Case Studies section of the Click and Learn can be used to refresh students about DSDs and the phenotypic presentations. Also acts as a good start to making predictions based on genetic and phenotypic differences associated with DSDs.
        - HHMI Biointeractive has a downloadable worksheet to accompany the Click and Learn. It can be accessed at https://www.hhmi.org/biointeractive/testing-athletes. The worksheet can be used as a pre-class assignment or as a scaffold/visual organizer for completing the group activity.

Differentiation for varied learners:

- Sex Development Powerpoint available to accompany the Sex Verification of Athletes Click and Learn if instructors choose to review in class
    - Students can use the powerpoint in notes mode to follow along with in class presentation
- HHMI Biointeractive has a downloadable worksheet to accompany the Click and Learn. It can be accessed at https://www.hhmi.org/biointeractive/testing-athletes. The worksheet can be used as a pre-class assignment or as a scaffold/visual organizer for completing the group activity.
- Prediction questions: If the instructor feels that students are not prepared for making the predictions asked of them in the activity, the data sheets (Part 2 of group activity) may be distributed with Part 1, allowing students to see the data while making their predictions.

COMMON MISCONCEPTIONS:

- Students may think that sex development is binary (ie, XX = female and XY = male).
- Students may have difficulty in distinguishing between gender and biological sex.
- Students may struggle with the idea of both a hormone and receptor being required for full physiological impact of the hormone.

SUGGESTED FORMATIVE/SUMMATIVE ASSESSMENTS:

- Pre-lesson quiz can be used as an online quiz to determine preparedness for the class activity.
- In-class quiz is intended to be filled out as information is presented in class.
- Think-Pair-Share questions at end of video can be used as a formative assessment for identifying misconceptions prior to the in-class activity
- In-class activity can serve as a scaffold for prediction and exploration of other DSDs as formative or summative assessments on exams.

ANSWER KEY

In-class activity

All Groups

1. Based on the graph above, please describe the trends you see in the testosterone levels of elite males and female athletes. (Who has more? What is the distribution?)
Biological males tend to have a higher level of serum testosterone than females, although there is significant overlap of the ranges of male and female levels.

2. What is the range of testosterone levels measured in the male athletes displayed in the graph? What is the range of testosterone levels measured for the female athletes? Do the ranges overlap? (Remember to use labels.)
Male Range: 1-41 nmol/L
Female Range: 0-32 nmol/L

3. a. If an individual had a serum testosterone level of 9 nmol/L, would you be able to definitively determine the individual's biological sex? Why or why not?
It would not be possible to definitely predict the biological sex of an individual with this testosterone level given that it falls within the range of both males and females.

b. What would your prediction of the individual's biological sex be? Please explain your answer.
Male. This level is more typical of a male and would be high for a female, but there are many females with testosterone serum levels at or above 9 nmol/L.

Group 1: 46, XY Androgen Insensitive Females

a. Given what you have learned, would these individuals have the SRY gene? Why or why not?
These individuals do have the SRY gene because they have a typical Y chromosome.

b. Would you predict that these individuals be able to produce levels of testosterone typical for biological males? Why or why not?
There is no information provided that would suggest there were issues with testosterone production and the individuals may be producing levels typical of individuals with an SRY gene. However, there is the possibility that a lack of positive feedback in testosterone signaling and differences in the development of the male gonads may result in lower levels of serum testosterone.

c. Looking at the range of testosterone levels from the graph above, please predict an approximate serum testosterone level for a 46, XY androgen insensitive female elite athlete. (This estimate need not be exact.) Please explain your reasoning.
There is not a right or wrong answer, but students should recognize that there is at least some testosterone present.

d. What is the range of serum testosterone levels that were measured for these individuals?
1.0-54.1 nmol/dL (53.1nmol/L spread)

e. What is the average of the serum testosterone levels measured for these individuals?
19.7 nmol/dL

f. How far off was your prediction from the average? Was it within the range?
This will depend upon each group.

g. Given what you have learned from the data, please explain why 46, XY androgen insensitive females have this level of testosterone.
46, XY androgen insensitive females have testosterone levels within the normal male range. These individuals have the SRY gene and there is no indication that there would be anything wrong with the testosterone production pathway, so we would expect male levels of testosterone. The individual is not female due to a lack of testosterone, but rather because is that there is no response to the testosterone that is there.

