Jennifer Kussmann, MS, CGC

Jennifer has 17 years of experience as a genetic counselor. She has dedicated the past 10 years of her practice to providing care in the Fetal Health Center and the NICU at Children's Mercy Hospital. She enjoys educating colleagues about the complexity and the always evolving world of genetic testing.

Genetics testing for the neonate

8th Annual Regional Neonatal Conference
April 29, 2022

Jennifer Kussmann, MS,CGC

Genetic Counselor

Children's Mercy Hospital

Genetic disease in the NICU

2022 study from Cincinatti Children's NICU

- 12% NICU patients had a genetic diagnosis
  - It's essential that neonatal providers understand the basics of genetics

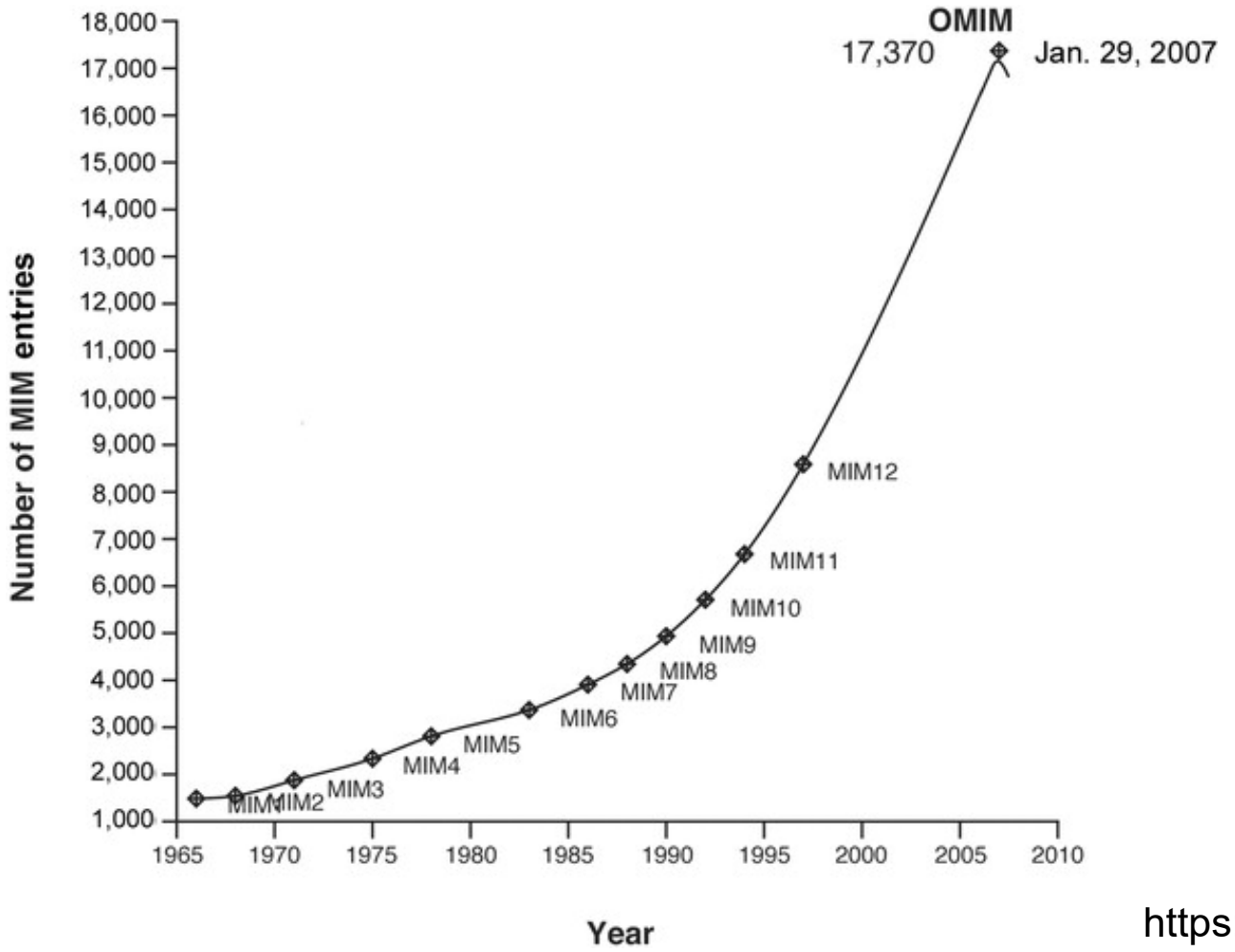
- 87% of these patients had NO family history
  - Families are overwhelmed – this is all new to them

- 70% of the diagnoses were only seen once in the 4 year study period
  - These conditions are rare
  - You need to know where to go for information

Hagen, L., Khattar, D., Whitehead, K. *et al.* Detection and impact of genetic disease in a level IV neonatal intensive care unit. *J Perinatol* (2022). https://doi.org/10.1038/s41372-022-01338-0d

Number of Entries in Catalog of Genetic Diseases in Humans

March 2022: 26334



https://www.omim.org/statistics/entry

Suspect a genetic etiology if . . .

