Understanding X and Y Variations: Genetic Diagnosis, Terminology & Next Steps Planning

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Important Terminology

Chromosome = structure that carries DNA

- Most people have 46 chromosomes total (23 pairs)

Aneuploidy = additional or missing chromosome(s)

Sex chromosome aneuploidies (SCA) = additional or missing X & Y chromosome(s)

Sex Chromosome Trisomies = 3 Sex Chromosomes
47,XXY = XXY = Klinefelter Syndrome (KS)
47,XYY = XYY = Jacobs Syndrome
47,XXX = XXX = Trisomy X Syndrome

Sex Chromosome Tetrasomies = 4 Sex Chromosomes
48,XXYY = XXYY
48,XXXY = XXXY
48,XXXX = XXXX

Understanding Chromosome Notation

47,XXY

Total number of chromosomes

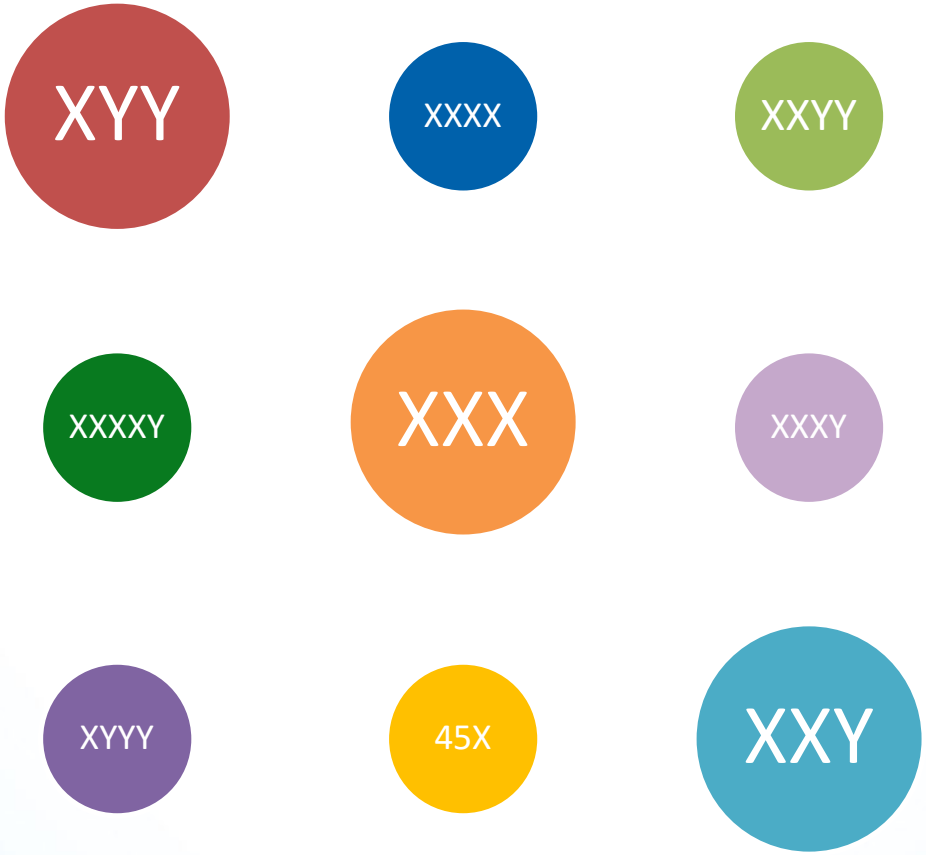
Which sex chromosomes are present;

This person has 2 X chromosomes and 1 Y chromosome

Many names, but all the same…

- Sex Chromosome Aneuploidy (SCA) - scientific term
- Sex Chromosome Abnormality
- Sex Chromosome Anomaly
- Sex Chromosome Disorders
- Sex Chromosome Variation
- X&Y Chromosome Aneuploidy
- X&Y Chromosome Variation - families often prefer

X&Y Variations



Incidence

MALES:

47,XXY = Klinefelter syndrome	1:650 males
47,XYY = Jacobs syndrome	1:1000 males
48,XXYY	1:18,000 males
48,XXXY	1:18,000 males
48,XYYY	1:80,000 males
49,XXXXY	1:250,000 males

FEMALES:

47,XXX = Trisomy X syndrome	1:1000 females
48,XXXX = Tetra X	1:25,000 females
49,XXXXX = Penta X	1:50,000 females
45,X = Turner syndrome	1:2500 females

Combined: ~1:400 live births

How are X&Y Variations Identified?

Historically:

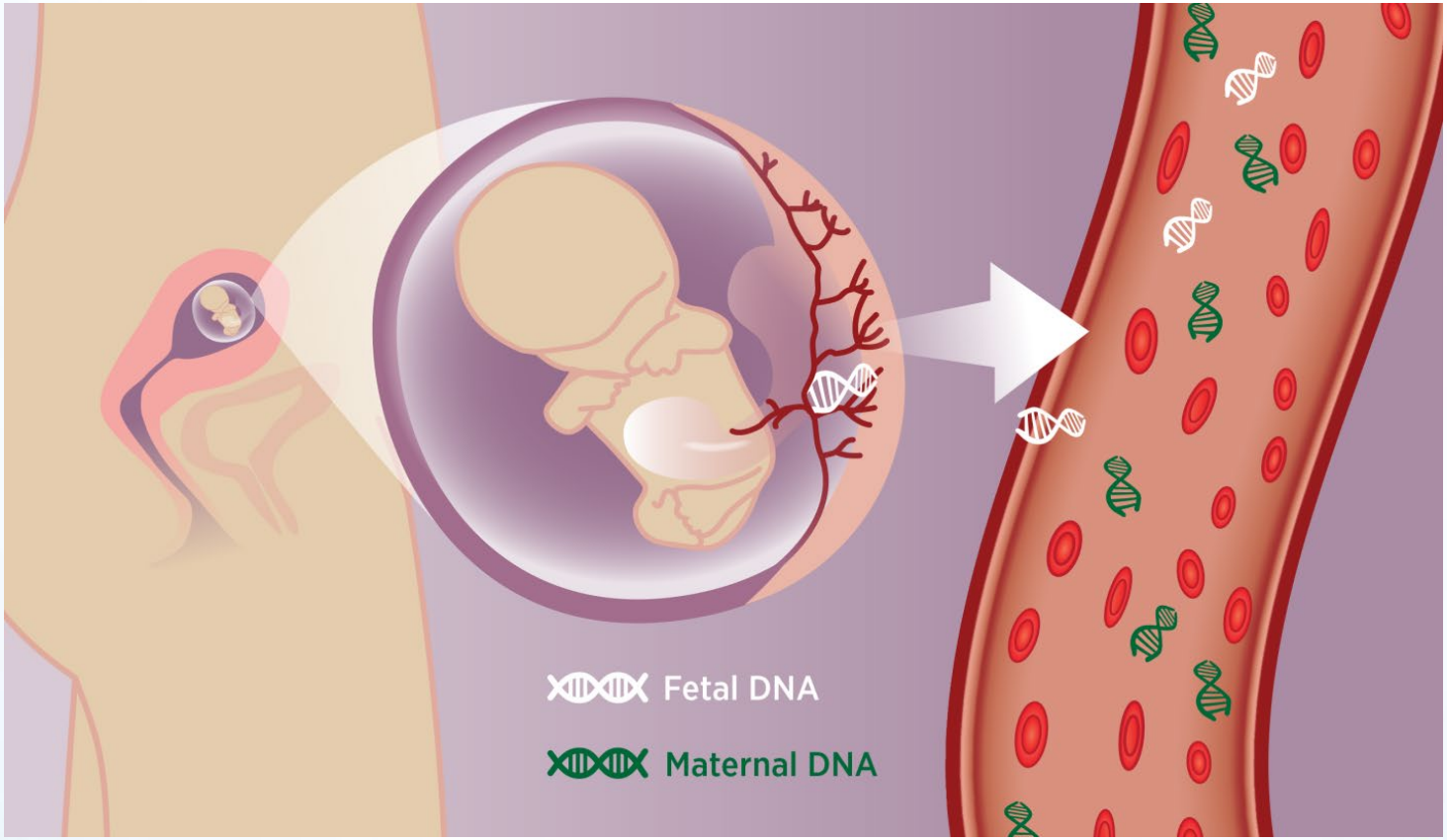
- Congenital abnormalities
- Global Developmental Delay
- Hypotonia
- Autism

More likely to be identified if more physical symptoms

Historically underdiagnosed - many features (ADHD & Anxiety) didn't trigger genetic testing

New Technology Identifying X&Y Variations Today

Prenatal cell-free DNA (cfDNA) Screening/Non-invasive prenatal screening



Prenatal cell-free DNA (cfDNA) Screening

- Other terms: NIPS or NIPT
- Commercially available in 2013
- ACOG recommended for all pregnant people as of 2020
- Performed as early as 10 weeks
- Possible results: positive, negative, or inconclusive
- Not Diagnostic – identifies risk for aneuploidy
- Risk for Discordance

Original Research

Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing

*Diana W. Bianchi, MD, Lawrence D. Platt, MD, James D. Goldberg, MD, Alfred Z. Abuhamad, MD, Amy J. Sehnert, MD, and Richard P. Rava, PhD, on behalf of the MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group**

PRENATAL **DIAGNOSIS**

ORIGINAL ARTICLE

Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma

Amin R. Mazloom[1†], Željko Dzakula[1†], Paul Oeth[1], Huiquan Wang[1], Taylor Jensen[1], John Tynan[1], Ron McCullough[1], Juan-Sebastian Saldivar[1], Mathias Ehrich[2], Dirk van den Boom[2], Allan T. Bombard[1,2], Margo Maeder[2], Graham McLennan[2], Wendy Meschino[3], Glenn E. Palomaki[4],

Jiang et al. BMC Medical Genomics 2012, 5:57
http://www.biomedcentral.com/1755-8794/5/57