Group 2: 46, XY Females with mutations in SRY/Testosterone Pathway Genes
a. Given what you have learned, would you expect these individuals to have a Barr body in their cells? Why or why not?
Since the individuals do not have two X chromosomes, we would not expect a Barr body.

b. Would you predict that these individuals be able to respond to the lower levels of testosterone that is produced? Why or why not?
You would expect a typical testosterone response given that there is nothing to indicate that the androgen receptors are mutated or that signaling is disrupted.

c. Looking at the range of testosterone levels from the graph above, please predict an approximate serum testosterone level for a 46, XY female elite athlete with one of these mutations in SRY or other testosterone production proteins. (This estimate need not be exact.) Please explain your reasoning.
Student should recognize that testosterone levels will be lower than typical for a male.

d. What is the range of serum testosterone levels that were measured for these individuals?
3.9-13.6 nmol/L (9.7nmol/L spread)

e. What is the average of the serum testosterone levels measured for these individuals?
9.4 nmol/L

f. How far off was your prediction from the average? Was it within the range?
This will depend upon each group.

g. Given what you have learned from the data, please explain why 46, XY females with mutations in SRY or another gene involved in testosterone production have this level of testosterone.
46, XY females have testosterone levels that are on the higher edge of the female range and the lower edge of the male range. This could be for many reasons, but is likely because the mutations in SRY/testosterone

production is mutation that complete prevents function. Additionally, SRY also induces expression of the testosterone receptor such that one of these individuals may have high testosterone but not be very responsive to it. The testosterone was likely below threshold levels at key points in development or not properly detected and responded to, but is still being produced to some degree.

Group 3: 46, XX SRY-Positive Males

a. Given what you have learned, would these individuals have functional androgen (testosterone) receptors and be able to respond to testosterone? Why or why not?
Yes, there is nothing to indicate that the receptor or signaling pathway is atypical.

b. Would you predict that these individuals be able to produce levels of testosterone typical for biological males? Why or why not?
The students have learned that SRY is driving male levels of testosterone production, although they may (correctly) predict that other genes on the Y chromosome/incomplete SRY genes/the presence of a second X chromosome results in testosterone levels on the lower end of the male spectrum.

c. Looking at the range of testosterone levels from the graph above, please predict an approximate serum testosterone level for a 46, XX SRY-positive male elite athlete. (This estimate need not be exact.) Please explain your reasoning.
The students should predict a level in the typical male range, possibly lower within the range.

d. What is the range of serum testosterone levels that were measured for these individuals?
3.3-13.0 nmol/L (9.7nmol/L spread)

e. What is the average of the serum testosterone levels measured for these individuals?
7.1 nmol/L

f. How far off was your prediction from the average? Was it within the range?
This will depend upon each group.

g. Given what you have learned from the data, please explain why 46, XX SRY-positive males have this level of testosterone.
The average testosterone level for 46, XX SRY-positive males is on the low end of the male range and the higher end of the female range. This is likely because SRY is not the only gene that induces testosterone production, often the entire SRY gene is not present in these individuals, and SRY also induces expression of the testosterone receptor such that even at low levels the individual would be more responsive to testosterone. While the presence of SRY would cause testosterone to be expressed at key times in key locations during development and the response to its expression may be stronger, the presence of the second X chromosome, the lack of other genes, and the possibility of an incomplete SRY gene would lead to lower levels of testosterone for a male.

Group 4: 47, XXY Males

a. Given what you have learned, would these individuals have functional androgen (testosterone) receptors and be able to respond to testosterone? Why or why not?

Yes, there is nothing to indicate that that receptor or signaling pathway is atypical. Also, given that they did develop into biological males, we would assume they were able to respond to hormones that drive male sexual differentiation.

b. Given what you have learned, would you expect these individuals to have a Barr body in their cells? Why or why not?

