Preconception genetic carrier screening and PGT

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Disclosures

- *Ferring*
- *Natara*

Objectives

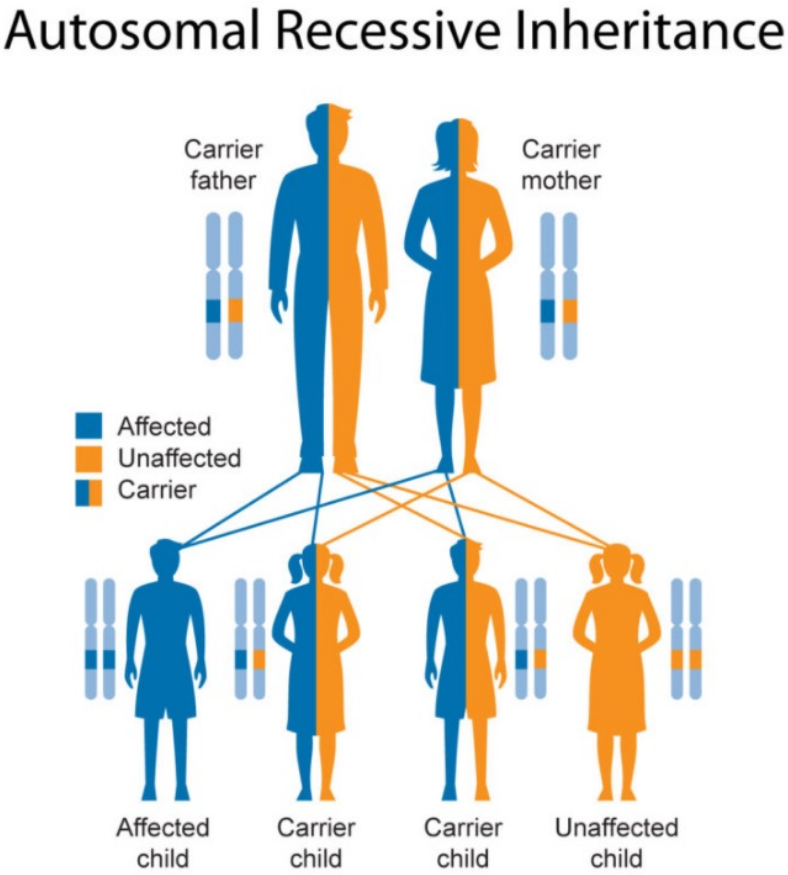
- *Address preconception genetic carrier screening*
- *Preimplantation Genetic Testing*
- *Prenatal Genetic Screening and Testing*
- *Utilization of genomics and technologies for pregnancy well being*

Carrier screening – identification of autosomal recessive disorders

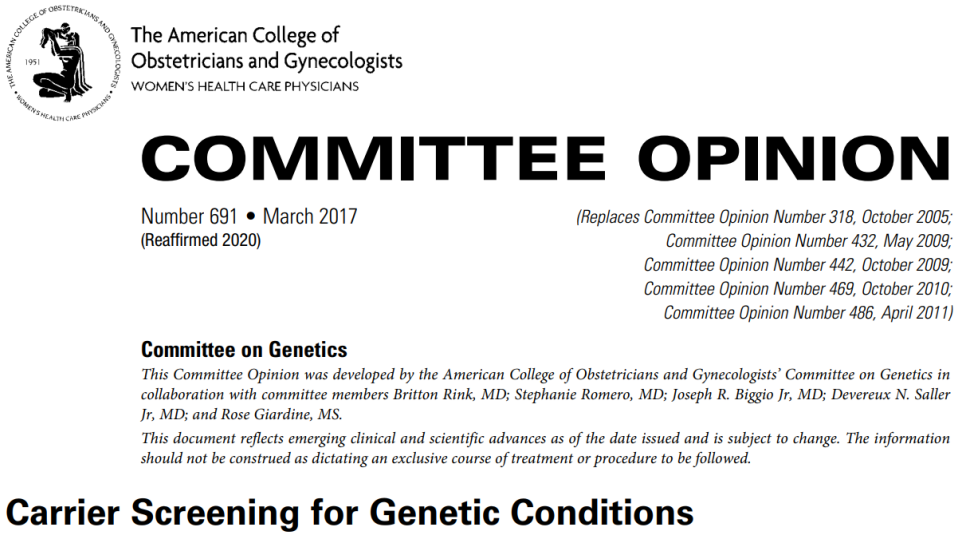
Cross: Aa x Aa

	A	a
A	AA	Aa
a	Aa	aa

Quora



Carrier screening for genetic conditions ACOG 2017



The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

COMMITTEE OPINION

Number 691 • March 2017
(Reaffirmed 2020)

(Replaces Committee Opinion Number 318, October 2005; Committee Opinion Number 432, May 2009; Committee Opinion Number 442, October 2009; Committee Opinion Number 469, October 2010; Committee Opinion Number 486, April 2011)

Committee on Genetics

This Committee Opinion was developed by the American College of Obstetricians and Gynecologists' Committee on Genetics in collaboration with committee members Britton Rink, MD; Stephanie Romero, MD; Joseph R. Biggio Jr, MD; Devereux N. Saller Jr, MD; and Rose Giardine, MS.

