

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

























MALES:

47,XXY = Klinefelter syndrome1:650 males47,XYY = Jacobs syndrome1:1000 males48,XXYY1:18,000 males48,XXXY1:18,000 males48,XYYY1:80,000 males49,XXXXY1:250,000 males

FEMALES:

47,XXX = Trisomy X syndrome1:1000 females48,XXXX = Tetra X1:25,000 females49,XXXXX = Penta X1:50,000 females45,X = Turner syndrome1:2500 females



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

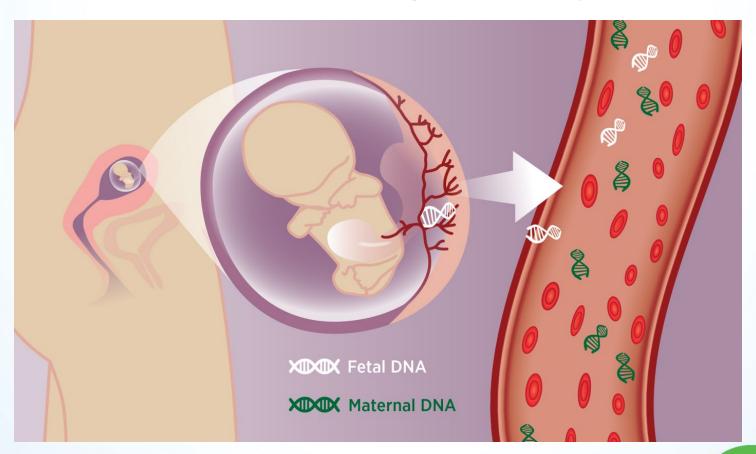






New Technology Identifying X&Y Variations Today

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 <u>risk</u> 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. Mazloom¹T, Želiko Džakula¹T, Paul Oeth¹, Huiquan Wang¹, Taylor Jensen¹, John Tynan¹, Ron McCullough¹, Juan-Sebastian Saldivar¹, Mathias Ehrich². Dirk van den Boom². Allan T. Bombard^{1,2}, Margo Maeder², Graham McLennan², Wendy Meschino³, Glenn E. Palomaki⁴,



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

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 Zhou³, Jiansheng Xie⁴, Shan Dan⁵, Yue Su⁵, Jianhong Xie³,



- 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/cm²)	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





Karyotype Result: 47,XXY

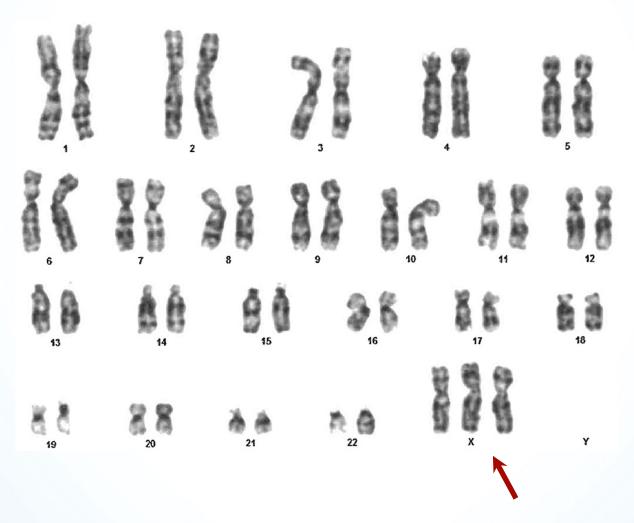
Karyotype from a male with Klinefelter syndrome (47,XXY)