BMC Medical Genomics

TECHNICAL ADVANCE **Open Access**

Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies

Fuman Jiang[1†], Jinghui Ren[2†], Fang Chen[1†], Yuqiu Zhou[3], Jiansheng Xie[4], Shan Dan[5], Yue Su[5], Jianhong Xie[3],

- No risk of miscarriage
- Identifies increased risk for trisomy 13, 18, 21, and SCA
- Not diagnostic = follow-up testing needed
- Poor positive predictive value (PPV) in SCAs (20%+)
  - *PPV = probability that a person who tests positive for condition actually has condition*

ACMG: Meta Analysis of 28 studies

	Sensitivity	Specificity	PPV Likelihood positive is TRUE positive	NPV	Accuracy
Overall	**99.63** (94.38-99.98)	**99.80** (99.69-99.88)	**43.13** (37.92-48.50)	**100** (99.99-100)	**99.78** (99.71-99.83)
45,X (Turner)	**97.68** (84.25-99.70)	**99.84** (99.67-99.92)	**29.52** (22.72-37.36)	**100** (99.98-100)	**99.82** (99.71-98.89)
47,XXY (Klinefelter)	**99.25** (78.13-99.98)	**99.99** (99.98-99.99)	**74.05** (59.47-84.73)	**100** (99.98-100)	**99.98** (99.98-99.99)
47,XYY (Jacobs)	**100** (0-100)	**99.99** (99.99-100)	**74.45** (58.40-85.81)	**100** (0-100)	**99.99** (99.99-100)
47,XXX (Trisomy X)	**100** (0-100)	**99.97** (99.96-99.98)	**53.95** (40.58-66.77)	**100** (0-100)	**99.97** (99.96-99.98)

Discordant Results

Example: cfDNA vs XXYY Diagnosis in eXtraordinarY Babies Study

12 Cases of cfDNA+ for Trisomy with Tetrasomy 48,XXYY Result

cfDNA Result	Cytogenetic Result	Fetal Fraction (%)	Maternal Pre-pregnancy BMI (kg/cm^2)	Maternal Age Range at Birth (yrs)
XXY	48,XXYY	7.0	26.5	30-34
XXY	48,XXYY	NR	22.4	30-34
XXY	48,XXYY	4.0	36.6	35-39
XYY	48,XXYY	6.0	25.6	20-24
XXY	48,XXYY	6.1	30.2	30-34
XXY	48,XXYY	NR	22.1	45-49
XXY	48,XXYY	13.0	28.3	30-34
XXY	48,XXYY	11.0	20.4	30-34
XXY	48,XXYY	4.8	27.2	20-24
XXY	48,XXYY	7.2	NR	35-39
XYY	48,XXYY	7.1	24	20-24
XXY	48,XXYY	19.0	37.5	35-39

NR = Not Reported, cfDNA = Prenatal Cell-Free DNA Screening, BMI = Body Mass Index

Diagnostic Genetic Testing Procedures:

Prenatal

- CVS (10-12 weeks gestation)
- Amniocentesis (15+ weeks gestation)

Postnatal:

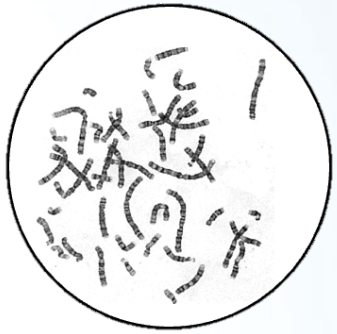
- Blood test or cheek swab/saliva test

Several genetic testing methods available

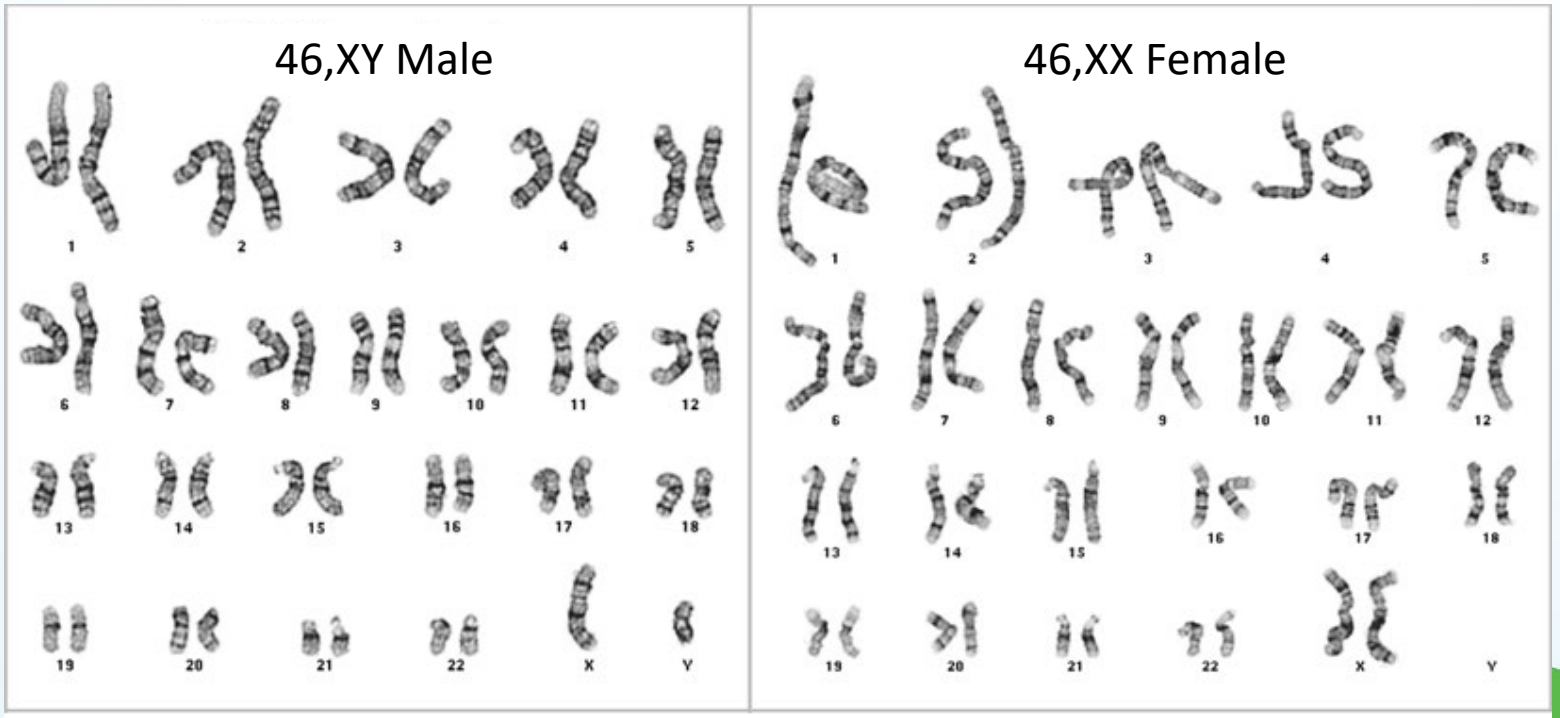
Genetic Testing Methods

Karyotype

- Count number of chromosomes
- Look at structure of chromosomes

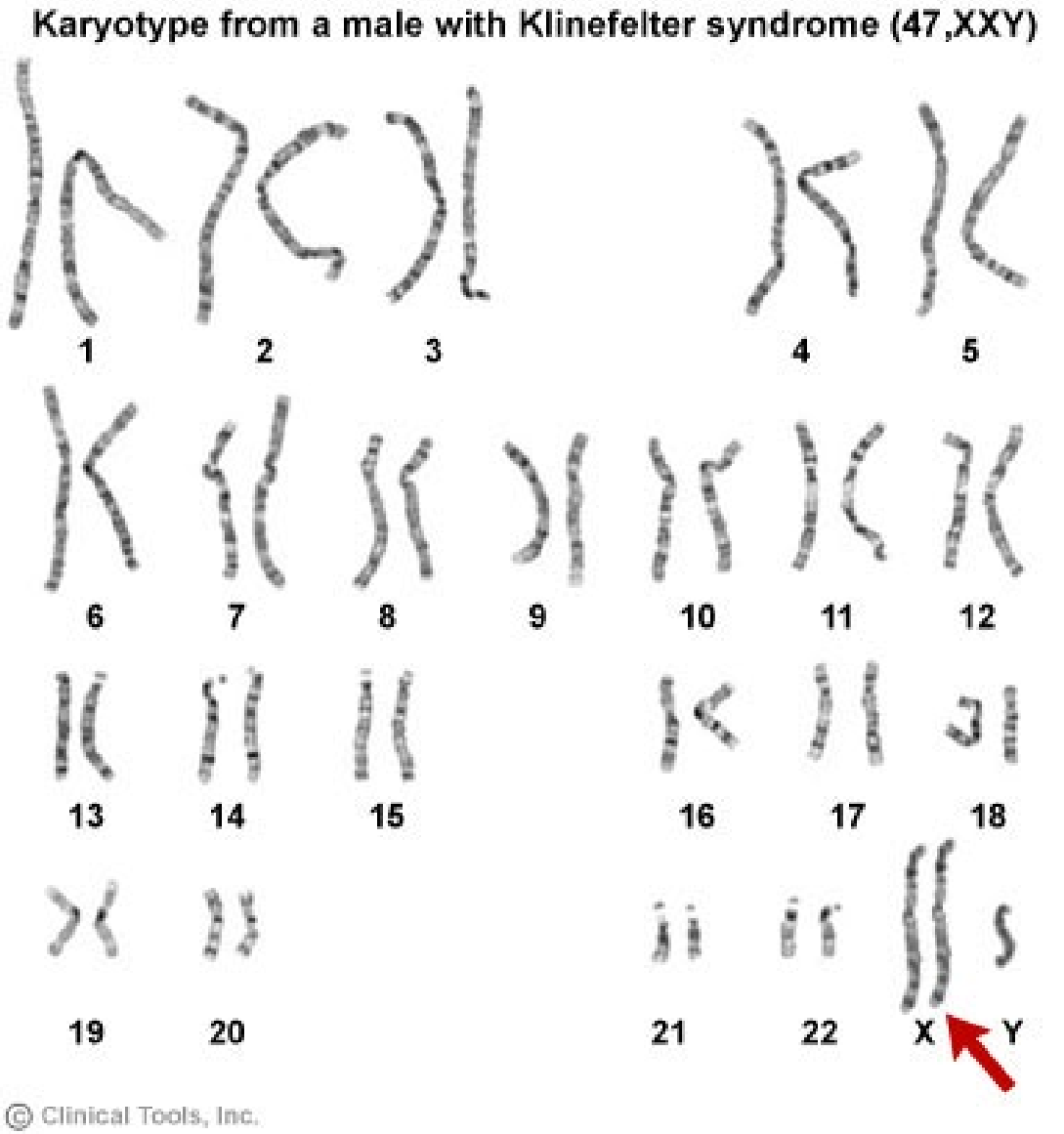


Before organization

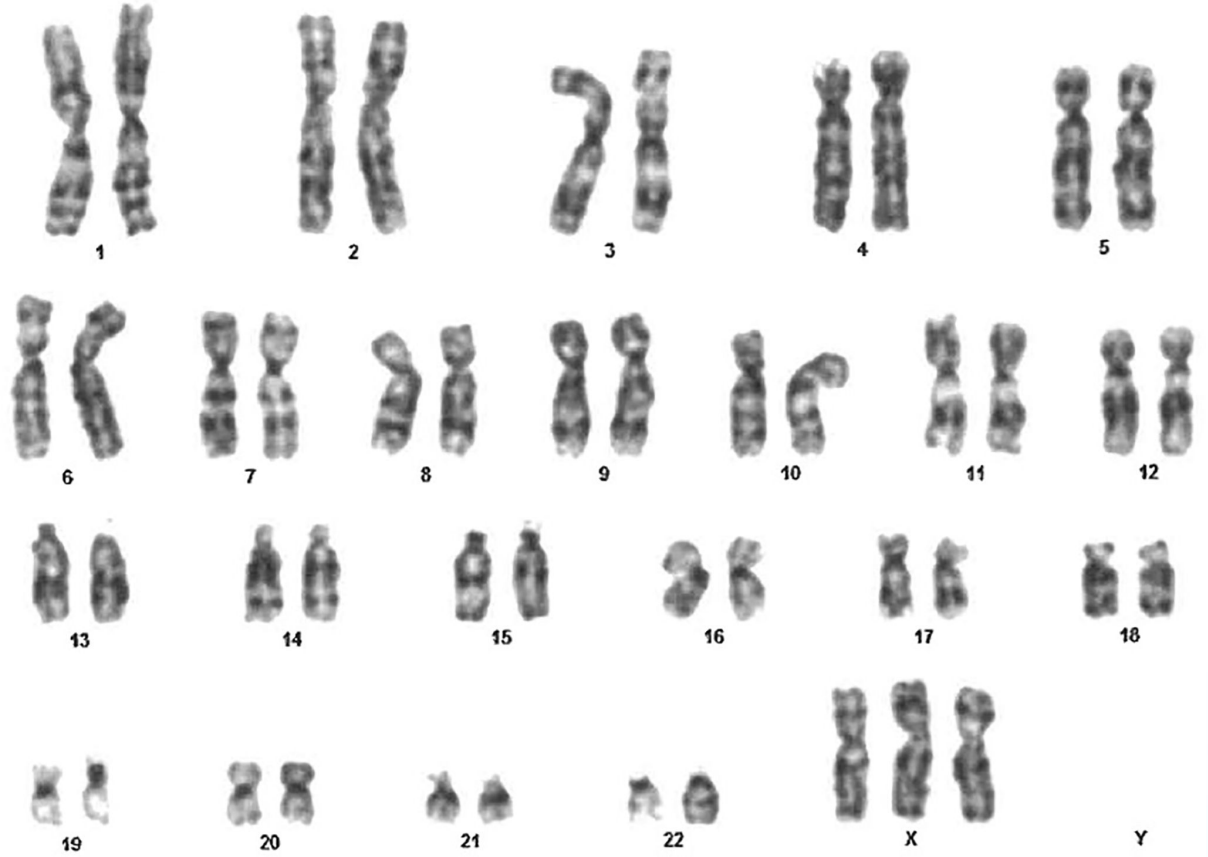


Source: W.W. Hay, M.J. Levin, R.R. Deterding, M.J. Abzug
Current Diagnosis & Treatment: Pediatrics, 24th Edition