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Carrier Screening for Genetic Conditions

- *Carrier screening to all couples, regardless of their race/ethnicity (ie, pan-ethnic carrier screening)*
  - *cystic fibrosis (CF)*
  - *spinal muscular atrophy (SMA)*
- *Carrier screening based on certain races/ethnicities*
  - *alpha thalassemia*
  - *Hb beta chain-related hemoglobinopathy (sickle cell disease)*
  - *Tay-Sachs disease*
  - *Canavan disease*
  - *familial dysautonomia*

ACOG Committee Opinion- Carrier Screening for Genetic Conditions No. 691, March 2017

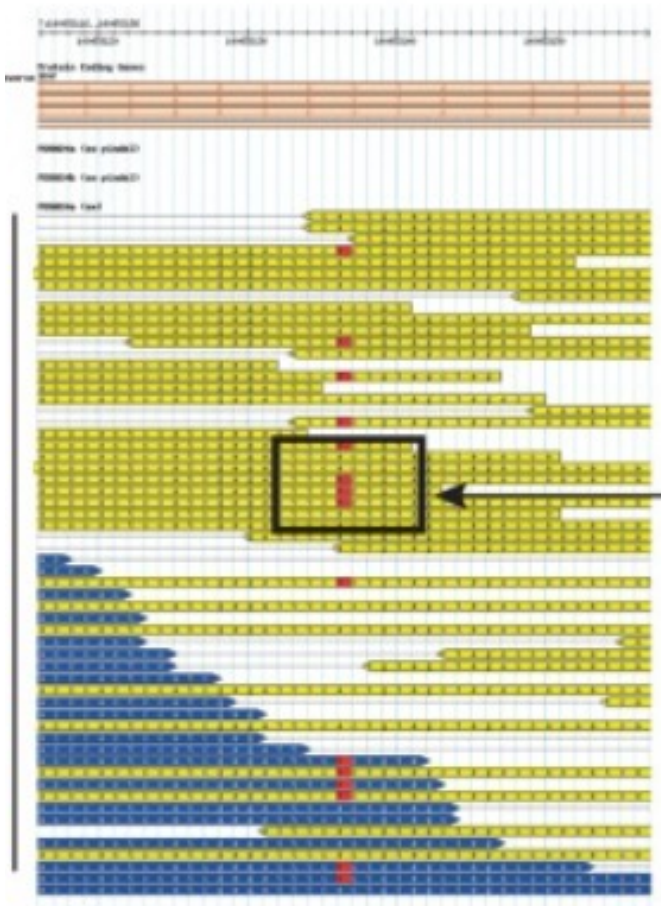
Preconception genetic carrier screening ACMG 2021

- *Clinical utility is measured by the fact that individuals or couples are informed and may alter reproductive decision making because of the carrier screening results.*
- *Clinical utility is represented by its ability to provide individuals an opportunity to discuss their risks and consider reproductive options that are available pre-pregnancy, during pregnancy, or after birth. Availability of reproductive options may depend on various socioeconomical, legal, and cultural factors in different regions.*
- *Examples of reproductive options include:*
  - *In vitro fertilization with preimplantation genetic testing for monogenic conditions*
  - *Use of donor gamete/embryo*
  - *Adoption*
  - *Prenatal diagnosis using chorionic villus sampling or amniocentesis followed by a decision to either prepare for an affected child including special care after birth or terminate the pregnancy*
  - *A decision not to have children*

Gregg AR, et al. ACMG Professional Practice and Guidelines Committee Genetics in Medicine (2021) 23:1793–1806; https://doi.org/10.1038/s41436-021-01203-

Next generation sequencing

- *NGS platforms perform sequencing of millions of small fragments of DNA in parallel.*
- *Bioinformatics analyses are used to piece together these fragments by mapping the individual reads to the human reference genome.*
- *Each of the three billion bases in the human genome is sequenced multiple times, providing high depth to deliver accurate data and an insight into unexpected DNA variation.*
- *NGS can be used to sequence entire genomes or specific areas of interest, including all 22 000 coding genes (a whole exome) or small numbers of individual genes.*



Genomic Coordinate

Amino Acids encoded by DNA codons

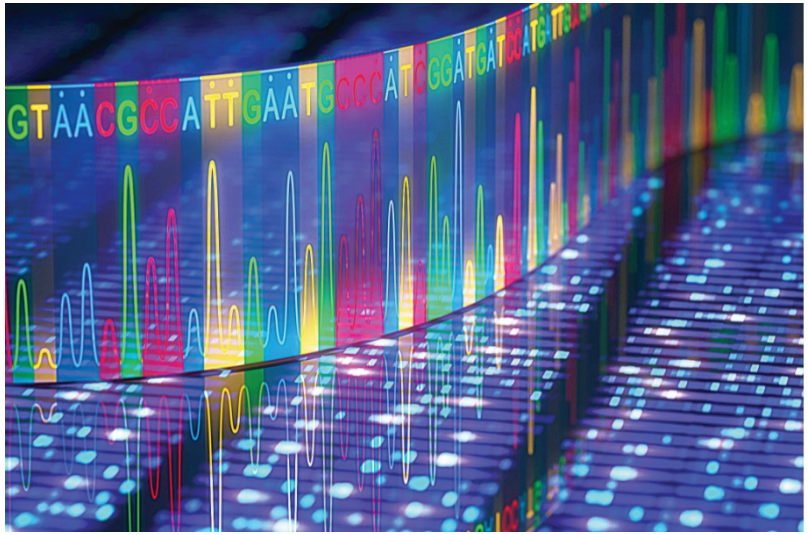
Bar represent a sequencing read, forward (blue) or reverse (yellow) direction. The DNA sequence on each row is the DNA sequence of a single fragment of DNA. The sum of reads covering the particular base is the sequencing depth in that position.

