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Genetic Testing and Congenital Heart Defects

Etiology of genetic disease

Dosage

- Trisomies
- Turner syndrome
- 22q11 deletion

Misspelling- single gene disorders

- Tuberous Sclerosis
- Noonan syndrome

Expression – genes not turned on/off at the correct time

- Prader Willi
- Beckwith Wiedemann

Diagnostic testing

Must obtain DNA from a CVS, amniocentesis or neonatal blood

Syndrome	Cardiac defects	Extracardiac anomalies
Trisomy 21	AVcanal, TOF, VSD	Thick nuchal, bowel atresia, short long bones
Trisomy 18	varied	IUGR, CNS, renal, omphalocele, clenched fists, polyhydramnios
Trisomy 13	varied	CNS, cleft, renal, omphalocele, polyhydramnios
Turner syndrome	HLHS, CoA	Cystic hygroma, renal
22q11 deletion	TOF, VSD	Cleft, renal, POLYHYDRAMNIOS
Williams syndrome	PPS, SVAS	IUGR

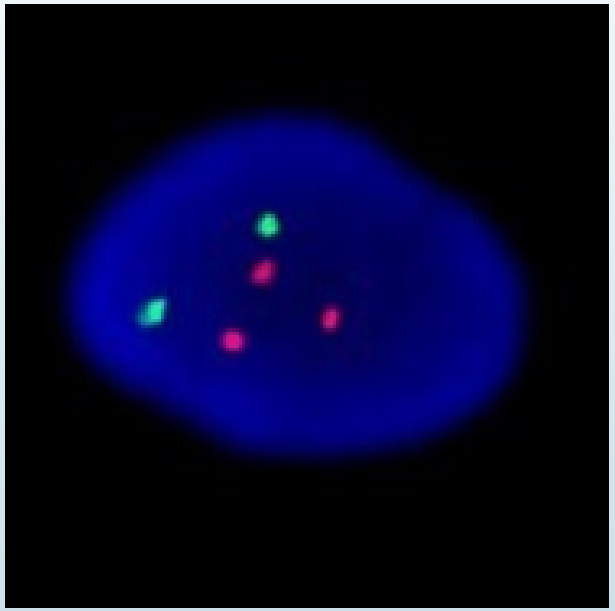
Dosage diseases often associated with cardiac findings

Dosage testing

- FISH
- Karyotype
- Chromosomal microarray

Dosage testing- FISH

- 1-2 days TAT
- Preliminary results
- Prenatally 13, 18, 21, X and Y are bundled
- Don't FISH for 22q11- array is better
- When should you order FISH?