- A child has a major anomaly or 2+ minor anomalies
- A child has growth problems
- A child has developmental delays/MR/ poor tone
- The child has ambiguous genitalia
- The child's features are not consistent with the family
  - Dysmorphic does NOT = ugly

Genetic testing in the NICU

- Cell free fetal DNA screening
  - NIPS
- FISH
- Karyotype
- Chromosomal microarray
- Exome sequencing
- Methylation studies

Prenatal testing
Cell free fetal DNA screening: NIPS

- Patients often are unsure about testing
- Patients and providers have misinformation
- Confirmatory testing is needed for all screening tests
  - Confirms diagnosis
  - Provides recurrence risk information
    - rr for DS ranges from 1%- 100%
- Best test to rule in/out a trisomy is a karyotype
  - Order FISH if you want a rapid preliminary results

What does the result mean?

Trisomy 18 positive predictive value (%)

Maternal age in years

Gestational age in weeks	20	25	30	31	32	33	34	35	36	37	38	39	40	41	42
10	10.2	11.3	16.2	18.2	20.8	24.0	28.0	32.7	38.2	44.3	51.0	57.6	64.3	70.6	76.3
12	8.3	9.3	13.4	15.2	17.4	20.2	23.7	28.0	33.1	39.0	45.4	52.1	59.1	65.8	71.9
14	7.0	7.8	11.3	12.8	14.8	17.3	20.4	24.3	29.0	34.4	40.7	47.3	54.3	61.2	67.8
16	5.9	6.6	9.7	11.0	12.7	14.9	17.7	21.2	25.5	30.6	36.5	43.0	49.9	57.0	64.0
20	4.4	4.9	7.3	8.3	9.6	11.4	13.6	16.5	20.1	24.4	29.6	35.6	42.2	49.3	56.4
40	1.2	1.4	2.1	2.4	2.8	3.4	4.1	5.1	6.4	8.1	10.3	13.0	16.5	20.8	26.0

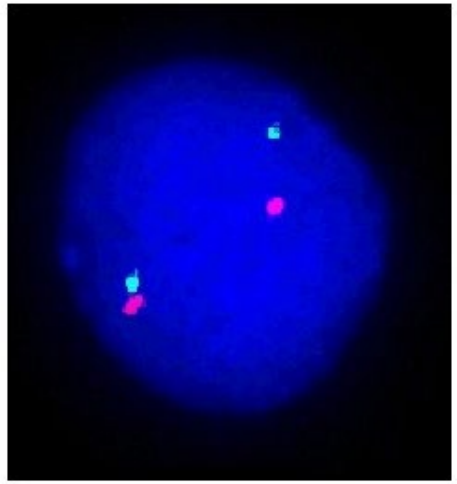
1. www.smfm.org/publications/183-cell-free-dna-screening-is-not-a-simple-blood-test

2. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol 2012, 119(5):890-901.

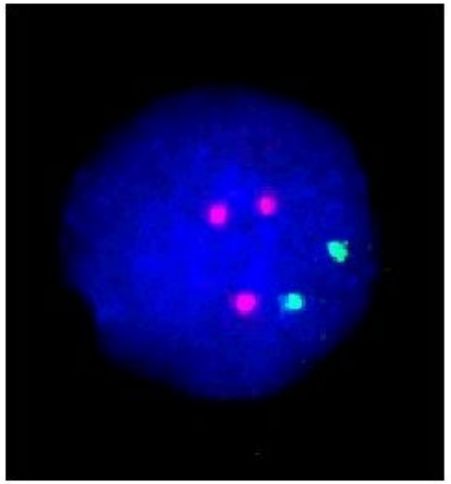
3. Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999;13:167-70.