Bases in yellow or blue are "normal", compared to the reference genome. Red indicates deviation from the reference genome, due to either a mutation or a sequencing artifact.

Sam Behjati and Patrick S Tarpey Arch Dis Child Educ Pract Ed. 2013 Dec; 98(6): 236–238.

Next Generation Sequencing for carrier screening - 2021

- *low cost*
- *high throughput identification of sequence variants across many genes simultaneously*
- *Allows equitable opportunities for patients to learn their reproductive risks using next-generation sequencing technology*
- *An improved understanding of this risk allows patients to make informed reproductive decisions*
- *Reproductive decision making is the established metric for clinical utility of population-based carrier screening*
- *Standardization of the screening approach will facilitate testing consistency*



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Gregg AR, et al. ACMG Professional Practice and Guidelines Committee Genetics in Medicine (2021) 23:1793–1806; https://doi.org/10.1038/s41436-021-01203-

Carrier Screening for Genetic Conditions American College of Medical Genetics and Genomics (ACMG) 2021

- *ACMG Goals*
  - *Develop carrier screening that is ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion*

Tables 1-4*

Public Database gnomAD v 2.0.2[49]; 415 autosomal recessive	+	Carrier frequency in gnomAD at least 1/200 for six ancestral populations where Pathogenic and Likely Pathogenic variants were considered[50]	**86 genes**

Table 5*

Carrier frequency known to be at least 1/200 however not captured in gnomAD v 2.0.2	or	Genes with at least a 1/200 carrier frequency of pathogenic or likely pathogenic variants in a subpopulation that has at least 1% representation in the US including US territories.	**9 genes**

Table 6*

X-linked phenotypes (N=355) were identified in the OMIM database (November 30, 2020)[55] (Table S2)	+	Prevalence of the OMIM phenotypes (Table S2) were determined using OMIM[55], Orphanet[63], MedlinePlus[64]; prevalence required was at least 1/40,000	**16 genes**

***All conditions included with at least moderate severity[5,65]**

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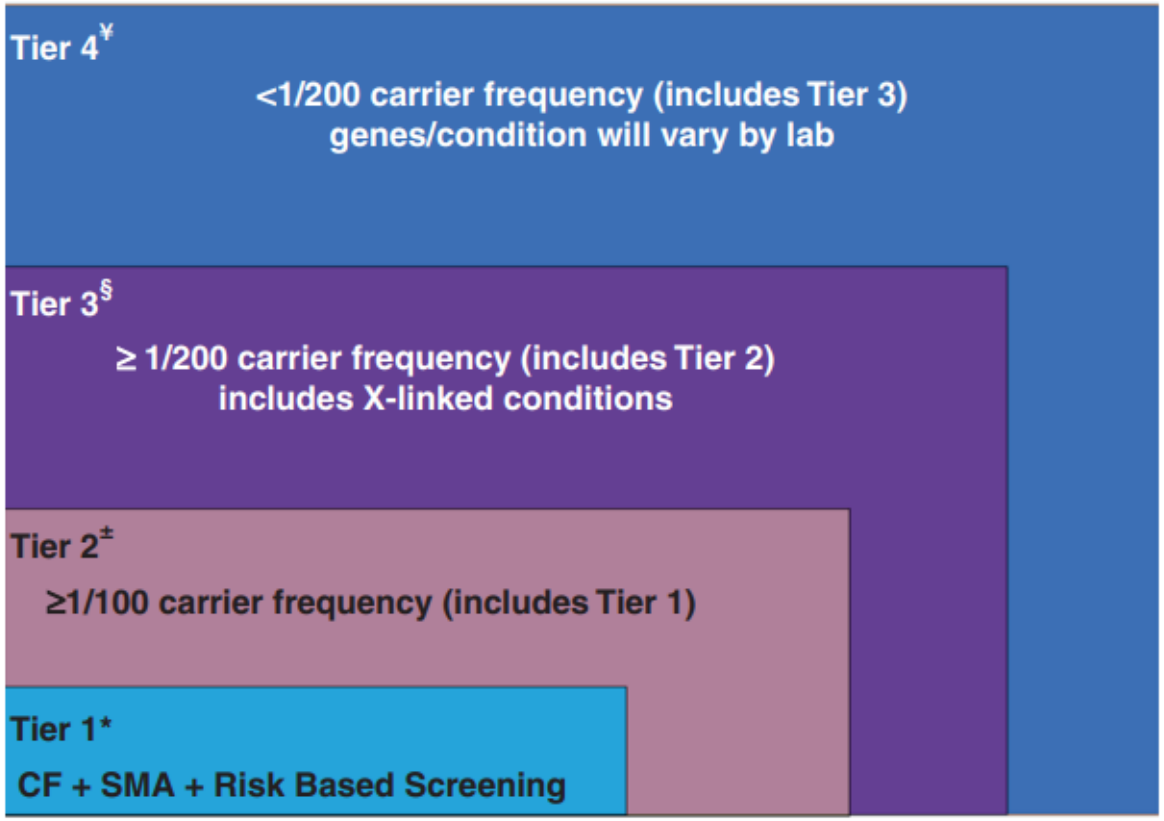
Preconception genetic carrier screening ACMG



Tier 4 screening should be considered for a pregnancy that stems from a known or possible consanguineous relationship (second cousins or closer) or when a family or personal medical history warrants.