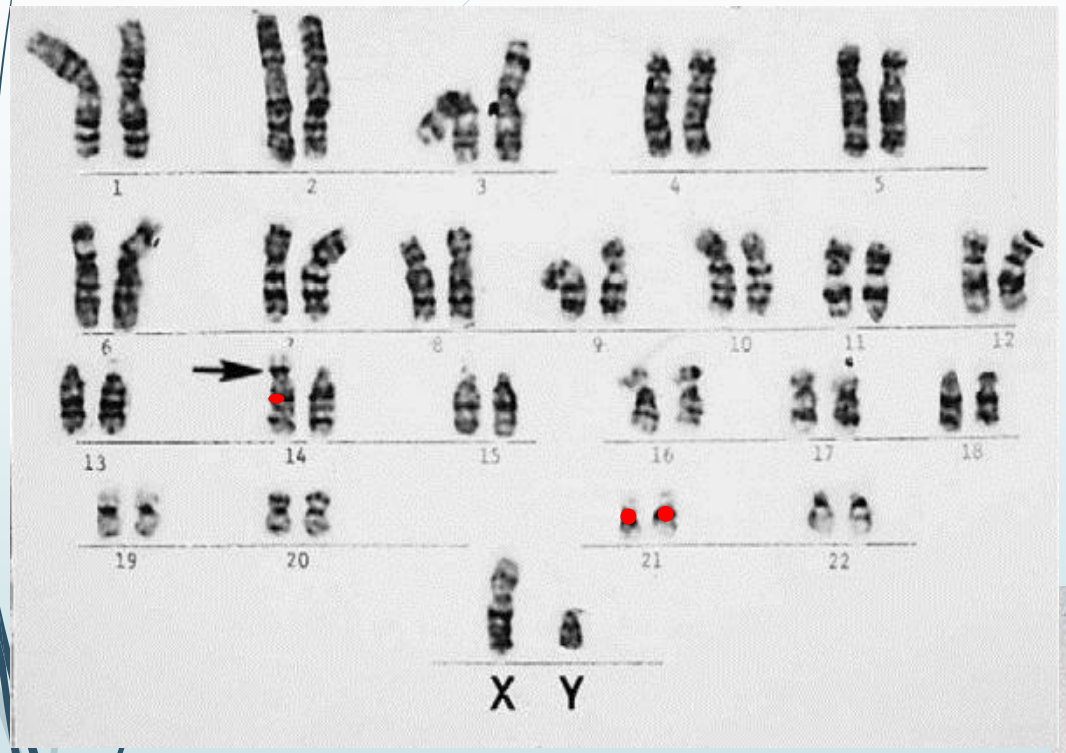


Dosage testing - Karyotype / chromosome analysis

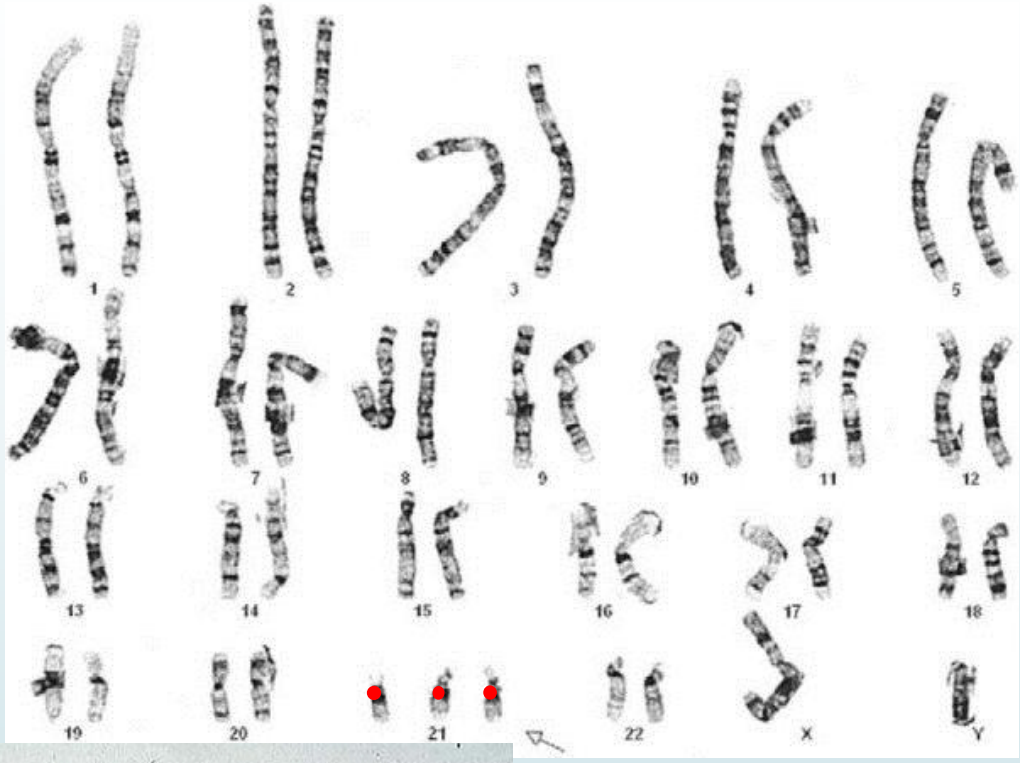
- 2 week TAT
- Can detect
  - whole extra/missing chromosome
  - Chromosome translocations
- Best test if we suspect a trisomy or Turner syndrome

Why order a karyotype if we have FISH?