FISH- rapid and preliminary

- Turnaround time:
  - 2-7 days
- Must be ordered on live cells
- Detects:
  - Presence or absence
  - **SPECIFIC region**
  - Chromosome location



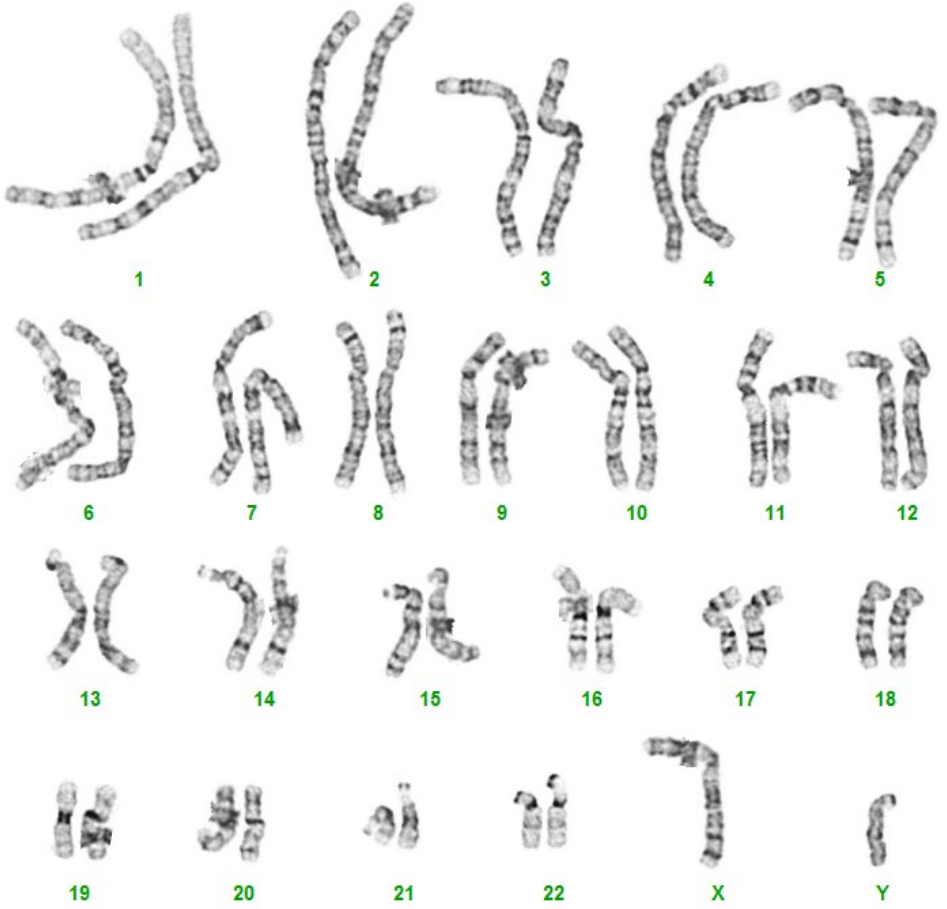
Normal



Down syndrome

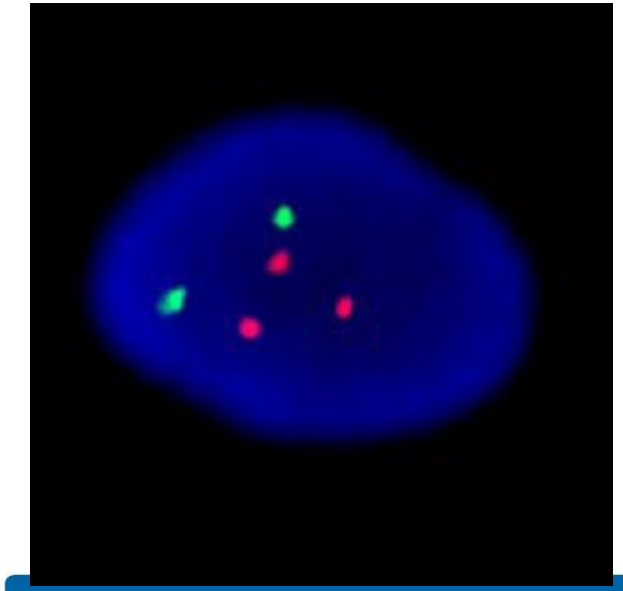