All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening which tests for 112 genetic conditions

Limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions

Carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations.

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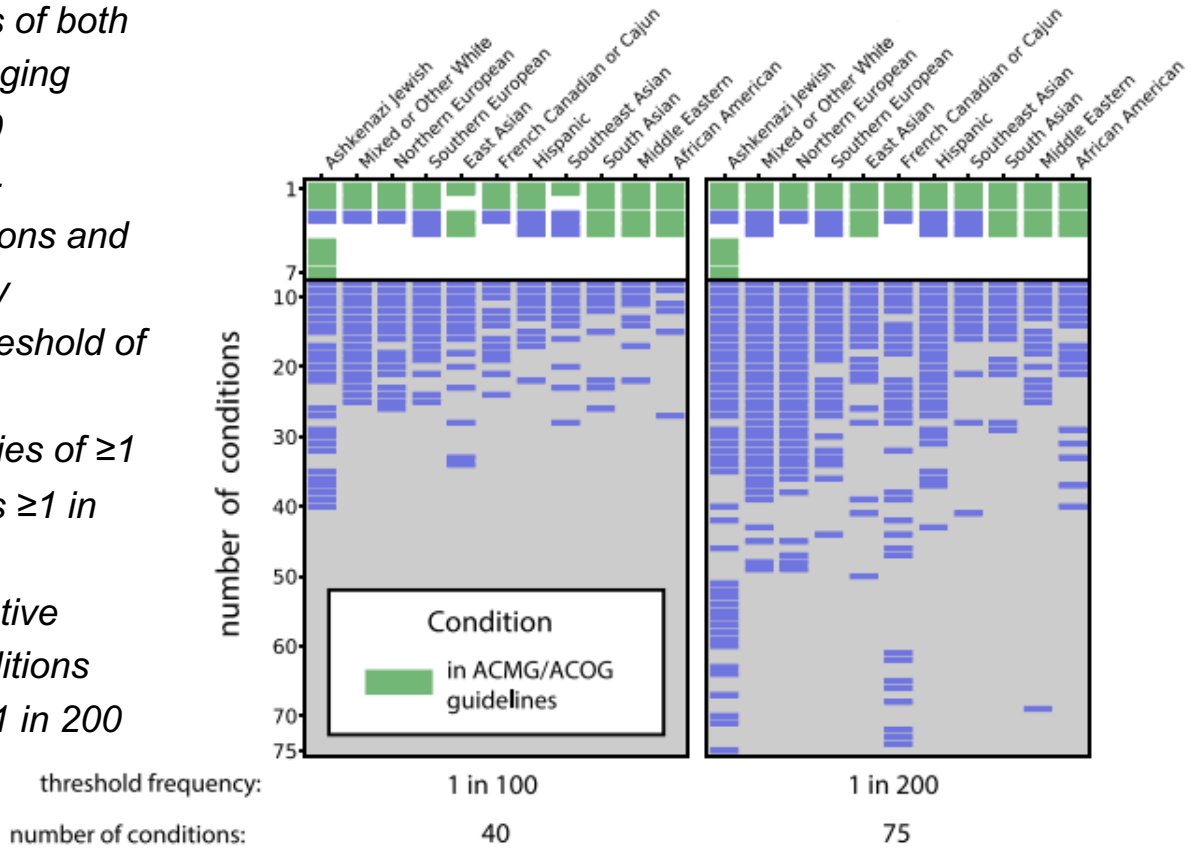
Gregg AR et al. ACMG Professional Practice and Guidelines Committee Genetics in Medicine (2021) 23:1793–1806; https://doi.org/10.1038/s41436-021-01203-

Carrier screening panel that supports equity across diverse populations

- *Using evidence-based interpretations of both ACOG and ACMG criteria and leveraging carrier frequency data from >460,000 individuals across 11 ethnicities (self-reported) which identified 176 conditions and applied criteria from ACOG frequency threshold of ≥1 in 100 and ACMG threshold of ≥1 in 200.*
- *Forty conditions had carrier frequencies of ≥1 in 100 and 75 had carrier frequencies ≥1 in 200*
- *Following severity criteria a conservative equitable panel consisting of 37 conditions and a more permissive panels and ≥1 in 200 consists of 74 conditions.*