Karyotype Result: 47,XXX

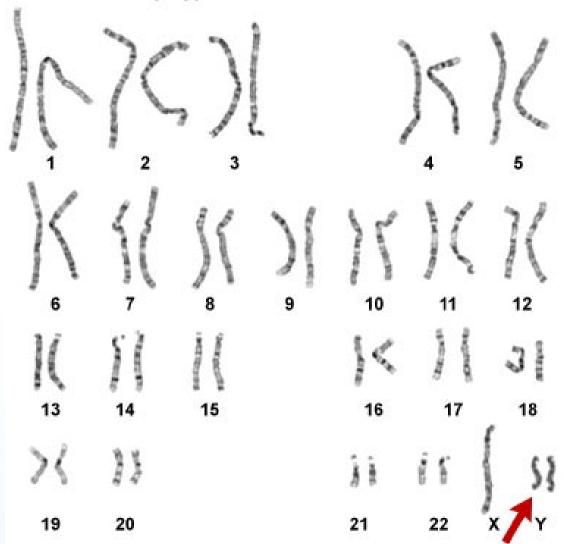






Karyotype Result: 47,XYY

Karyotype from a male with 47,XYY

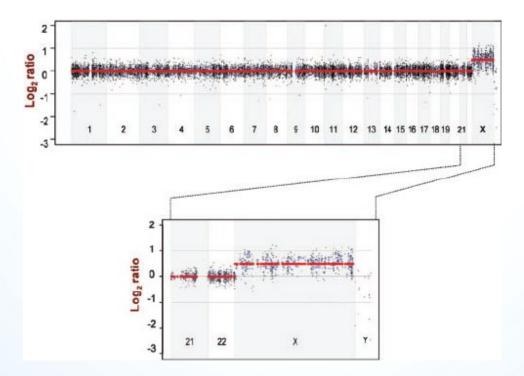




Genetic Testing Methods

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

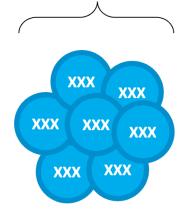
- Presence of multiple genetically different cell lines:
 - Example: mos 47,XXY [17]/48,XXYY[3] (85% XXY, 15% XXYY)
- Prognosis depends on cell lines present not always milder
- Different parts of the body may have different ratios of the cell lines
- Important to have follow-up FISH testing if mosaicism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

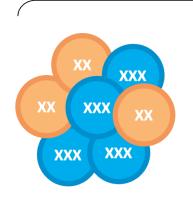
Non-Mosaic Cells

All cells are genetically identical.

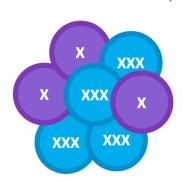
Mosaic Cells

There are two or more genetically different cells.





Example of mosaicism with aneuploidy cells (XXX) and nonaneuploidy cells (XXX)



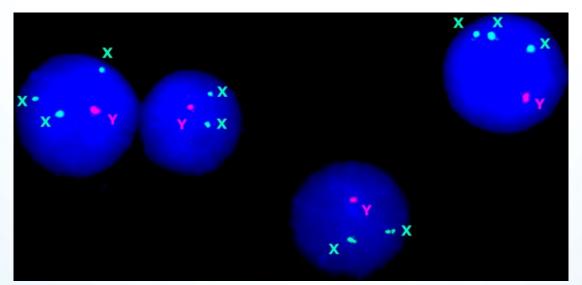
Example of mosaicism with with two aneuploidy cell lines (X and XXX)



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!



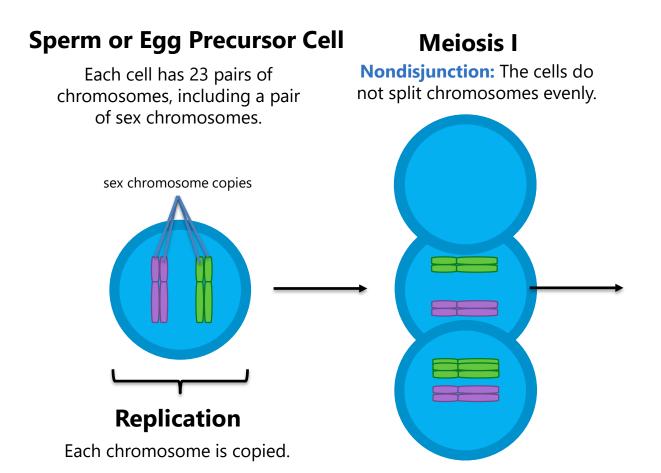




Nondisjunction in Meiosis I

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

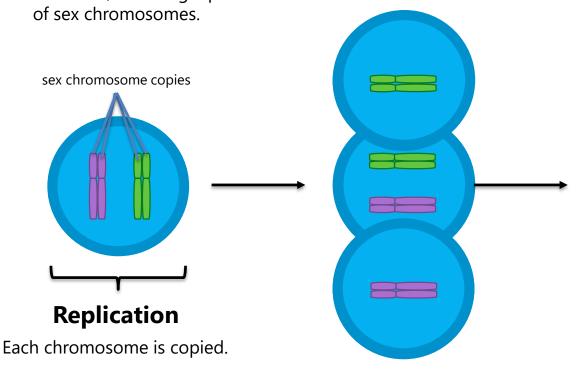
Nondisjunction: The cells do not split chromosomes evenly.