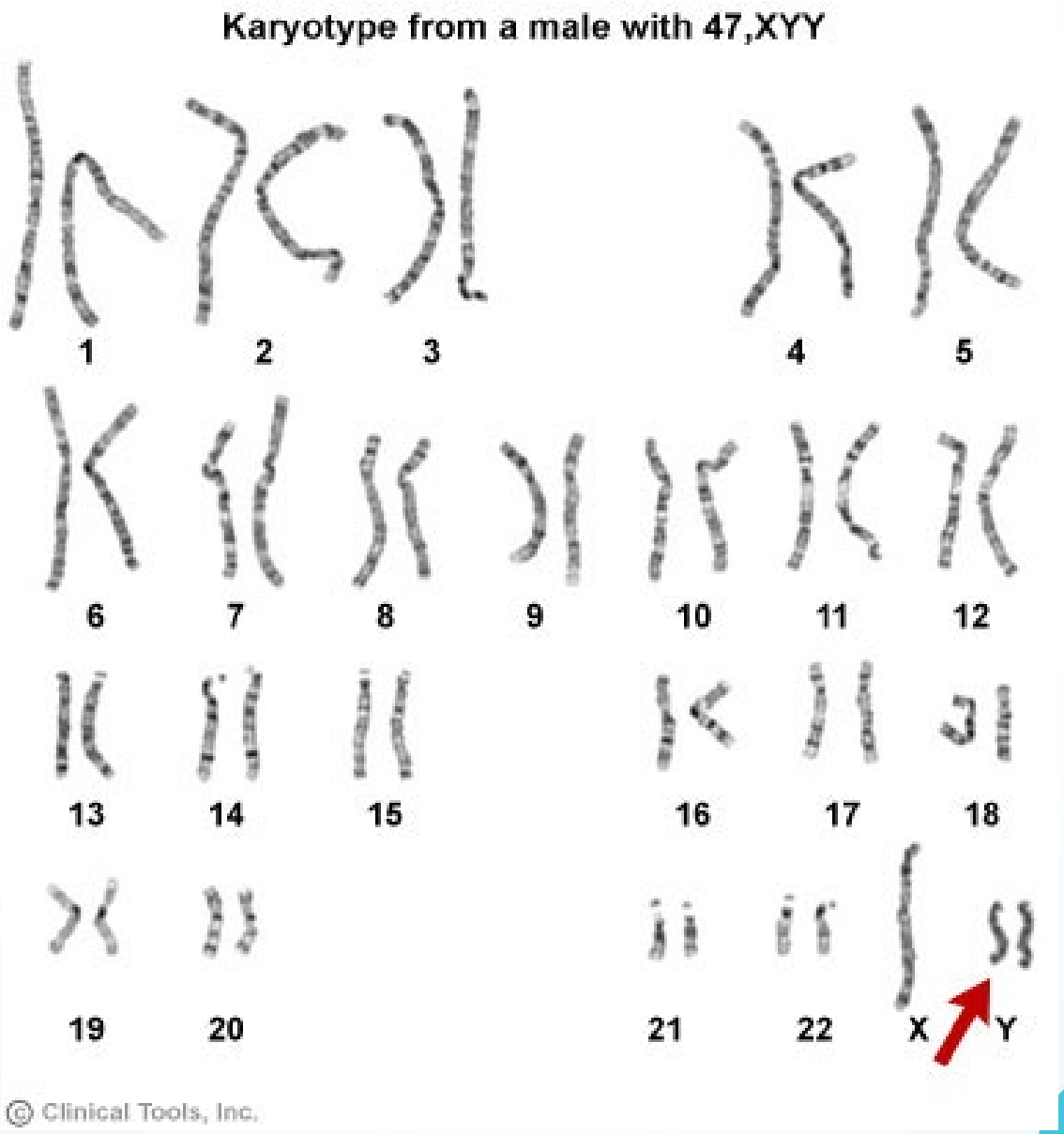
Karyotype Result: 47,XXY



Karyotype Result: 47,XXX



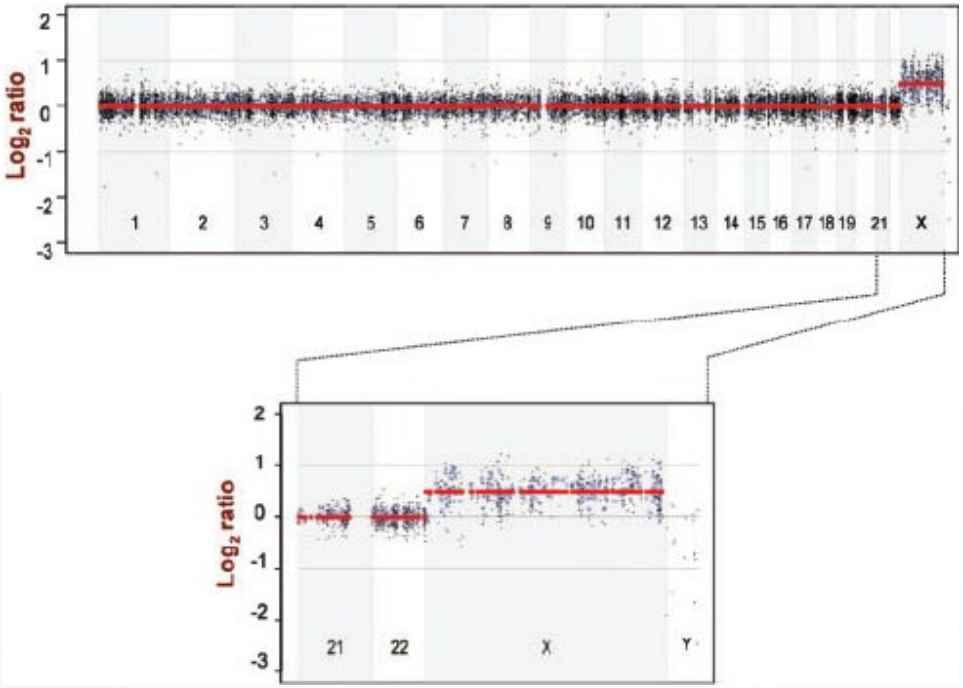
Karyotype Result: 47,XYY



Genetic Testing Methods

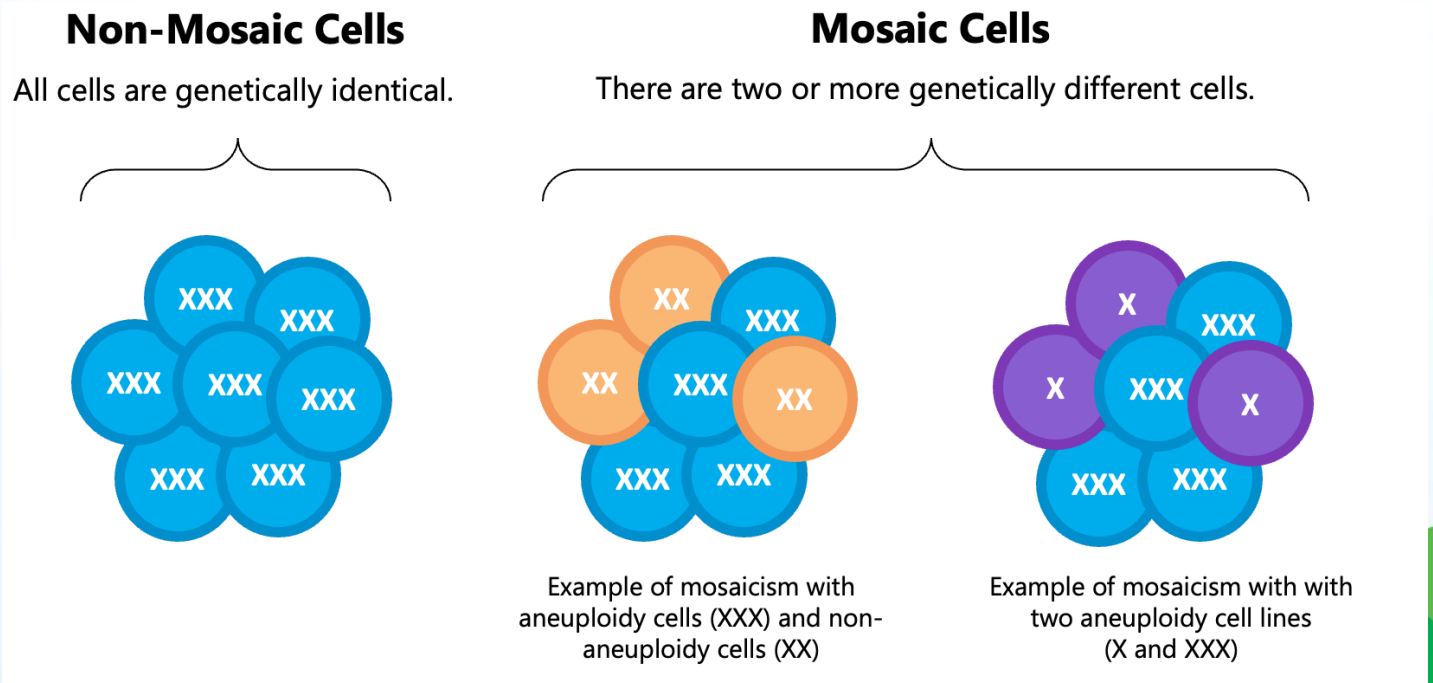
Microarray

- Compare sample DNA to a reference to see if there are missing or extra pieces of DNA
- Can find breakpoints, deletions, duplications
- Does not detect balanced translocations



Mosaicism

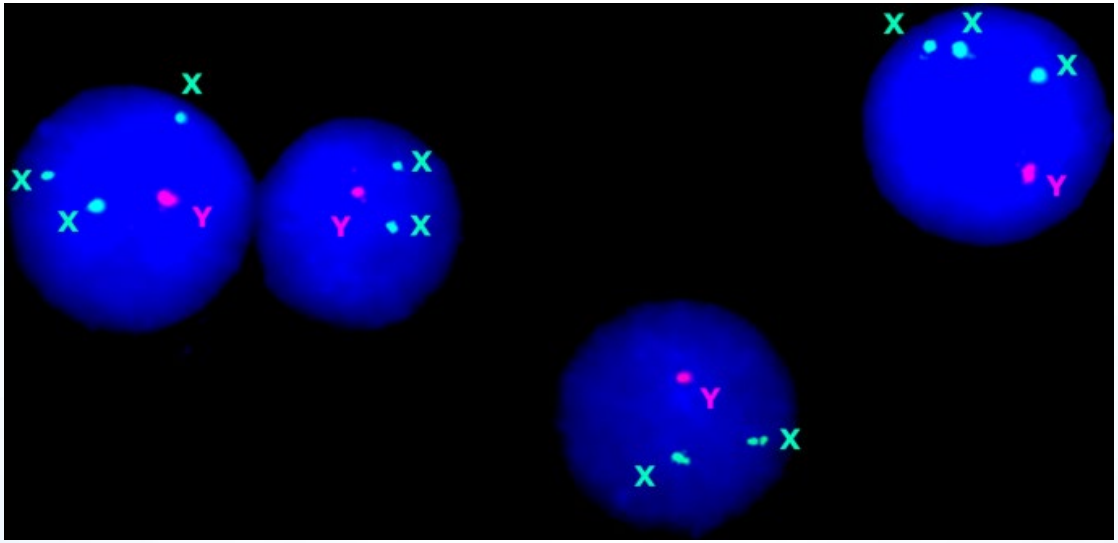
- Presence of multiple genetically different cell lines:
  - *Example: mos 47,XXY [17]/48,XXYY[3] (85% XXY, 15% XXYY)*
- Prognosis depends on cell lines present - not always milder
- Different parts of the body may have different ratios of the cell lines
- Important to have follow-up FISH testing if mosaicism is indicated
  - *Standard Karyotype looks at ~20 cells, mosaic testing looks at ~200+ cells*



Genetic Testing Methods

Florescent In Situ Hybridization (FISH)

- Uses probes that bind to a specific chromosomes and glow
- Can identify what chromosomes are present by number of glowing spots
- Evaluate 200+ cells
- More sensitive for detecting mosaicism



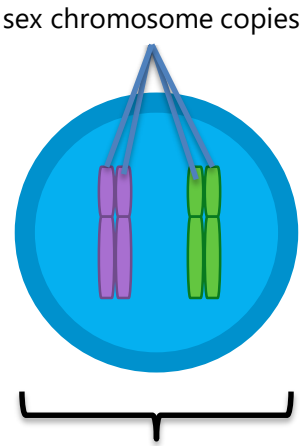
What causes X&Y Variations?

- **Nondisjunction:** When chromosomes fail to separate properly during cell division.
  - During formation of sperm or egg (meiosis)
  - Or occurs after conception (during mitosis) – leads to mosaicism
- **Not caused by something that a parent did or did not do before or during pregnancy.**
  - This is not your fault!

Nondisjunction in Meiosis I

Sperm or Egg Precursor Cell

Each cell has 23 pairs of chromosomes, including a pair of sex chromosomes.

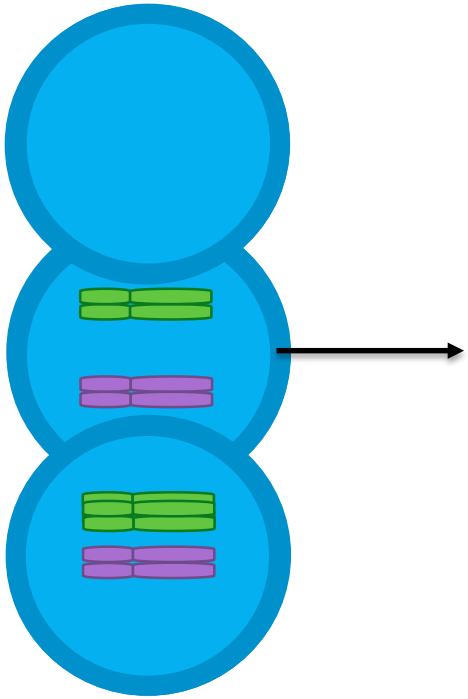


Replication

Each chromosome is copied.

Meiosis I

Nondisjunction: The cells do not split chromosomes evenly.



Meiosis II

The cells divide again, splitting chromosomes evenly.

These cells have one extra sex chromosome.

If combined with a sperm or egg, this will produce a trisomy.

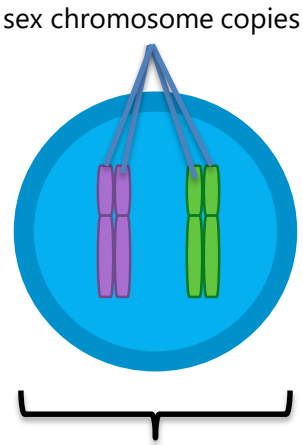
Nondisjunction in Meiosis II

Meiosis II

Nondisjunction: The cells do not split chromosomes evenly.

Sperm or Egg Precursor Cell

Each cell has 23 pairs of chromosomes, including a pair of sex chromosomes.