Taber, et al. Genetics in Medicine (2022) 24, 201–213

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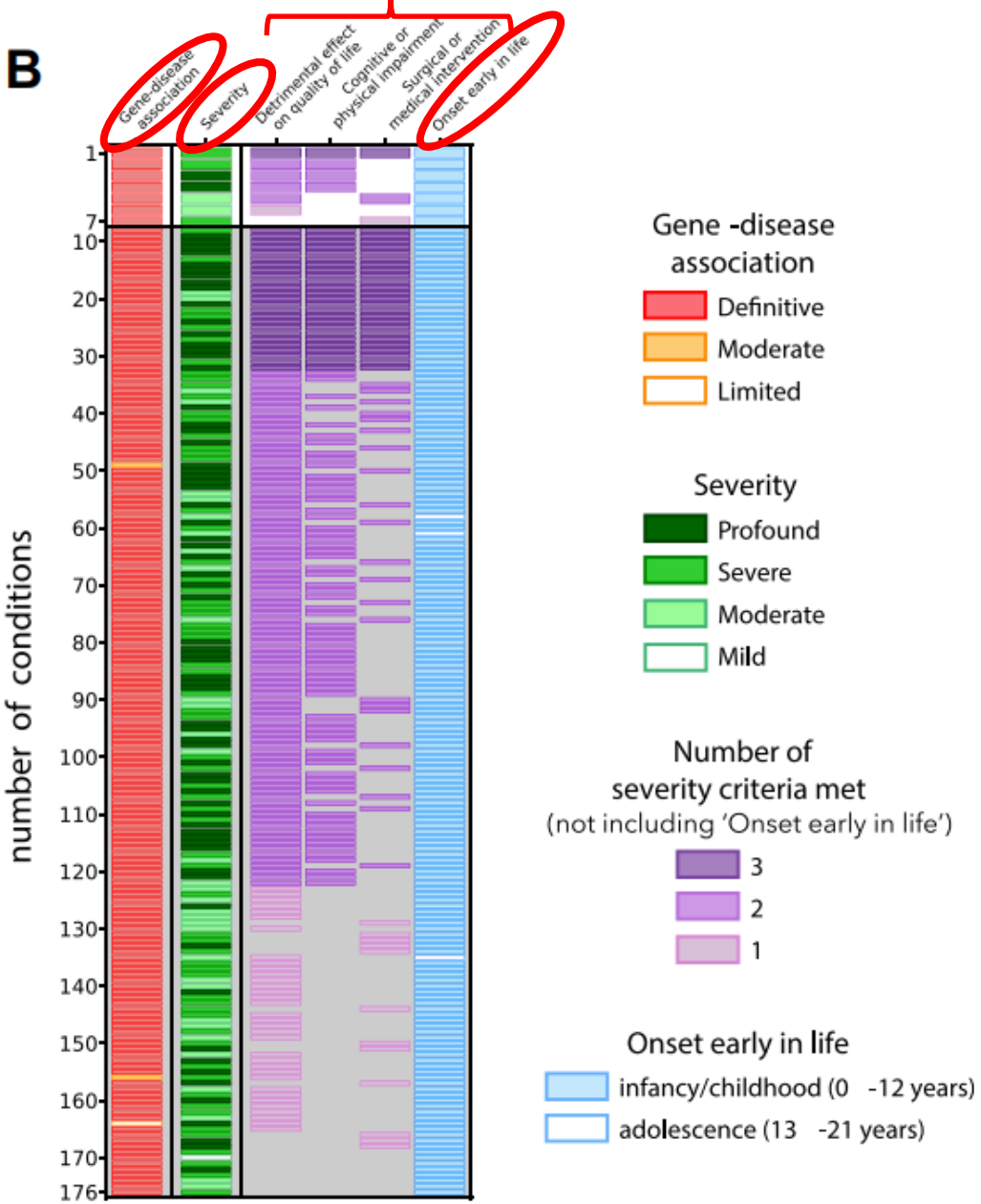
176 Panel

Moderate or higher gene–disease association – 175 of 176 conditions (99.4%) Captures 99.8% of carriers and >99.9% of ARCs compared with a 176-condition panel

Moderate severity - 175 of 176 conditions (99.4%)

ACOG severity criterion, 165 of 176 - Captures 94.2% of carriers and 92.3% of ARCs

Age of onset (infancy/childhood) - 165 of 176 conditions (93.8%)



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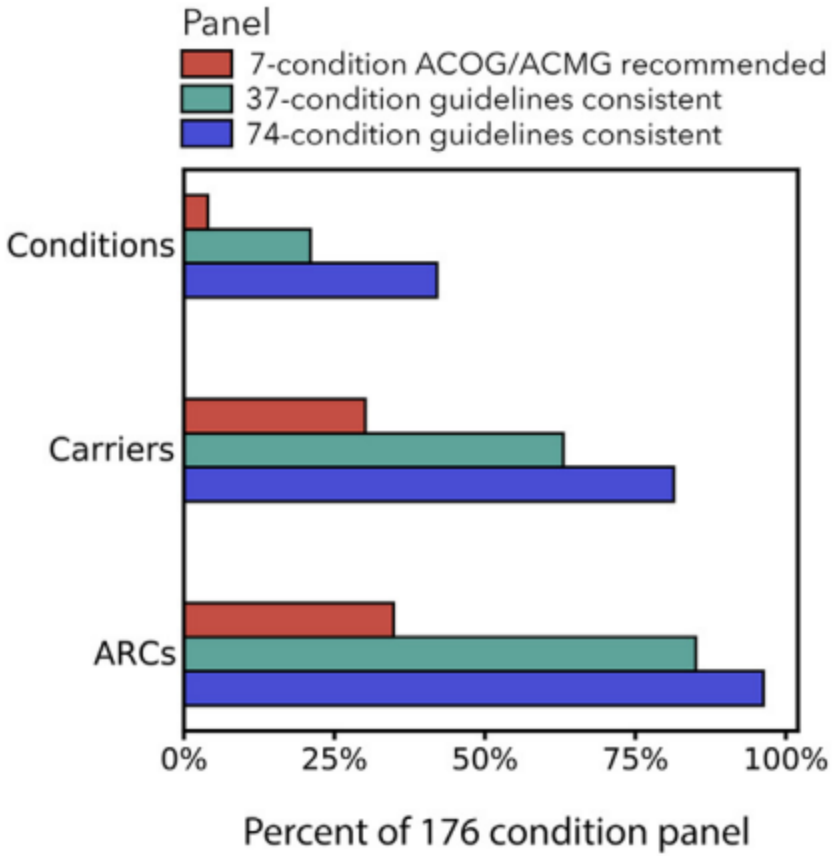
Carrier screening panel that supports equity across diverse populations