Karyotype

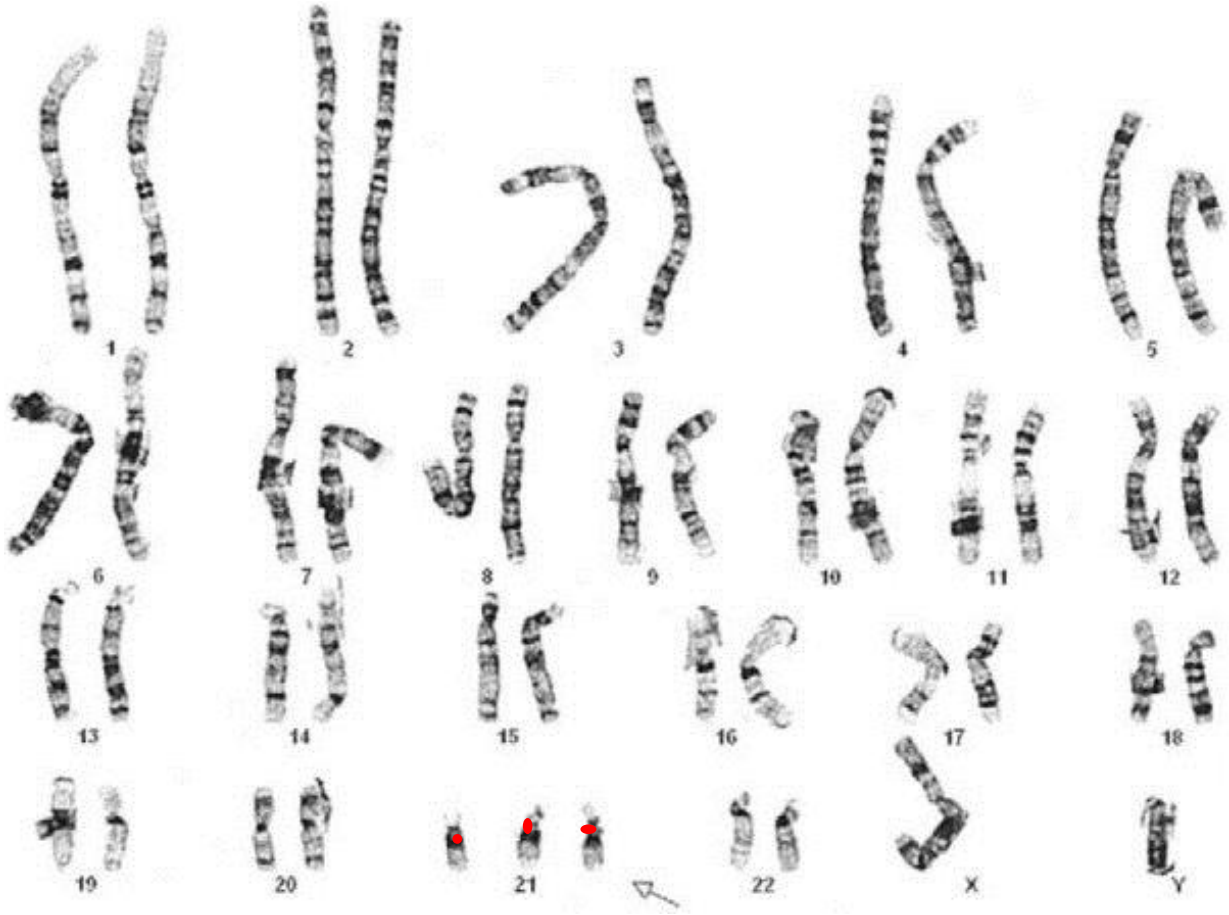
- Best test if you suspect a trisomy
- Turnaround time:
  - 2 weeks
- Must be done on live cells
  - Do not order on a deceased baby
- Detects:
  - Whole extra/missing
  - Very large extra/missing
  - Rearrangements