Sperm or Egg Precursor Cell

Each cell has 23 pairs of chromosomes, including a pair

Meiosis I

The cell divides into two, splitting chromosomes equally.



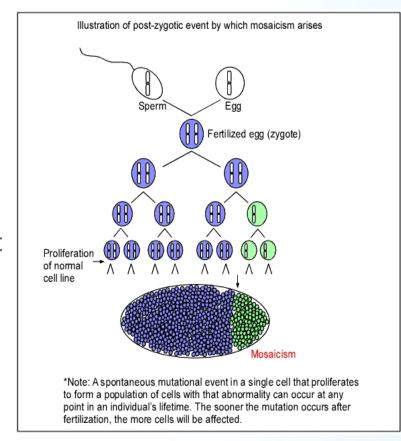
This cell has one extra sex chromosome.

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





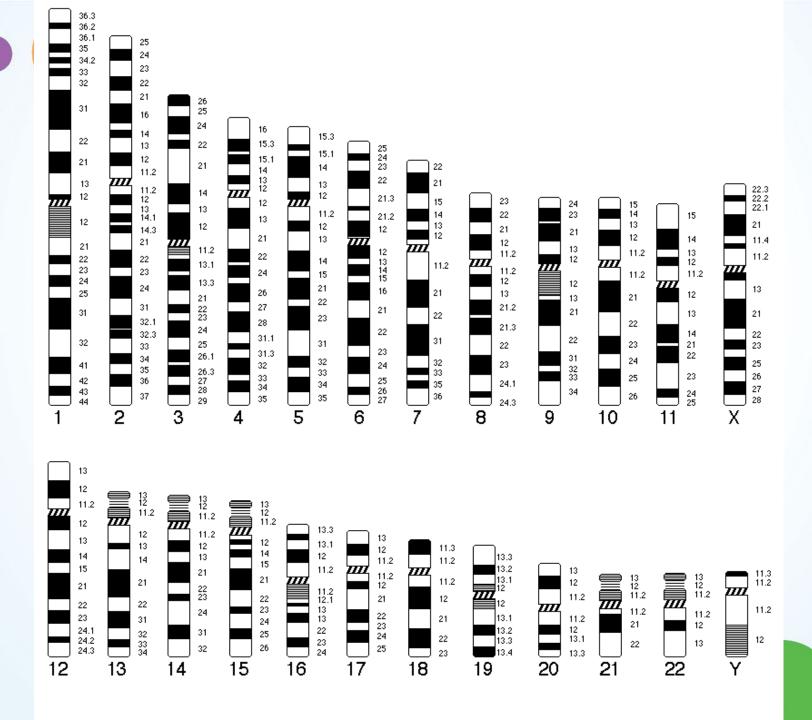


Common Counseling Points

- 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









Short stature, idiopathic familial Leri-Welli dyschondrosteosis Langer mesomelic dysolasia ternia, acute myeloid, M2 type Chondrodysplasia punctata Kalimann syndrome ism. Nettleship-falls type -facial-digital syndrome Nance-Horan cataract-dental syndrome Heterocellular hereditary persistence of fetal hemoglobin Pyravote dehydrogenase deficiency Glycogen storage disease Coffin-Lowry syndrome Spondyloepiphysial dysplasia tarda Parenysmal noctumal hemoglobinuria Infantile spasm syndrome Aicardi syndrome Deafness, sensorineural Simpson-Golabi-Behmel syndrome, type 2 Adrenal hypoplasia, congenital Dosage-sensitive sex reversal Dealness, congenital sersorineusal Retinitis pigmentosa Wison-Tumer syndrome Cone dystrophy Aland island eye disease (ocular albinism) Optic atrophy Night blindness, congenital stationary, type 1 Erythroid-potentiating activity Arthrogryposis multiplex congenito Night blindness, congenital stationary, type 2 Brunner syndrome Wiskett-Aldrich syndrome Thrombocytopenia Dent disease Nephrolithiasis, type I Hypophosphatemia, type III Proteinuria Aremia, sideroblastic/hypochromic Cerebellar ataxia Renal cell carcinoma, papillary Diabetes mellitus, insulin-dependent Sutherland Haan syndrome Cognitive function, social Mental setardation, nonspecific Menkes disease Occipital hors syndrome FG syndrome Immunodeliciency, moderate and severe Miles-Carpenter syndrome Charcot Marie-Tooth neuropathy, dominant X-inactivation center Promoture exorian failure Arts syndrome Cleft palate and/or ankyloglossia Megalocomes Epilepsy (Juberg-Hellman syndrome) Polizacus-Merzbacher diseaso Sportic parapleolo Alport syndrone Cowchock syndrome Hypertrichesis, congenital generalized Prosis, hereditary congenital Apaptosis inhibitor Panhypopituitarism Thoraccabdominal syndrome Simpson-Golabi-Behmel syndrome, type 1 Solit hand/foot malformation, type 2 Hypoparathyroidism Mental retardation, Shashi type Lesch-Nyhan syndrome MPRT-related pout Lowe syndrome Borjeson-Forssman-Lehmann syndrome Testicular germ cell tumor Hemophilia B Warfarin sensitivity Osseous dysplasia (male lethal), digital Adrenaleukodystrophy Adrenomyeloneuropathy Colorbindness, blue monochromatic Cardiac valvular dysplasia Emery-Dreifuss muscular dystrophy Heterotopia, periventricular Hemolytic anemia Calorblindness, green cone pigment Incontinentia pigmenti, type II MASA syndrome Spartic paraplegia Rett syndrome Mature T-cell proliferation Myapia (Bomholm eye disease) Mental retardation with psychosis