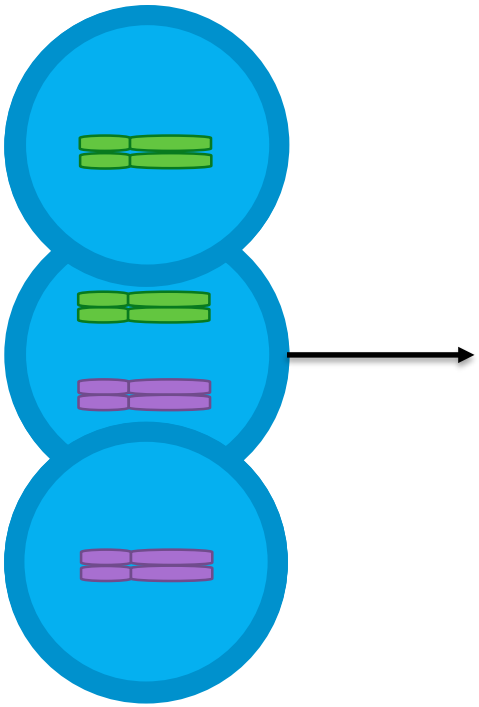


Replication

Each chromosome is copied.

Meiosis I

The cell divides into two, splitting chromosomes equally.

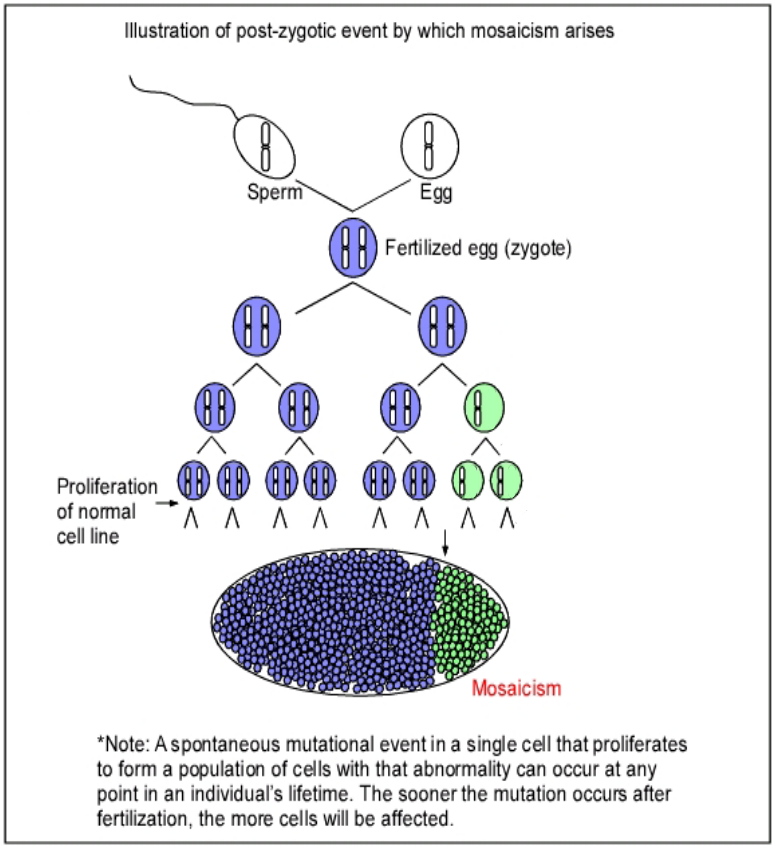


This cell has one extra sex chromosome.

If combined with sperm or egg, will produce a trisomy.

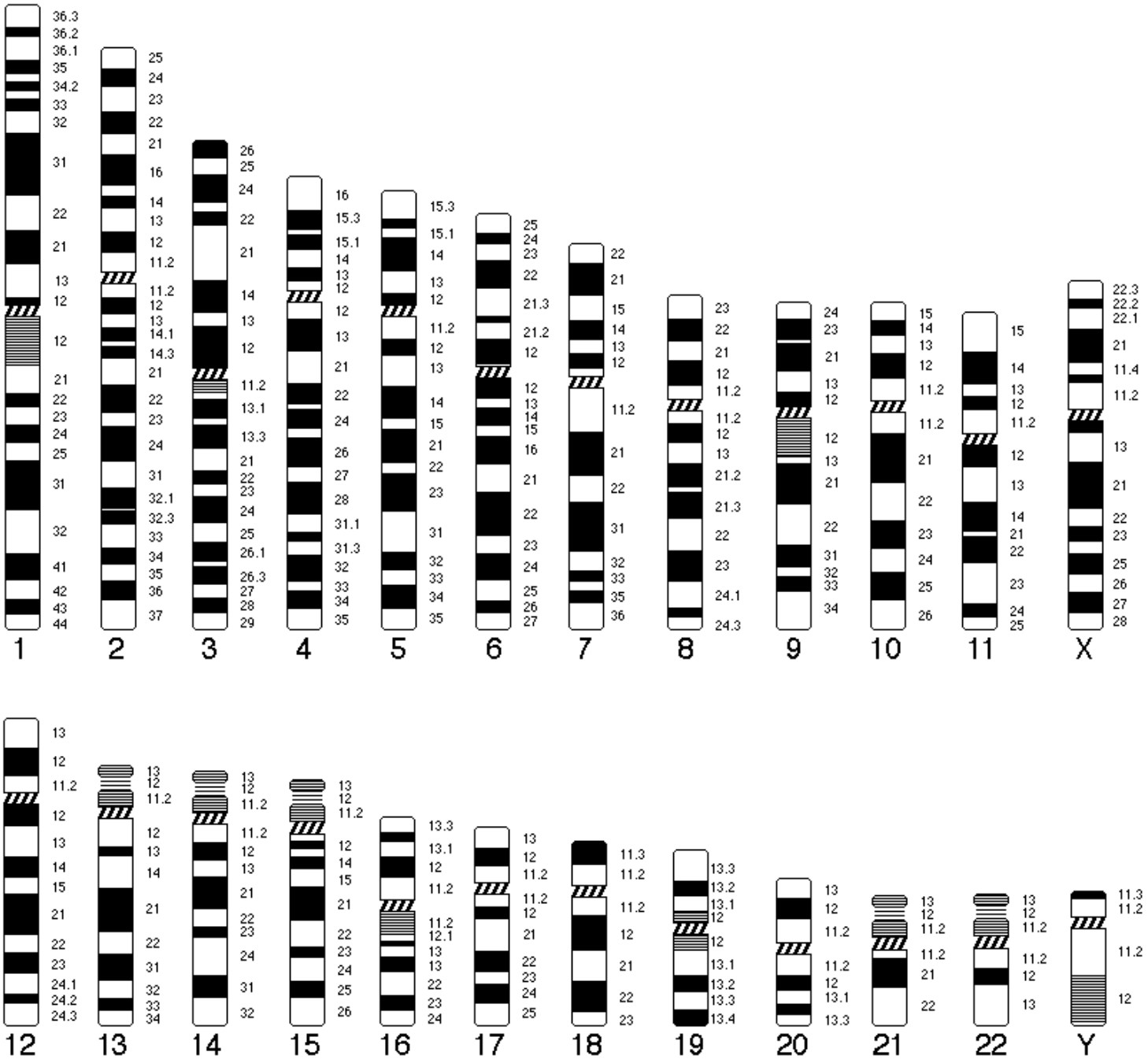
Mosaicism

- Mosaicism occurs when nondisjunction occurs after conception (during mitosis)
  - This is called post-zygotic nondisjunction
  - Cells before nondisjunction do not have aneuploidy; cells after nondisjunction do
  - End up with two genetically different cells in same person



Common Counseling Points

1. cfDNA needs to be confirmed. Always.
2. Keep a copy of the lab report
   - Helpful to know aneuploidy vs. translocations vs. mosaic
3. Couples who have a child with a SCA typically have a <1% risk of recurrence (or AMA risk)
   - Rarely inherited (passed on), although this is possible if rare parental rearrangements or translocations are present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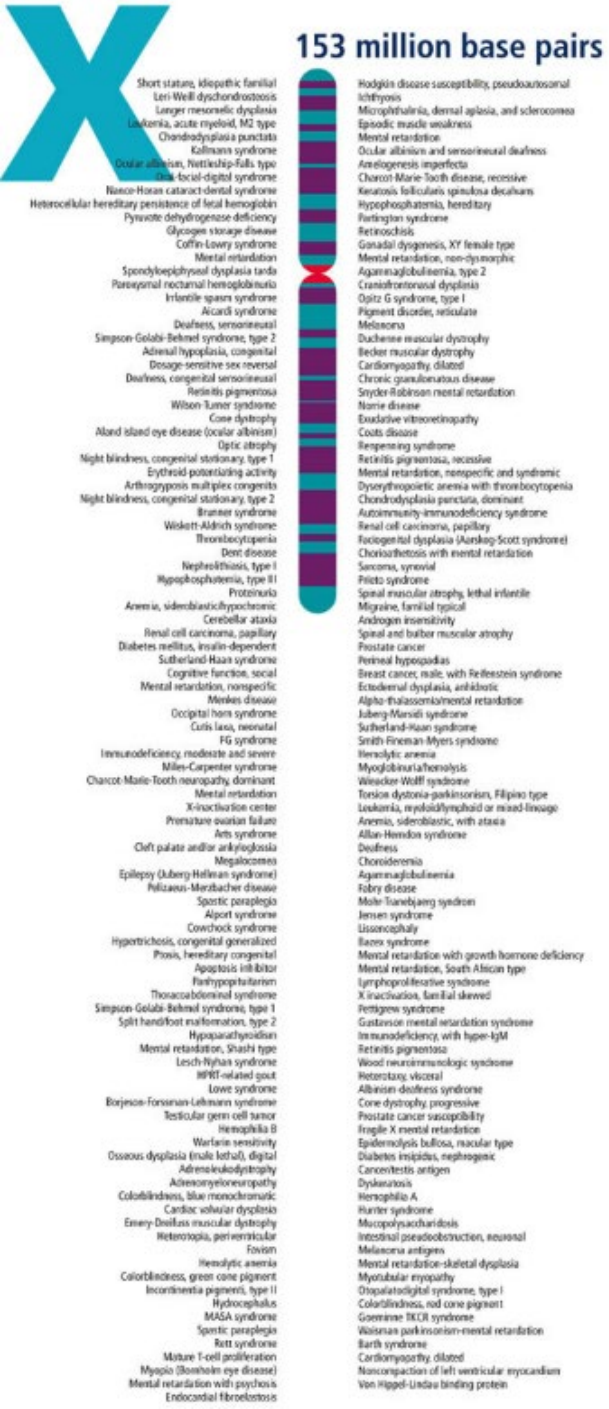
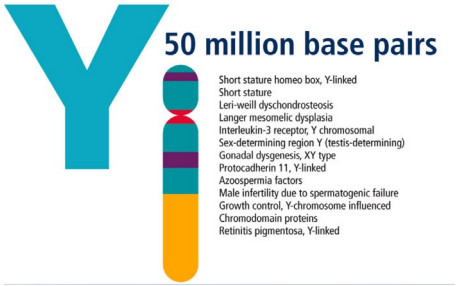
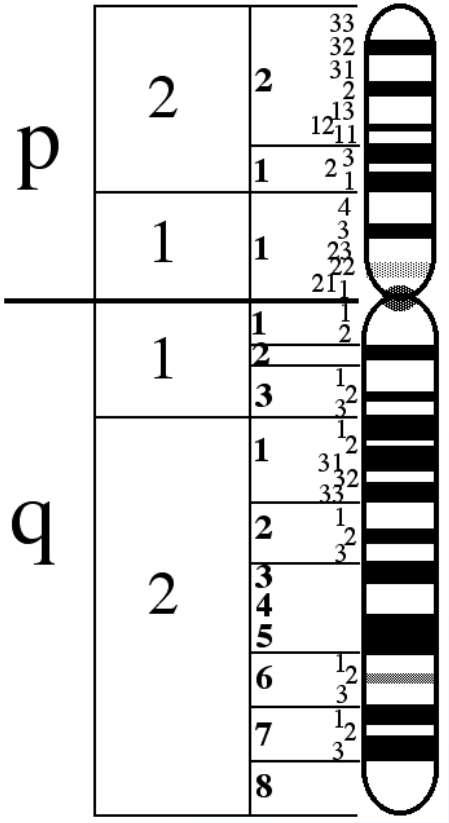
Understanding Chromosomes