Down syndrome: Trisomy 21

- 95% sporadic
- Nondisjunction
- Recurrence risk
  - 1% or maternal age risk



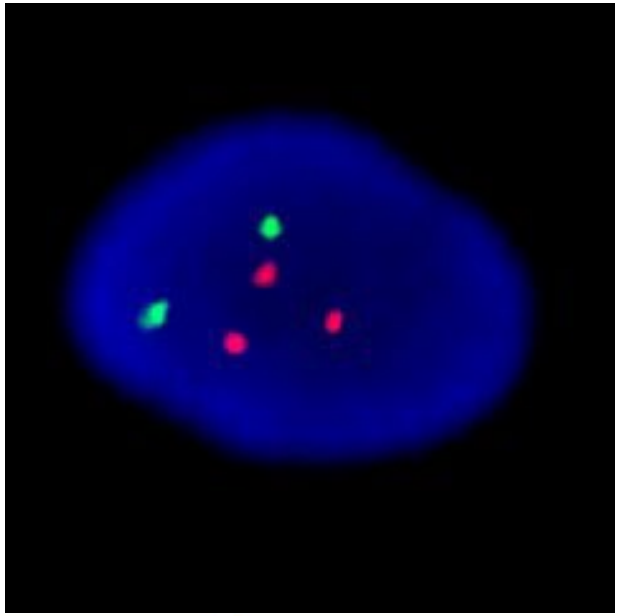


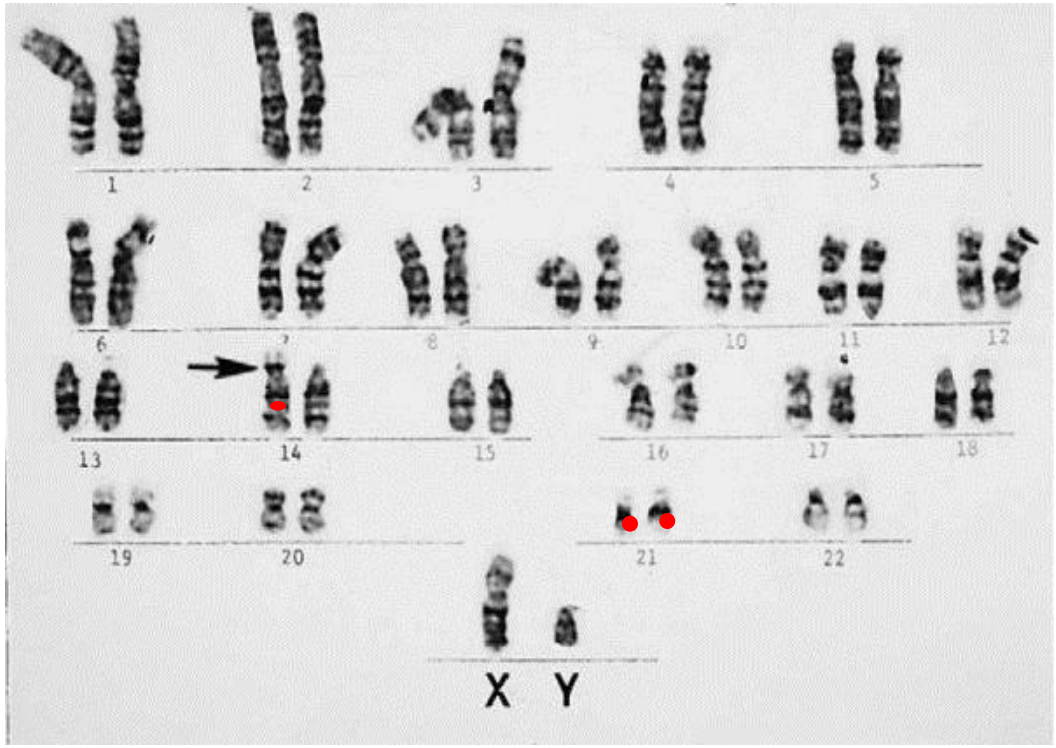
Down syndrome: Trisomy 21

- ~5% of DS caused by a translocation

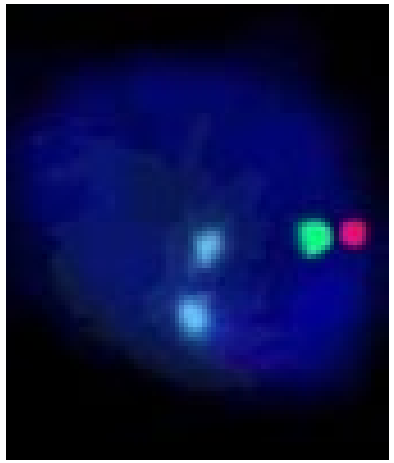
- 25% are familial translocations

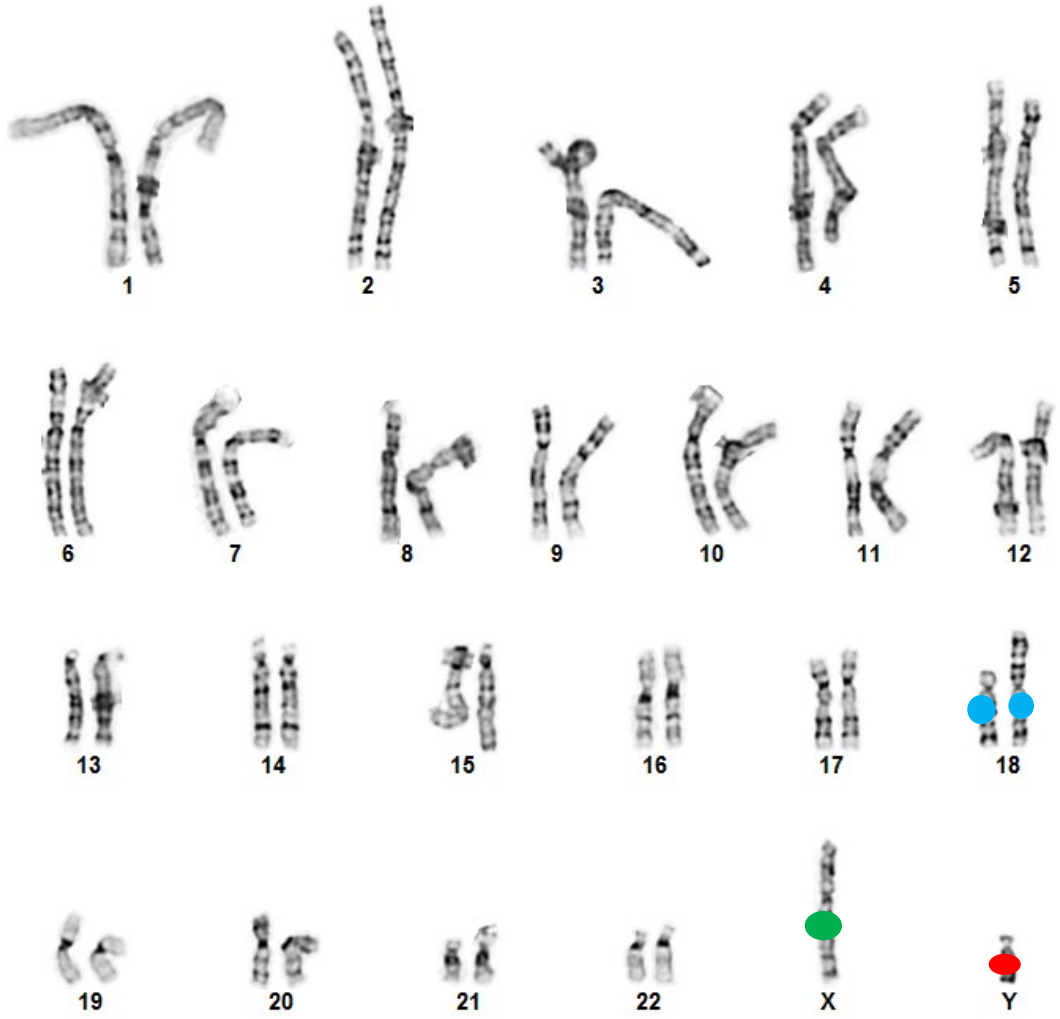
- Recurrence risk depends on the chromosomes involved and who carries it (<1%- 100%)





A word of caution…





Trisomy 18!!!

Chromosomal microarray

Detects:

- Deletion or duplications
- Trisomy
- VUS: variants of unclear significance
- CNV: Copy number variants

Does not detect:

- Balanced changes
- translocations
- Single gene disorders (Noonan)
- Small deletions (like SMA)

Chromosomal Microarray

- Recommended as 1st tier testing by the ACMG
  - ALL children with autism, developmental delays and MCA
    - 15-20% detection rate
- Recommended by ACOG
  - ultrasound anomalies