- *Compared to the 176 conditions panel*
  - *37 conditions panel would capture 63.0% of carriers and 84.6% of ARCs*
  - *74 conditions panel would capture 81.4% of carriers and 96.6% of at risk couples (ARCs)*

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Taber, et al. Genetics in Medicine (2022) 24, 201–213

Genetic carrier screening – Impact on decision making (3 studies)

- *47% - screening was to spare a future child a life with a severe disorder*
- *Higher anxiety in high-risk and pregnant respondents*
- *100% would opt for the test again*
- *Reproductive decision making was more common when patients received results **before an established pregnancy** (62–77%).*
  - *The most common decisions were*
    - *59% in vitro fertilization with preimplantation genetic diagnosis*
    - *20% diagnostic test during pregnancy*
    - *7.7% use of a donor gamete*
    - *5.1% consider adoption*
- ***Testing during pregnancy***
  - *16-36% had an affected fetus of those performing diagnostic testing*
  - *40-67% discontinued their pregnancy*

Ivy van Dijke, et al European Journal of Human Genetics (2021) 29:1252–1258;
Ghiossi, C. E., et al. J. Genet. Couns. 27, 616–625 (2018).
Johansen Taber, K. A.,et al Genet. Med. 21, 1041–1048 (2019)
Gregg AR, et al ACMG Professional Practice and Guidelines Committee Genetics in Medicine (2021) 23:1793–1806

Carrier screening ACMG – Recommendations Tier 3 or Tier 4

- *Carrier screening (Tier 3) is optional and can be performed at any time*
- *Preconception screening is recommended over prenatal screening*
  - *less stressful on patients with positive screening*
  - *allows for the full complement of reproductive decision making*
- *If done in pregnancy, concurrent partner testing should be offered*
- *When a reproductive partner has changed, carrier screening should be readdressed*
- *Carrier screening is not a test for all genetic conditions*
  - *will not identify de novo variants in the offspring*
  - *does not replace newborn screening*
- *When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered*
- *Consanguineous couples should have Tier 4 screening*
- *If family history warrants, additional genes may be considered*
- *Negative test reduces but does not eliminate the risk of an affected child*

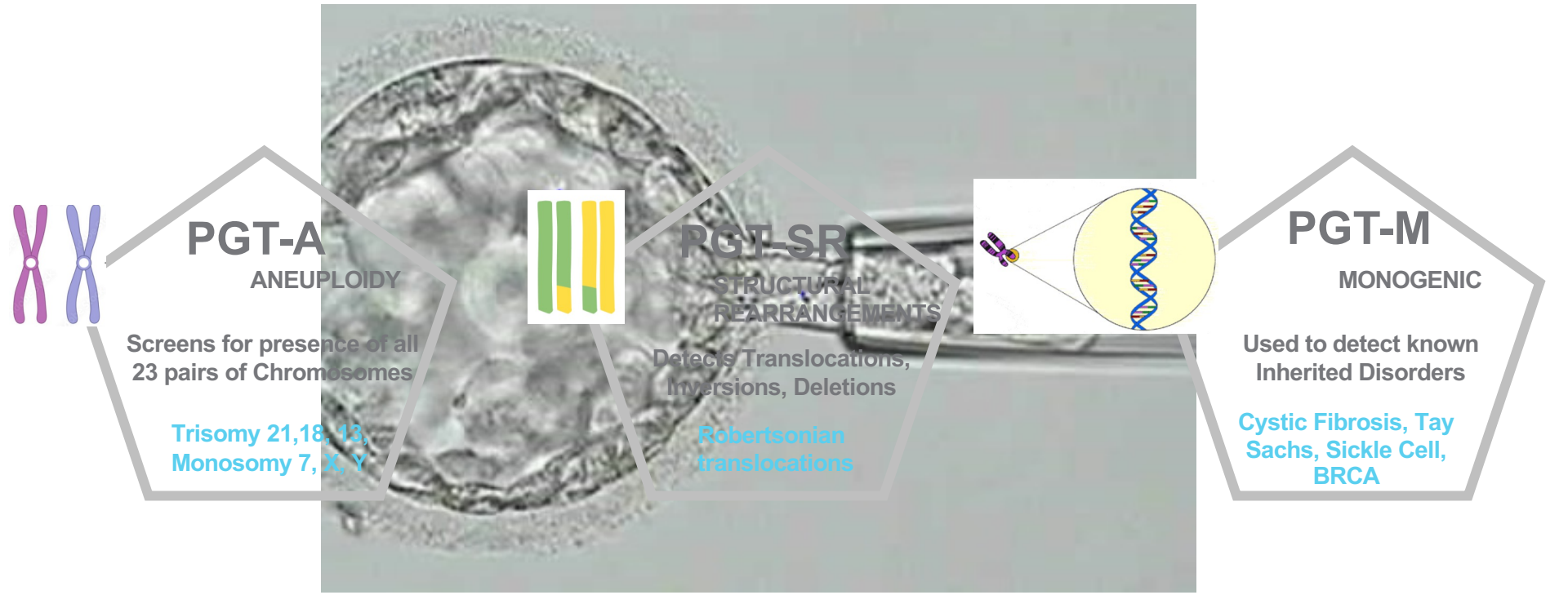
Gregg AR et al. ACMG Professional Practice and Guidelines Committee Genetics in Medicine (2021) 23:1793–1806; https://doi.org/10.1038/s41436-021-01203-