- Need to check for a translocation

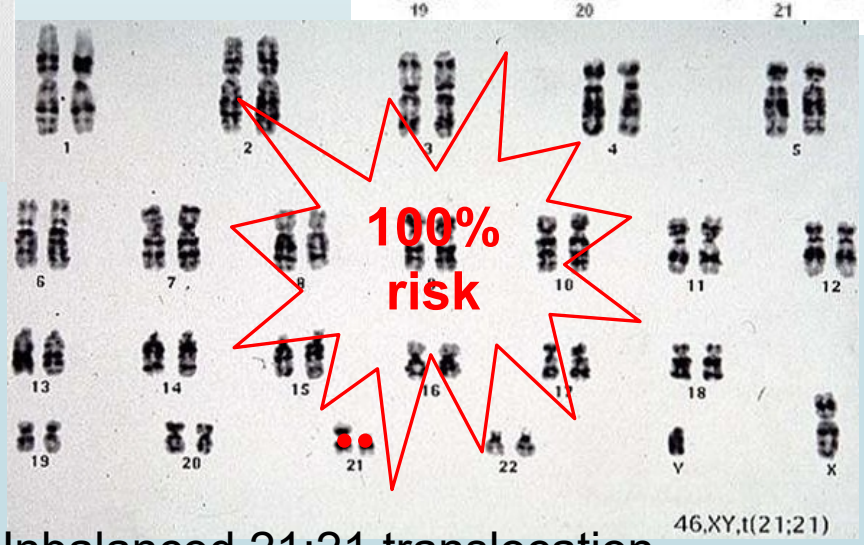


Unbalanced 14:21 translocation



Trisomy 21

False Neg



Unbalanced 21:21 translocation

Dosage testing- chromosomal microarray

- Turnaround time:
  - 2-4 weeks
- Detects:
  - Deletion or duplications
  - CNV of unclear significance
  - **Consanguinity**
- Does not detect:
  - Single gene disorders
- Best test for 22q11 deletion

Sample report 22q11 del

SPECIMEN TYPE
Blood

RESULTS
MICROARRAY ANALYSIS REPORT: CYTOSCAN HD CN+SNP ARRAY
Genome Build GRCh37 (hg19)
Genotypic Gender: Male

INTERPRETATION
Pathogenic Variant - Loss
arr[GRCh37] 22q11.21(18916843_21465659)x1

Sample report VUS

Variant of Unknown Significance - Gain
arr[GRCh37] 12q24.31(124115035_124207388)x3

Misspellings- single gene disorders

- Single gene testing
  - Tuberous Sclerosis (2 genes)
  - Familial mutation

2-6 weeks
Rarely the best choice

- Panel testing
  - Noonan syndrome panel- 14 -18 genes
  - Cardiomyopathy panel 5-121 genes
  - Arrhythmia panel 7-80 genes

Need to understand the test
4-8 weeks
Variants of unclear significance

- Exome sequencing panel
  - Generalized symptoms- hydrops, MCA

Exome Sequencing

How many misspellings would we find?

- Exome
  - 150,000
- Whole genome
  - 4,000,000

Symptom driven testing

Trio testing help reduce VUS

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

Prenatal 3 week TAT
Postnatal 12 week TAT
Postnatal STAT testing 4 week TAT

Only detects misspellings. Will not detect:
triplet repeat disorders- Fragile X, myotonic dystrophy, Fredreich's ataxia methylation issues- Prader Willi syndrome, Beckwith Weideman
large chromosome abnormalities- 22q11 deletion, Down syndrome

Prenatal Screening

Cell free fetal DNA screening

Aka: NIPS

Cell free fetal DNA screening

- Maternal blood sample in specialized tubes
- Degraded placental DNA (syncytiotrophoblast)
- Amount fetal DNA is variable
  - Gestational age (9+ weeks)
  - Maternal weight
  - Placental function
- Screens for T13, T18, T21
  - Some labs offer microdeletions including 22q11