  - stillbirths, fetal demise
  - for any women having invasive diagnostic testing

Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. Committee Opinion No. 682. American College of Obstetricians and Gynecologists. Obstet Gynecol 2016;128:e262–8
Miller, D. T. et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am. J. Hum. Genet.* **86**, 749–764 (2010)

Symptom driven exome sequencing
AKA: NGS testing

Detects:

- Single gene disorders
  - Noonan syndrome
  - CHARGE syndrome

Does not detect:

Triplet repeat disorders: myotonic dystrophy
Methylation issues: PWS, BWS
Chromosome anomalies: 22q11 del, T21
Small deletions: SMA

Spell checks the DNA
Only analyze genes associated with the baby's phenotype
The more we know about a baby the higher the yield

How many misspellings are in YOUR exome?

Correct answer:

>10,000

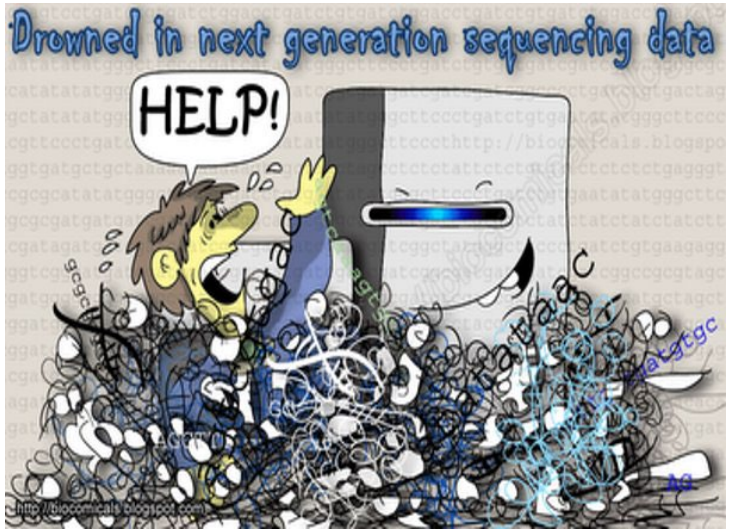
150,000 in the exome

4 million in the genome

NGS testing- it's a lot of data!