Endocardial fibraelastosis

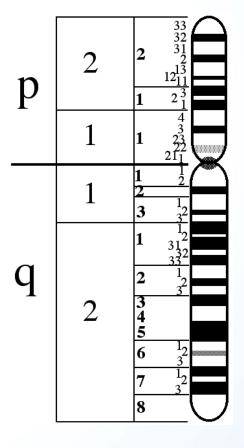
153 million base pairs Hodgis disease secreptibility, pseudoautosomal

Microphthalmia, dermal aplasia, and sclerocomea Episodic muscle usukness Mental retardation Ocular albinism and sensorineural deafness Amelogenesis imperfecta Charmt-Marie Tooth disease, recessive Kesatosis follicularis spinulosa decalsars. Hypophosphatemia, hereditary Fartington syndrome Retinoschisis Gonadal dysgenesis, XY female type Mental retardation, non-dysmorphic Agammaglobulinemia, type 2 Craniofrontorusal dysplania Opitz G syndrome, type I Pigment disorder, reticulate Melanoma Duchenne muscular dystrophy Becker muscular dystrophy Cardiomyopathy, dilated Chronic granulomatous disease Snyder-Robinson mental retardation Exudative vitreoretinoparty Coats disease Respensing syndrome Retinitis pigmentosa, recessive Mental retardation, nonspecific and syndromic Dyserythropoietic anemia with thrombocytopenia Chondrodysplasia punctata, dominant Autoimmunity-immunodeficiency syndrome Renal cell carcinome, papillary Fociogenital dysplasia (Aarskog-Scott syndrome) Chorioarthetosis with mental retardation Sacona, synoxial Prieto syndrome Spinal muscular atrophy, lethal infantile Migraine, familial typical Androgen insensitivity Spinal and builbor muscular atrophy Prostate cancer Perineal hypospadias Breast cancer, male, with Reifenstein syndrome Ectodemul dysplasia, anhidrotic Alaba-fraissomis/mental retardation Juberg-Marsidi syedrome Sutherland-Huan syndrome Smith Fineman Myers syndrome Herolytic anemia Myoglobinuria/hemolysis Wiescker Wolff syndrome Torsion dystonia-parkinsonism, Filipino type Leukamia, myoloidfymphoid or mixed-lineage Anemia siderablastic with attack Allan-Herndon syndrome Deafness Choroideremia Agammaglobulisemia Fobry disease Mole Transbiarro syndrom Jensen syndrome Bazex syndrome Mental retardation with growth hormone deficiency Mental retardation, South African type Lymphoproliferative syndrome X inactivation, familial skewed Fettigrew syndrome Gustavson mental resurdation synchome Immunadeficiency, with hyper-IgM Retinitis pigmentosa Wood neuroimmunologic syndrome Reterotoxy visceral Albinism deafness syndrome Cone dystrophy, progressive Prostate cancer susceptibility Fragile X mental retardation Epidermolysis bullosa, reacular type Diabetes insigidus, nephrogenic Cancentestis antigen Herrophilio A Hunter syndrome Mucopolysaccharidosis Intestinal pseudoobstruction, neuronal Mental retardation-skeletal dysplasia Myotubular myopathy Otopalatodigital syndrome, type I Colorblindness, red cone pigment Goeminne TKCR syndrome Waisman parkinsonism-mental retardation Barth ondrone Cardiomyogarity, dilated Noncompaction of left ventricular myocardium