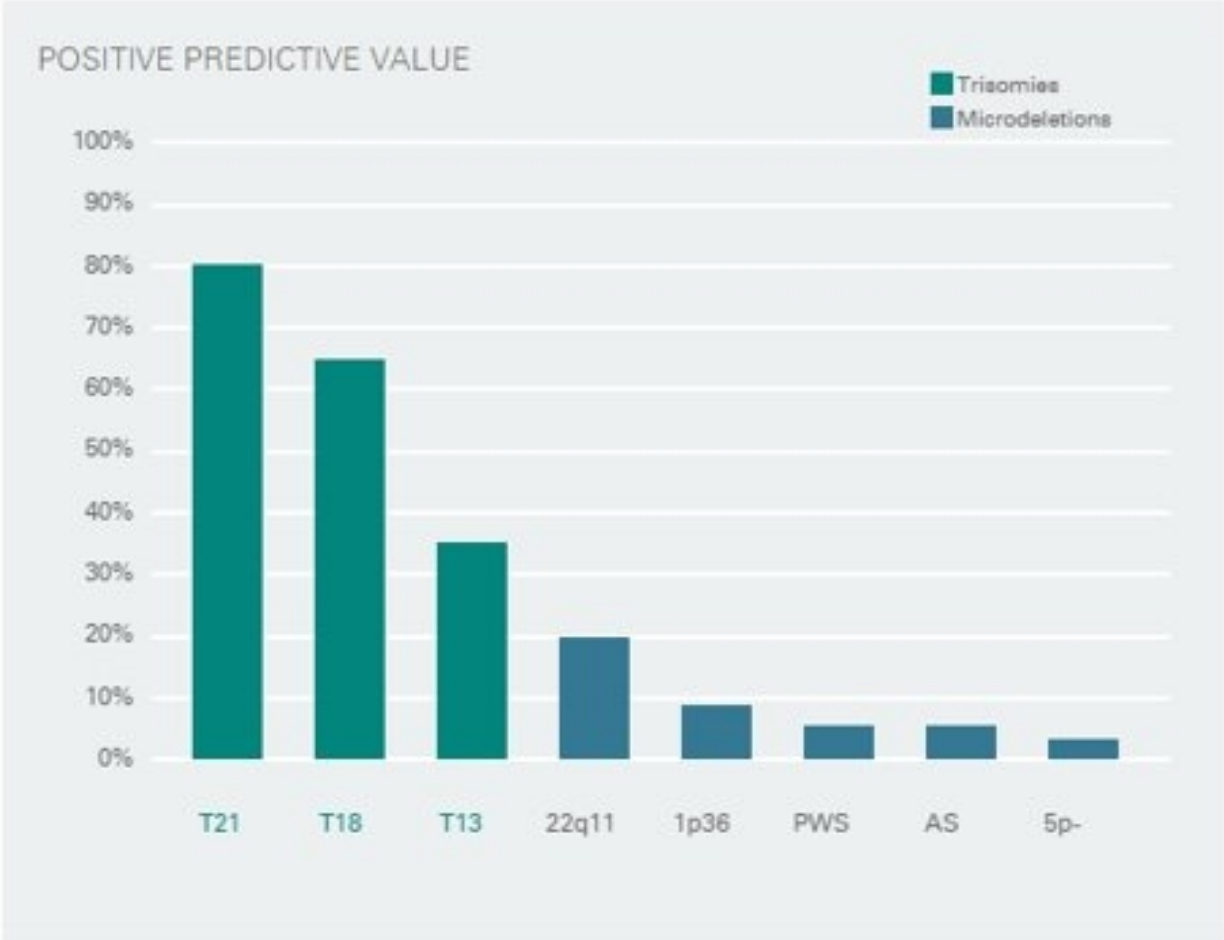
Marketing of NIPS

https://www.natera.com/womens-health/panorama-nipt-prenatal-screening/

Condition	*Sensitivity (95% CI)*	*Specificity (95% CI)*
Trisomy 21[1,2,3,4]	>99% (CI 97.8-99.9)	>99% (CI 99.7-100)
Trisomy 18[1,2,3,4]	98.2% (CI 90.4-99.9)	>99% (CI 99.7-100)
Trisomy 13[1,2,3,4]	>99% (CI 87.2-100)	>99% (CI 99.8-100)
Monosomy X[1,2,3,4]	94.7% (CI 74.0-99.9)	>99% (CI 99.7-100)
Triploidy[5,6]	>99% (CI 66.4-100)	>99% (CI 99.5-100)
XXX, XXY, XYY[4]	N/A-Reported when identified	N/A-Reported when identified
22q11.2 deletion syndrome[7,8,9]	90.0% (CI 55.5-99.7)	>99% (CI 98.6-99.9)
1p36 deletion syndrome[7,8]	>99% (CI 2.5-100)	>99% (CI 99.1-100)
Angelman syndrome[7,8]	95.5% (CI 77.2-99.9)	>99% (CI 99.1-100)
Cri-du-chat syndrome[7,8]	>99% (CI 85.8-100)	>99% (CI 99.1-100)
Prader-Willi syndrome[7,8]	93.8% (CI 69.8-99.8)	>99% (CI 99.1-100)
Female	>99.9% (CI 99.4-100)	>99.9% (CI 99.5-100)
Male	>99.9% (CI 99.5-100)	>99.9% (CI 99.4-100)

Interpreting NIPS results

POSITIVE PREDICTIVE VALUE (PPV) OF THEORETICAL GENETIC TEST WITH FALSE-POSITIVE RATE (FPR) OF 0.1%*



http://www.ariosadx.com/healthcare-professionals/performance/

Trisomy 21 positive predictive value (%)

Gestational age in weeks	Maternal age in years																	