- Parental samples:
  - reduce VUS
  - increase diagnostic yield

- Pretest counseling is essential
  - Adult onset conditions
  - Non-paternity



www.biocomicals.com, Alper Uzun, PhD

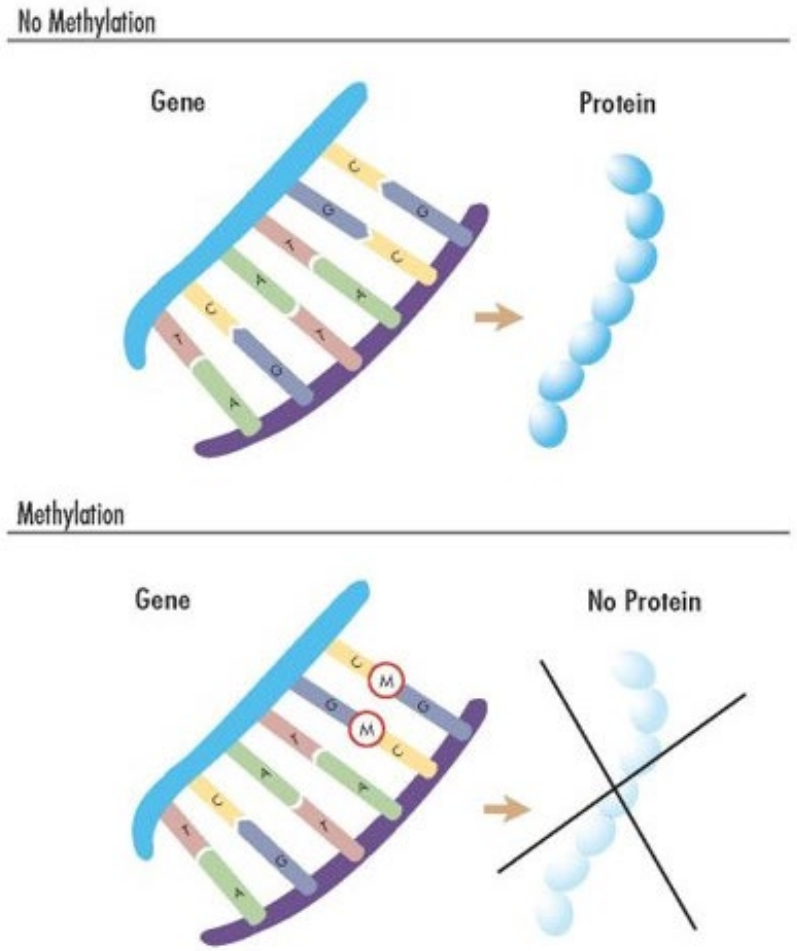
Exome Sequncing (ES) in the NIC

- ACMG recommends ES as 1st or 2nd tier testing for patients with congenital anomalies, dev delay or ID
- Diagnostic yeild in the NICU is 30-40%
- Clinical management changed for 10-20% of diagnosed patients

Manickam, K., McClain, M.R., Demmer, L.A. *et al.* Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* **23,** 2029–2037 (2021). https://doi.org/10.1038/s41436-021-01242-6