Von Hippel-Lindau binding protein

Understanding Chromosomes







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:

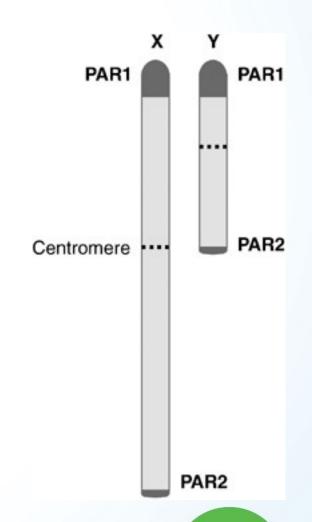
PAR1 is on the short-arm tips of both X & Y chromosomes

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)







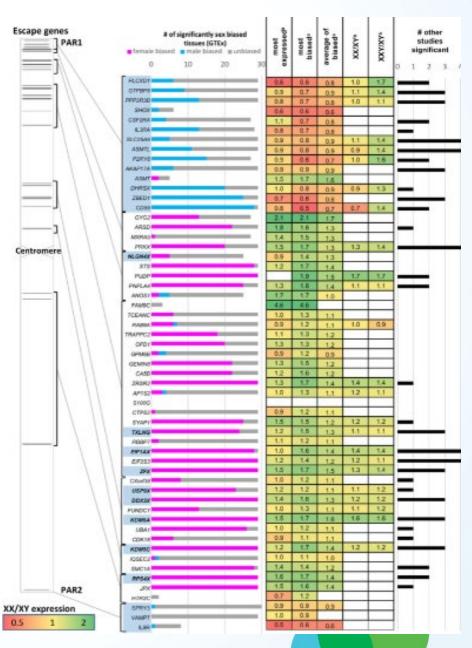
Prior Hypothesis: Gene Expression

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







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 (CAGCAGCAGCAG)
 - 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

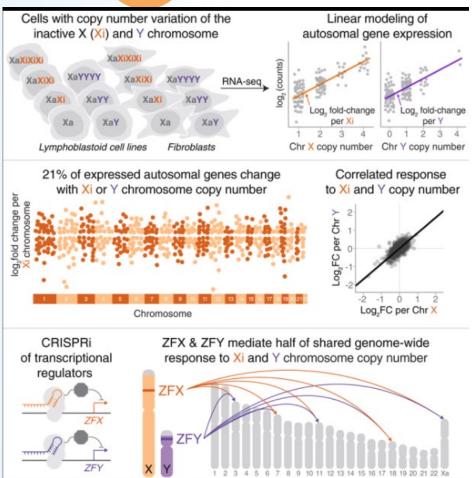


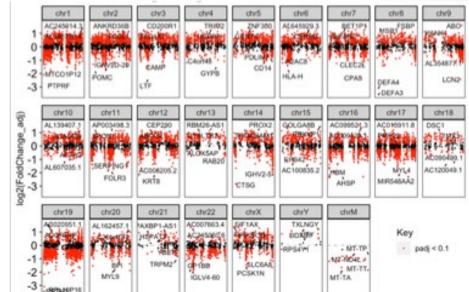






More Recent Hypothesis





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...