Humangenomeproject.gov

X-inactivation

- Typically, males have one X chromosome, and females have two
  - To compensate for this difference, in XX females one X chromosome is “inactivated” and turns off gene expression
- Occurs early in embryonic development
- It is typically random as to which X-chromosome is inactivated in each cell (~50/50)

However – not 100% of X chromosome is inactivated

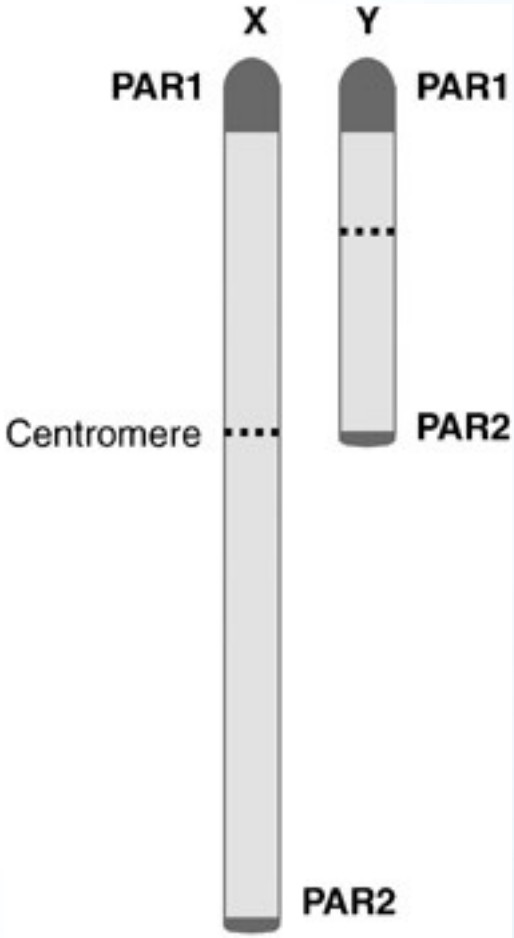
Pseudoautosomal Regions (PARs)

Both the X and Y chromosome have two pseudoautosomal regions:

- PAR1 is on the short-arm tips of both X & Y chromosomes
- PAR2 is on the long-arm tips of both the X & Y chromosomes

These regions are NOT inactivated on X chromosomes

- One X chromosome is kept active; all other extra X chromosomes are typically randomly inactivated
- However, each inactive X-chromosome continues to express genes in the Pseudoautosomal Regions (PARs)
  - For XXY and XXX there is an “extra dose” of genes expressed from the PARs



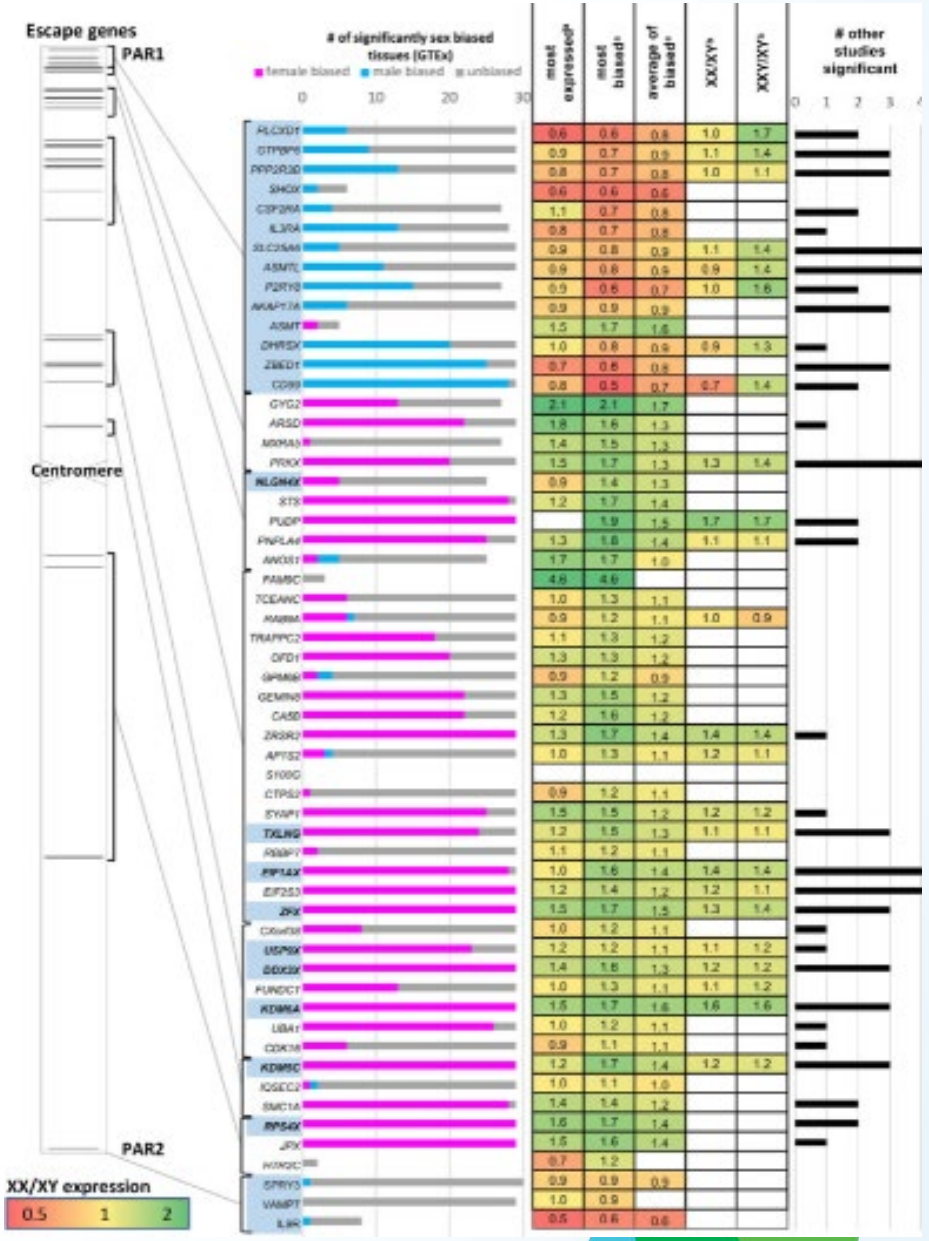
http://www.elsevierimages.com/images/vpv/000/000/028/28512-0550x0475.jpg 7/28/2011

Prior Hypothesis: Gene Expression

Over-expression of X-chromosome genes which escape inactivation is hypothesized to cause the differences in children with extra X chromosomes