Since the individuals do not have two X chromosomes, we would not expect a Barr body.

c. Looking at the range of testosterone levels from the graph above, please predict an approximate serum testosterone level for a 47, XXY male elite athlete. (This estimate need not be exact.) Please explain your reasoning.
These individuals may have lower than typical levels of male hormones, although many different hypotheses could be justified.

d. What is the range of serum testosterone levels that were measured for these individuals?
1.4nmol/L-25.3nmol/L (23.9nmol/L spread)

e. What is the average of the serum testosterone levels measured for these individuals?
9.5nmol/L

f. How far off was your prediction from the average? Was it within the range?
This will depend upon each group.

g. How does the average and range of serum testosterone levels for 47, XXY males compare to those of typical males and females?
Males with 47, XXY are on the low end of the male range for testosterone but above average for females. They are generally infertile. There are hypotheses that this is due to incomplete X activation, but scientists still do not fully understand why.

Readiness Assessment Quiz (OPTIONAL--Accompanies the Test Your Knowledge of Sex Development Click and Learn):
Answers in **BOLD *Italics:***

1. A human who is XX and has male genitalia, would likely have:
   a. an additional X chromosome.
   b. a piece of the Y chromosome containing the SRY gene.
   c. any piece of the Y chromosome.

2. In order to have an XY mammal develop as a female:
   a. the X chromosome would have to be removed.
   b. the X and Y chromosomes would both have to be removed.
   c. the part of the Y chromosome containing the SRY gene would have to be removed.
   d. there is no way of making this mouse develop female.

3. Climate change experts suggest that temperatures are increasing, what could happen to reptiles?
   a. There will be more females than males.
   b. There will be more males than females.
   c. The numbers of males and females will become equal.
   d. Temperature does not impact reptile sex determination.

4. Individuals with Triple X syndrome have an extra X chromosome, thus an individual with this disorder will likely develop biologically as:
   a. male.
   b. female.

c. both male and female.

5. A fruit fly has the genotype XXY. It will develop as:
   a. male.
   b. female.
   c. both male and female.
   d. neither male nor female.

In-class Quiz (OPTIONAL--accompanies the Sex Verification in Athletes Click and Learn)
Answers in **BOLD *Italics:***

1. What is testosterone?
   a. a disaccharide (carbohydrate)
   b. a steroid (lipid)
   c. a polypeptide (protein)
   d. a form of RNA (nucleic acid)

2. What is an autosome?
   a. A full 23 pairs of chromosomes
   b. The X chromosome
   c. The Y chromosome
   d. Any chromosome other than the X or Y chromosome

3. What is a Barr body?
   a. The active X chromosome of a pair
   b. The inactivated X chromosome of a pair
   c. A mutated X chromosome with an SRY gene
   d. A mutated Y chromosome missing the SRY gene

4. Where are Barr bodies typically found?
   a. in the nucleus
   b. in the nucleolus
   c. on a ribosome
   d. floating in the cytoplasm

5. Where are the precursors to the female reproductive system?
   a. Wolffian ducts
   b. Mullerian ducts
   c. The ureter
   d. Cloaca

6. What type of protein does the SRY gene encode?
   a. a transcription factor
   b. a hormone
   c. a structural protein

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Optional Resources

- The Test Your Knowledge of Sex Determination Click and Learn Activity (http://www.hhmi.org/biointeractive/test-your-knowledge-sex-determination) can be used to introduce students to other, non-mammalian mechanisms of sex determination.
- Sex Verification of Athletes Click and Learn Activity and accompanying worksheets (https://www.hhmi.org/biointeractive/testing-athletes) prepare students for in-class activity.
- Sex Development Powerpoint can be used to introduce/review concepts in class prior to introducing the activity.

Authors

Jessica Allen, Columbia College of South Carolina, jallen@columbiasc.edu, South Carolina, USA
Christie Landry, Nicholls State University, christie.landry@nicholls.edu, Louisiana, USA
Laura Pickell, Heritage College, lpickell@cegep-heritage.qc.ca, Quebec, CA
Deborah Taylor, University of Kansas, dtaylor@ku.edu, Kansas, USA