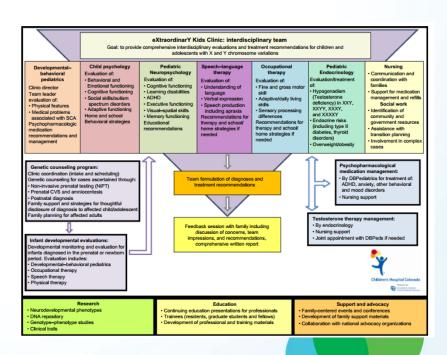
Next Steps Planning

- 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 El team
- <u>Speech/language</u> 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







Developmental Pediatrician



Endocrinologist



Speech Therapist



Therapist

Physical Therapist



Psychologist







Psychologist



Education Specialist



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





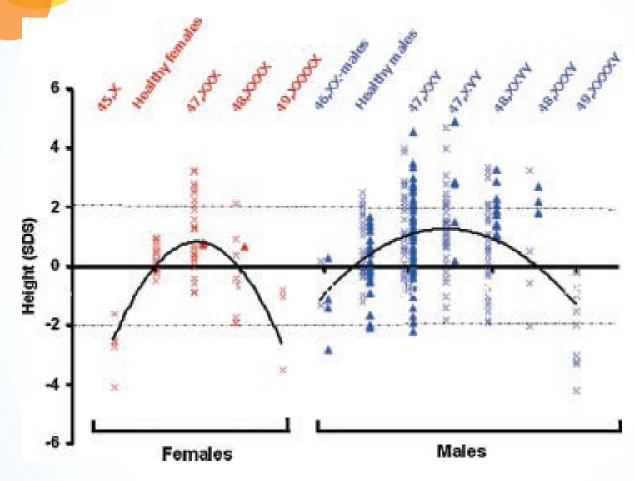


QUESTIONS?





eXtraOrdinarY Kids Clinic & Research Program www.extraordinarykidsclinic.org



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







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%	

Parent of Origin

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)



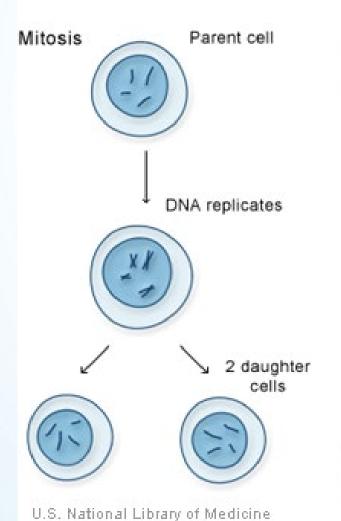
Jacobs and Hassold et al, Ann Hum Genet. 1988 May;52(Pt 2):93-109

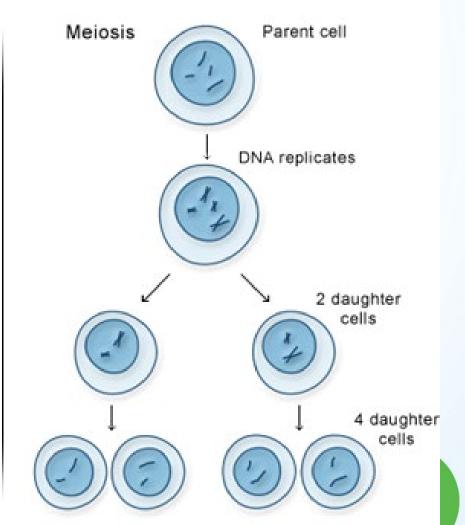


Review of Cell Division

Mitosis – makes new cells, used for growth and repair

Meiosis – makes egg and sperm cells







Nondisjunction in **Tetrasomies**

Meiosis II

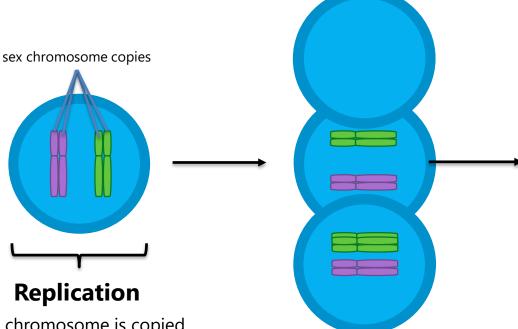
Nondisjunction: The cells do not split chromosomes evenly.

Precursor Cell

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

Meiosis I

Nondisjunction: The cells do not split chromosomes evenly.



Each chromosome is copied.

This cell has two extra sex chromosomes.

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