Over-expression = more genes making protein = more protein

Also applies to extra Y chromosome gene expression

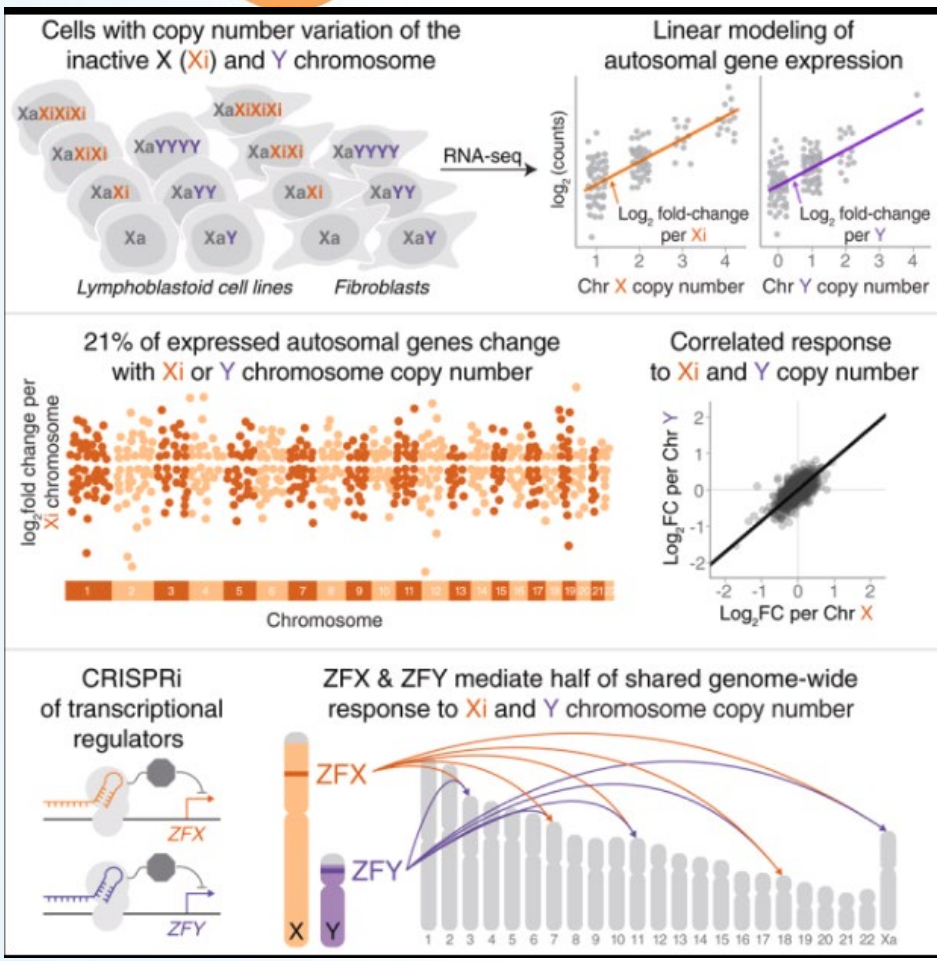


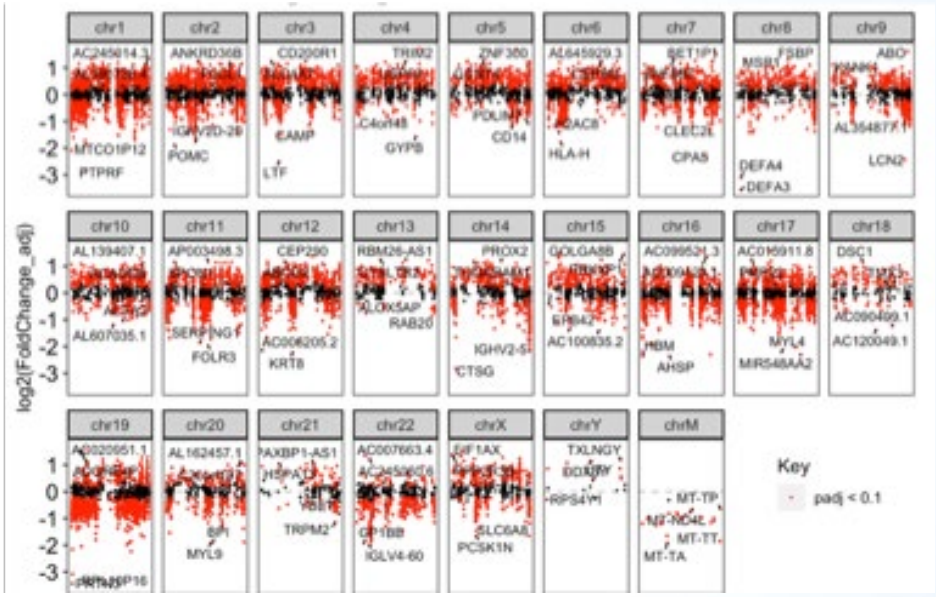
Navarro-Cobos et al 2020

Prior Hypothesis: Androgen Receptor (AR) Gene

- Makes AR protein - causes physiological changes in response to androgens (hormones like testosterone)
- On X Chromosome
- Has a CAG repeat sequence (CAGCAGCAGCAGCAG)
  - Number of CAG repeats is inversely correlated with the functional response of the androgen receptors to androgens
  - Longer repeat length associated with increased body height, decreased bone density, decreased testicular volume, and gynecomastia
  - Fewer number of CAG repeats may have better response to Testosterone therapy

More Recent Hypothesis





San Roman et al 2024

Likely it's not simple…

Biological Factors

- Epigenomics
- Methylomics
- Metabolomics
- Transcriptomics
- Proteomics
- Microbiomics

Environmental Factors

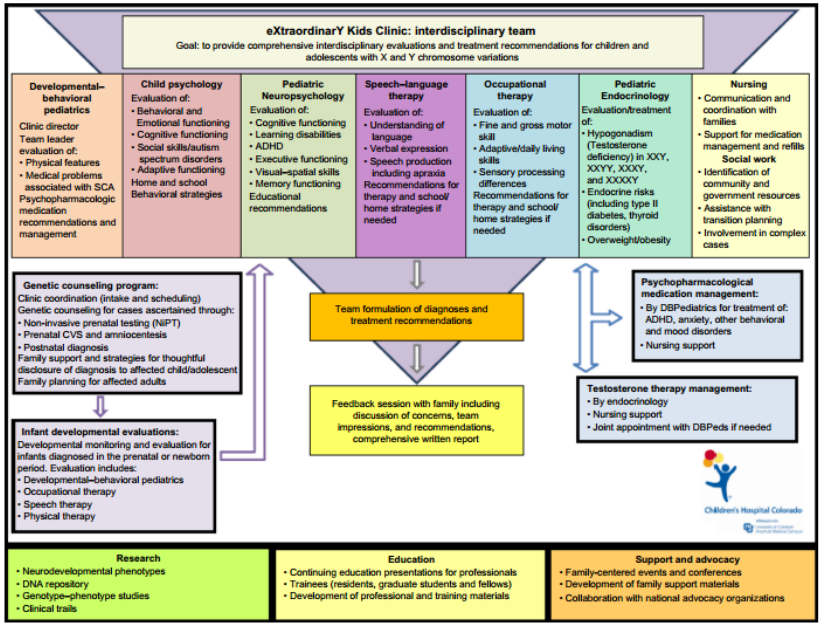
- Prenatal environment
- Nutrition
- Education
- Physical Activity
- Healthcare
- Combination of multiple!

We are more than just our genes! Many biological and environmental factors influence us

Focusing on what matters…

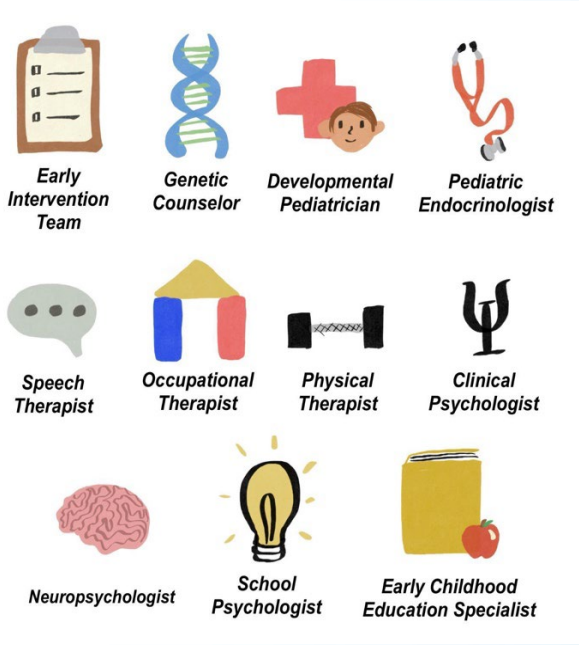
Next Steps Planning

- Once a diagnosis is made, then what?
- Children are individuals and this diagnosis does not define who they are.
  - Understanding strengths and weaknesses = what they need
    - Evaluations based on age, concerns, history
- Evaluating:
  - Physical features
  - Medical features
  - Developmental features
  - Psychological features
    - Learning
    - Behavior



Supporting Kids with SCA

- Development – can be monitored + supported by **developmental pediatrician, child psychologist, or EI team**
- Speech/language – can be monitored + supported by **speech language pathologist**
- Motor skills - can be monitored + supported by **occupational therapist and/or physical therapist**
- Learning and behavior - can be monitored + supported by **psychologist or neuropsychologist**
- Growth and puberty – can be monitored + supported by a **pediatric endocrinologist**



Other resources too (EI, school supports, private therapists, tutors, etc.)

Focusing on Strengths

Lots of strengths we see in people with SCA

Positive Character Strengths Reported by Parents of Children with SCA	
Kindness	Love of learning
Eagerness to please	Creativity
Perseverance	Teamwork

Thompson, T., Davis, S., Takamatsu, S., Howell, S., & Tartaglia, N. (2021). Exploring academic and character strengths in students with sex chromosome aneuploidies. Journal of Positive School Psychology, (FirstView articles), 1-13

"He is polite, gentle, and shares things with others"

"She is very creative and thinks 'outside the box''

"He is like a sponge and soaks up information he hears on subjects that he likes"