	20	25	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
10	29.2	31.8	41.4	44.8	48.9	53.6	58.6	64.0	69.4	74.5	79.1	83.4	86.9	89.8	92.3	94.2	95.5	96.7
12	27.5	30.0	39.4	42.8	46.9	51.5	56.6	62.1	67.5	72.9	77.8	82.2	85.8	89.0	91.6	93.5	95.3	96.4
14	26.3	28.7	37.8	41.2	45.2	49.8	55.0	60.5	66.1	71.5	76.6	81.2	85.1	88.4	91.0	93.3	94.9	96.2
16	25.3	27.7	36.6	40.0	44.0	48.6	53.7	59.2	64.9	70.5	75.7	80.4	84.4	87.9	90.6	92.9	94.6	96.0
20	23.9	26.1	34.9	38.2	42.1	46.7	51.8	57.4	63.1	68.8	74.2	79.1	83.4	86.9	90.0	92.3	94.2	95.8
40	21.0	23.1	31.2	34.4	38.1	42.6	47.7	53.3	59.2	65.1	71.0	76.2	80.9	84.9	88.2	91.0	93.3	94.9

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.

NIPS for microdeletions

Anomaly	Incidence	Sensitivity +	Specificity+	PPV
22q11 deletion *	1/4000	60-86%	>99%	1.5%-3.5%
5q deletion	1/50000	85-90%	>99%	0.17%-0.3%
15q deletion	1/28500	60-86%	>99%	0.21%-0.5%
1p36 deletion *	1/10000	60-86%	>99%	0.6%-1.4%
Trisomy 16	1/50000	>99%	>99%	0.2%
Trisomy 22	1/40000	>99%	>99%	0.25%

PPV decreases as the incidence of the disease decreases
If mom is affected the status the baby is not detectable with NIPS
MaterniT GENOME report anything ≥7 Mb

Low PPV for Turner syndrome

Comparison between NIPS and karyotyping for detecting foetal SCAs based on stratification of demographic characteristics.