Methylation testing

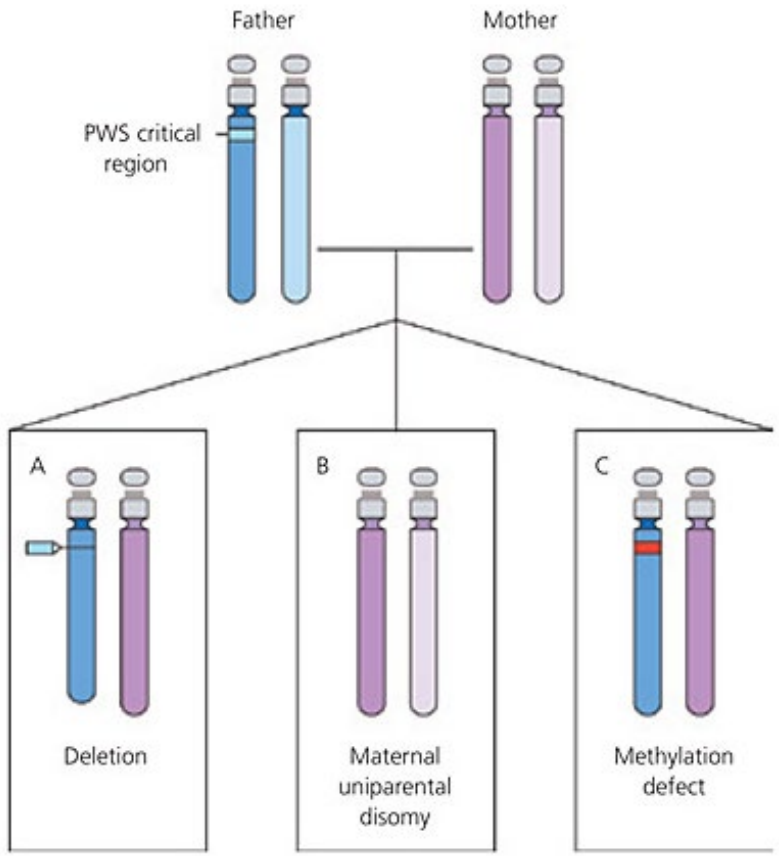
- Methylation= turning off a gene
- Testing determines if the gene is "off" or "on"
- Conditions caused by errors in methylation:
  - Prader Willi syndrome
  - Angelman syndrome
  - Beckwith Weideman syndrome
  - Russel Silver syndrome
  - Uniparental disomy (UPD)



Methylation testing

- Must be ordered independently
- Yes/no a diagnosis
- Can't determine mechanism
  - UPD (separate test)
  - Deletion (microarray)
  - Mutation (sequencing)

Mechanism determines recurrence risk



Am Fam Physician. 2005 Sep 1;72(5):827-830

Other tests

- Condition specific testing
- SMA- need to order deletion testing for SMN1 and SM2
  - SMA is treatable so fast diagnosis is essential
- Triplet repeat testing
  - Myotonic dystrophy

Metabolic testing

Genereviews

- Clinically useful information about common syndrome

- www.genereviews.org

- Example: 22q11 deletion
- https://www.ncbi.nlm.nih.gov/books/NBK1523/?report=classic

Questions?

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Call us at 816-302-3290

Appendix: Nomenclature Resources

Cytogenetic nomenclature

- **Chromosome analysis**
  - 46,XX or 46,XY (normal)
  - 47,XX,+21
    - Female with Down syndrome
  - 46,XX,del(3)(p12)
    - Female with 46 chromosomes with a deletion of part of one chromosome 3 on the short arm (p) at band 12.
  - 46,XY,dup(14)(q22q25)
    - Male with a duplication of part of one chromosome 14 on the long arm (q) involving bands 22 to 25.
  - Other abbreviations include “t,” “inv,” “r” “mar” “der” and many more

Cytogenetic nomenclature

- **Array CGH results**
  - arr (1-22,X)x2 *(normal female)*
  - arr(1-22)x2,(XY)x1 *(normal male)*
  - arr 4q28.3qter(134,293,639-qter)x3 *(duplication of 4q)*
  - arr 12q24.33qter(131,203,633-qter)x1 *(deletion of 12q)*
- **FISH results**
  - 46,XX.ish Xp22(SHOXx2),Xp11.1q11.1(DXZ1x2)[20] nuc ish(SHOX,DXZ1)x2[200] *(normal)*
  - 46,XY.ish del(22)(q11.2q11.2)(HIRA-)[20] nuc ish(HIRAx1)[10] *(22q deletion)*

Molecular Genetic Nomenclature

- **All sequence variants are described at the DNA level, in relation to a coding reference sequence.**
  - c.83G>A means the “G” that should be at the 83rd position has been changed to an “A.”
- **Sequence variants are also described at the protein level, in relation to the protein reference sequence.**
  - p.Val312Ala or p.V312A means that the valine that should be the 312th amino acid has been changed to an alanine.

- **Online Mendelian Inheritance in Man (OMIM) (http://omim.org/)**
  - Contains information on all known Mendelian disorders and over 12,000 genes
  - Provides synonyms for genes and conditions
  - Provides historical overview of published cases but usually no summary
  - Clinical synopsis option helpful for looking at clinical symptoms
  - Can search for a combination of symptoms to generate a differential diagnosis list


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