Summary

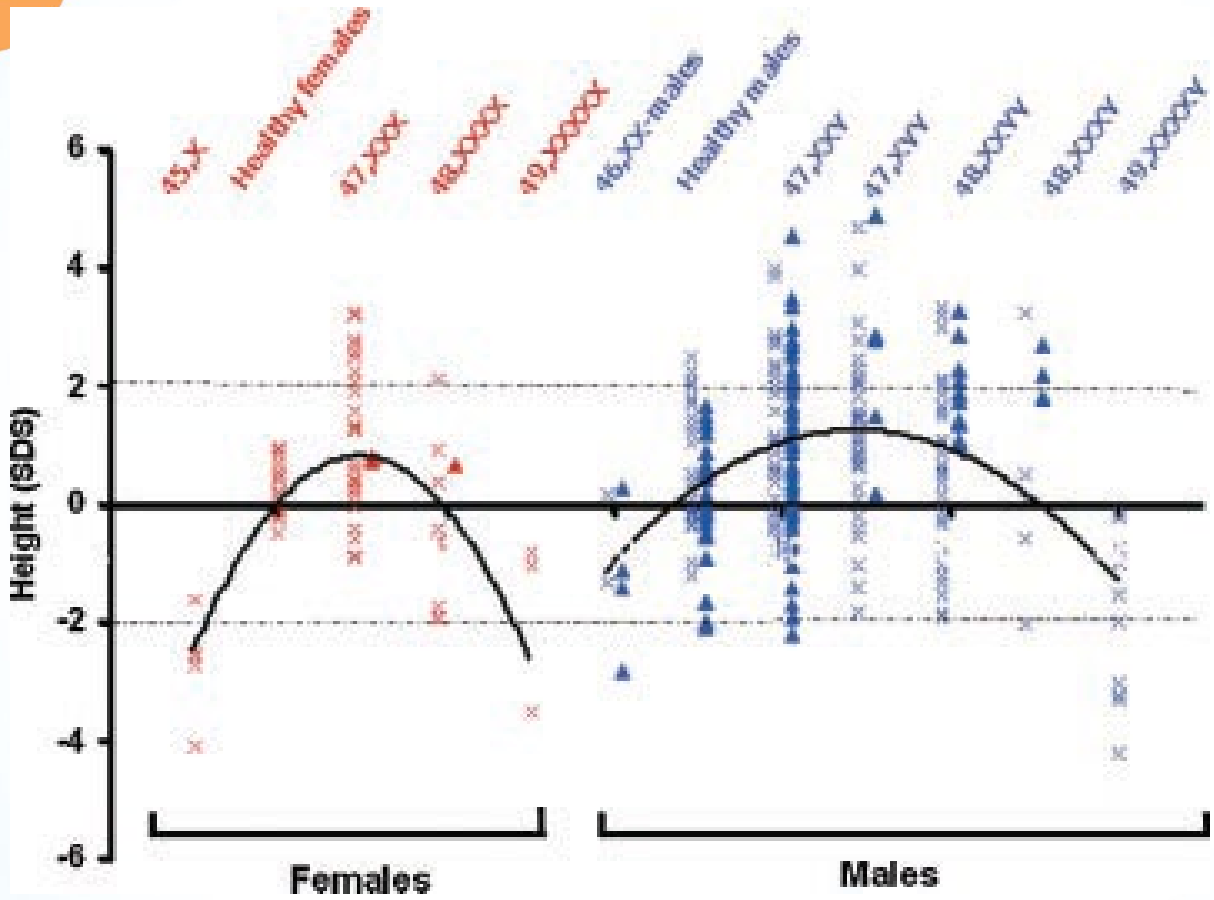
- Genetics are complicated
- Highly variable conditions
- A lot to learn from children identified by cfDNA
- Family background genes and environment matter
- Proactive approach when identified early
- Diagnosis does not define you – helps you get the care and support you need
- Many strengths we see in our patients

QUESTIONS?

eXtraOrdinarY Kids Clinic &
Research Program
www.extraordinarykidsclinic.org

Children's Hospital Colorado
Here, it's different.

Affiliated with
School of Medicine
UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS



sex chromosome-related short stature homeobox-containing gene **(SHOX)** located in the pseudoautosomal region (PAR1) = not inactivated and therefore gene expression is related to number of X chromosomes

Parent of Origin

47,XXY	Maternal	Paternal
Overall	~50%	~50%
Meiosis I	34% (AMA)	90+% (NO APA)
Meiosis II	9%	0%
Post-zygotic mitotic	3-10%	

Jacobs and Hassold, 1995; MacDonald et al., 1994; May et al., 1990

47,XXX	Maternal	Paternal
Overall	90%	10%
Meiosis I	58-63% (AMA)	0%
Meiosis II	16-17.4%	100%
Post-zygotic mitotic	18-19.6%	

Jacobs and Hassold et al, Ann Hum Genet. 1988 May;52(Pt 2):93-109

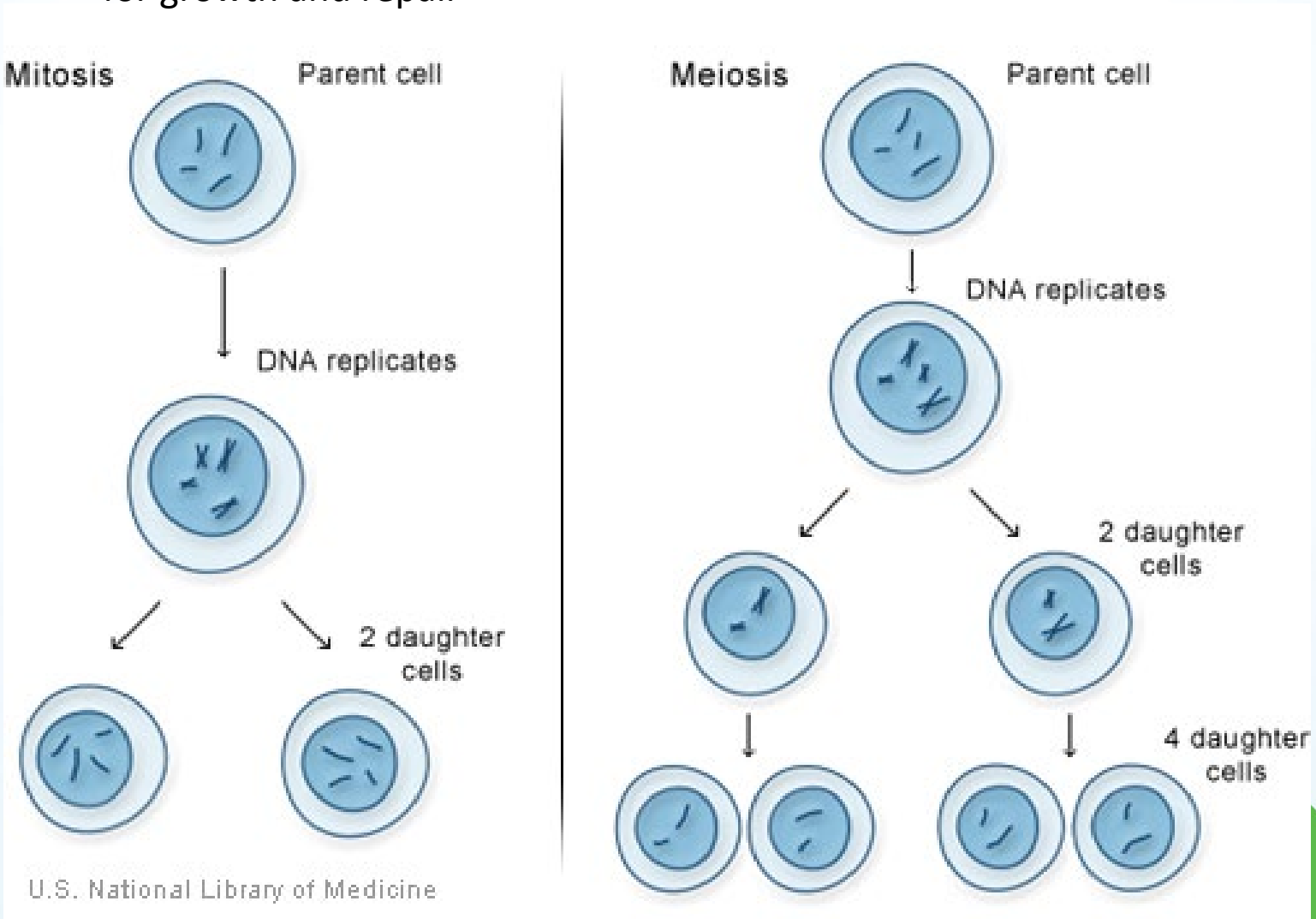
47,XYY	Maternal	Paternal
Overall	0%	100%
Meiosis I		0%
Meiosis II		16/19
Post-zygotic mitotic		3/19

Current Opinion in Genetics & Development (0959-437X), 16 (3)

Review of Cell Division

Mitosis – makes new cells, used for growth and repair

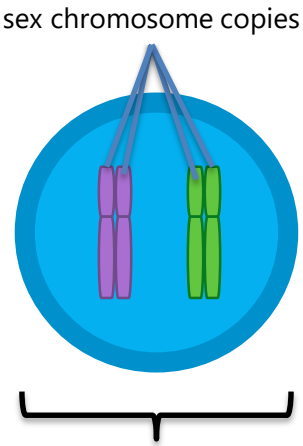
Meiosis – makes egg and sperm cells



Nondisjunction in Tetrasomies

Precursor Cell

Each cell has 23 pairs of chromosomes, including a pair of sex chromosomes.

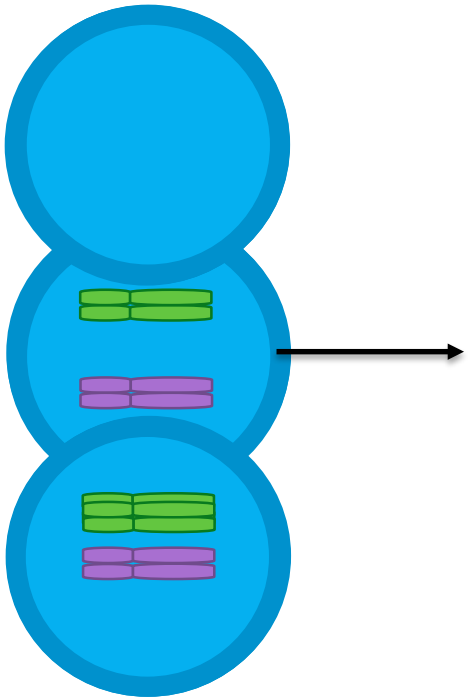


Replication

Each chromosome is copied.

Meiosis I

Nondisjunction: The cells do not split chromosomes evenly.



Meiosis II

Nondisjunction: The cells do not split chromosomes evenly.

This cell has two extra sex chromosomes.

If combined with a sperm or egg, this will produce a tetrasomy.