Carrier screening- Greater Expanded Panel (176 plus)

- *Larger panels that include ACOG and ACMG criteria should be considered*
  - *More ethnically inclusive panel*
  - *Moderate or higher gene–disease association – 175 of 176 conditions (99.4%)*
  - *Moderate to severe disease severity - 175 of 176 conditions (99.4%)*
  - *ACOG severity criterion*
    - *Determinantal effect on quality of life*
    - *Cognitive or physical impairment*
    - *Surgical or medical intervention*
  - *Onset early in life*

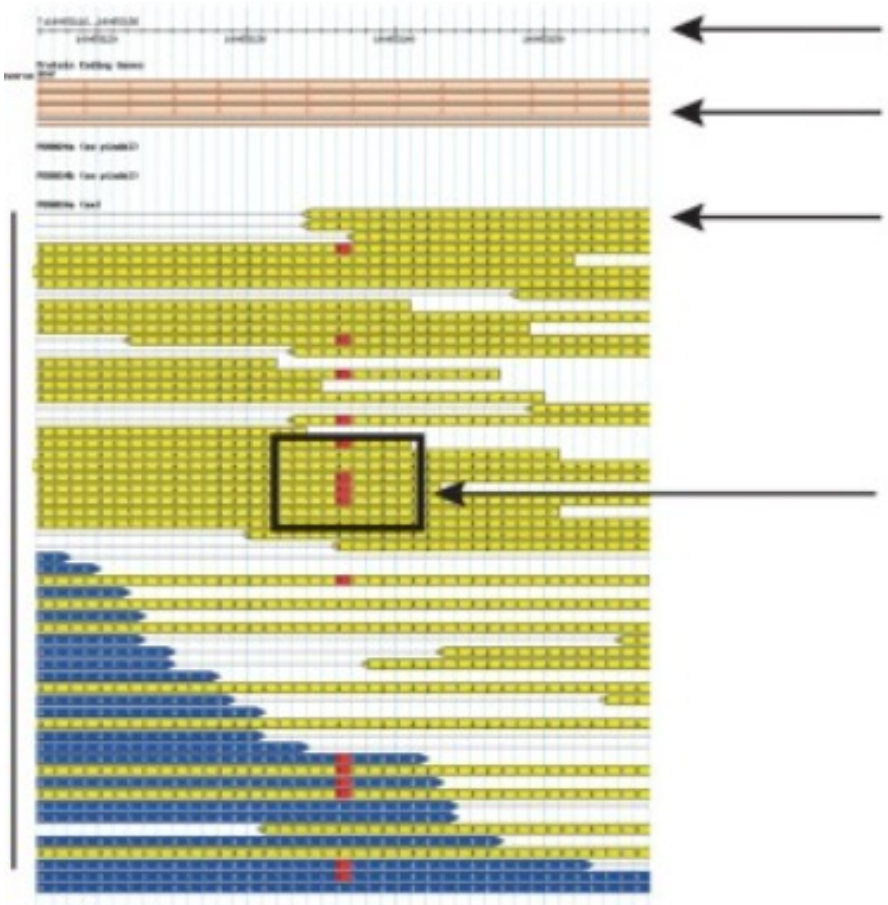
Taber, et al. Genetics in Medicine (2022) 24, 201–213

Preimplantation genetic testing
PGT-A, PGT-SR, PGT-M



Preimplantation genetic testing platforms

- *NGS allows for direct reading of sequenced DNA fragments and their quantification based on sequence read numbers*
- *whole chromosome aneuploidy (PGT-A)*
- *medium size deletions or insertions in chromosomes (PGT-SR)*
- *detection of single gene disorders (PGT-M)*



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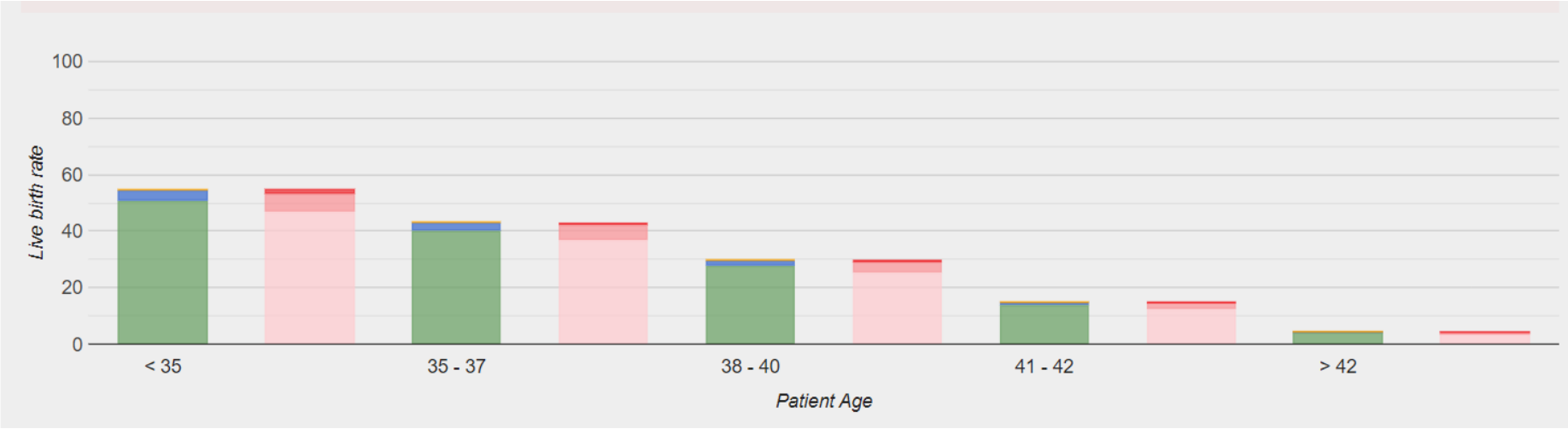
ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations† ESHRE PGT-SR/PGT-A Working Group 2020
Sam Behjati and Patrick S Tarpey Arch Dis Child Educ Pract Ed. 2013 Dec; 98(6): 236–238.