Characteristic	n	NIPS positive	Without karyotype validated	Karyotype validated[a]		PPV (%)
				TP	FP	
Prenatal biochemical screening						
High risk	4260	28	15	5	8	38.46
Intermediate risk	18860	108	43	22	41	34.92
Low risk	8082	55	22	8	23	25.81
Not performed	19099	117	42	24	51	32.00
Maternal age						
<35 years	41394	250	98	49	101	32.67
≥35 years	8907	58	24	10	22	31.25
Gestational age						
12–22 + 6 weeks	43931	251	96	54	97	35.76
≥22 + 6 weeks	6370	57	26	5	26	16.13
BMI						
<18.5	1095	17	5	5	6	45.45
18.5–27.9	48550	269	107	52	108	32.50
≥28	656	22	10	2	9	18.18

Clinical application of noninvasive prenatal screening for sex chromosome aneuploidies in 50,301 pregnancies: initial experience in a Chinese hospital
Sci Rep. 2019; 9: 7767.

NIPS summary

- The best screening test we have to offer
- It's far from perfect
- Clinical interpretation is necessary
- Proper counseling is essential

Recurrence risks for isolated CHD

Typically a low recurrence risk

Table 5
Recurrence risks for isolated (nonsyndromic) CHDs

Defect	Father Affected (%)	Mother Affected (%)	1 Sibling Affected (%)	2 Siblings Affected (%)
ASD	1.5–3.5	4–6	2.5–3	8
AVSD	1–4.5	11.5–14	3–4	10
VSD	2–3.5	6–10	3	10
AS	3–4	8–18	2	6
PS	2–3.5	4–6.5	2	6
TOF	1.5	2–2.5	2.5–3	8
CoA	2–3	4–6.5	2	6
PDA	2–2.5	3.5–4	3	10
HLHS	21[48]		2–9[a]	6
TGA	2[97]		1.5	5
L-TGA	3–5[97]		5–6	NR
EA	NR	6[97]	1	3
TrA	NR	NR	1	3
TA	NR	NR	1	3
PA	NR	NR	1	3

Merged cells indicate recurrence when 1 parent is affected, irrespective of gender, and are used in the absence of gender-stratified risks.

Abbreviations: AVSD, atrioventricular septal defect; EA, Ebstein anomaly; L-TGA, congenitally corrected transposition of the great arteries; NR, not reported/insufficient data; PA, pulmonary atresia; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TA, tricuspid atresia; TGA, transposition of the great arteries.

[a] Eight percent recurrence risk HLHS, up to 22% recurrence risk for any CHD.[48]

Data from Refs.[52–54] except where otherwise noted.

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Congenital Heart Disease

Hypoplastic Left Heart Syndrome Is Heritable

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Table 1 Relatives of 38 HLHS Probands Affected With CVM

Relationship	Normal	Affected	Total
Fourth-degree plus	12	5	17
Third-degree	8	12	20
Grandparent	15	4	19
Avuncular	9	2	11
Half sibling	5	1	6
Parent	63	12	75
Sibling	35	10	45
Total	147	46	193*

*Excludes participants not genetically related to the proband.
CVM = cardiovascular malformation; HLHS = hypoplastic left heart syndrome.

Table 3 Hypoplastic Left Heart Syndrome and CVM λ_R

	HLHS	CVM
Frequency in first-degree relatives	3.5%	18.3%
Frequency in siblings	8%	22%
Frequency in siblings with 1 affected parent	21%	26%
First-degree relative λ_R	175	9
Sibling λ_R	515	11
Sibling λ_R with 1 affected parent	1,050	13

CVM = cardiovascular malformation; HLHS = hypoplastic left heart syndrome; λ_R = recurrence risk ratio.

20-25% recurrence risk for sibs to have a CHD
Echos for siblings and parents
Most common defect found was a BAV 10%
Others had HLHS, dilated aorta, CoA, ASD and VSD

Resources

- Genetic Counselors at CMH
  - Internally call 15240
  - Externally- call transport (1-800-GO MERCY) and ask to speak to a GC
- **Genereviews** www.genereviews.org
  - Expert-authored peer-reviewed disease descriptions for clinical professionals
  - Is NOT exhaustive but an excellent resource for many conditions
  - Lists possible differential diagnoses for each condition listed
  - Testing, management and surveillance info
  - Patient resources