PGT-A to improve IVF outcomes –live births

National assisted reproductive technology (ART) surveillance systems (SART)

Data 2019

293, 672 Total Cycles



PGT-A - Time to pregnancy and advanced reproductive age > 37 yo

- *Analysis of data from national assisted reproductive technology (ART) surveillance systems*
- *PGT-A is not associated with improved rates of clinical pregnancy or live birth after fresh autologous blastocyst transfer among women aged <37 years*
- ***PGT-A of embryos appeared to improve the likelihood of having a live birth among women >37 years***
- *Cycles that were intended for PGT-A were more likely to reach embryo transfer in all age groups, but more significantly in women aged >37*
- *RCT that focused on women with advanced maternal age (38-41 years old) demonstrated a significantly higher live birth rate with PGT-A group per cycle (36% vs 21.9%, P<031) and a lower miscarriage rate (2.7% vs 39%, P<0007)*

Chang et al. Fertil Steril. 2016; 105(2): 394–400.
Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology Fertil Steril 2018;109:429–36.

Live birth with and without PGT-A for < 38 yo (RCT)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Live Birth with or without Preimplantation Genetic Testing for Aneuploidy

J. Yan, Y. Qin, H. Zhao, Y. Sun, F. Gong, R. Li, X. Sun, X. Ling, H. Li, C. Hao, J. Tan, J. Yang, Y. Zhu, F. Liu, D. Chen, D. Wei, J. Lu, T. Ni, W. Zhou, K. Wu, Y. Gao, Y. Shi, Y. Lu, T. Zhang, W. Wu, X. Ma, H. Ma, J. Fu, J. Zhang, Q. Meng, H. Zhang, R.S. Legro, and Z.-J. Chen

- *20 and 37 years of age*
- *three or more good-quality blastocysts*
- *Good Prognosis*

Table 3. Cumulative Live-Birth Rate and Secondary Outcomes.*

Outcome	PGT-A Group (N=606)	Conventional-IVF Group (N=606)	Absolute Difference (95% CI)	Rate Ratio (95% CI)
Primary outcome				
Cumulative live-birth rate — no. (%)†	468 (77.2)	496 (81.8)	−4.6 (−9.2 to −0.0)	0.94 (0.89 to 1.00)
Singleton	462 (76.2)	478 (78.9)	−2.6 (−7.3 to 2.1)	0.97 (0.91 to 1.03)
Twin	6 (1.0)	18 (3.0)	−2.0 (−3.5 to −0.4)	0.33 (0.13 to 0.83)
Secondary outcomes				
Cumulative biochemical pregnancy — no. (%)	526 (86.8)	571 (94.2)	−7.4 (−10.7 to −4.2)	0.92 (0.89 to 0.96)
Cumulative clinical pregnancy — no. (%)	505 (83.3)	556 (91.7)	−8.4 (−12.1 to −4.7)	0.91 (0.87 to 0.95)
Cumulative ongoing pregnancy — no. (%)	479 (79.0)	514 (84.8)	−5.8 (−10.1 to −1.5)	0.93 (0.88 to 0.98)
Birth weight				
Singleton				
No. of observations	462	478		
Mean weight — g	3417±488	3449±488	−32 (−95 to 30)	
Twin				
No. of observations	12	36		
Mean weight — g	2500±714	2605±420	−105 (−444 to 235)	
Cumulative pregnancy loss — no./total no. (%)				
Biochemical	31/526 (5.9)	41/571 (7.2)	−1.3 (−4.2 to 1.6)	0.82 (0.52 to 1.29)
Clinical	46/526 (8.7)	72/571 (12.6)	−3.9 (−7.5 to −0.2)	0.69 (0.49 to 0.98)
First trimester	37/526 (7.0)	60/571 (10.5)	−3.5 (−6.8 to −0.1)	0.67 (0.45 to 0.99)
Second trimester	9/526 (1.7)	12/571 (2.1)	−0.4 (−2.0 to 1.2)	0.81 (0.35 to 1.92)
Good birth outcome — no. (%)‡	378 (62.4)	385 (63.5)	−1.2 (−6.6 to 4.3)	0.98 (0.90 to 1.07)
Features of live births				
Duration of pregnancy — wk	39.2±1.7	39.1±1.6	0.0 (−0.2 to 0.2)	
No. of embryos transferred	1.2±0.4	1.3±0.6	−0.2 (−0.2 to −0.1)	
No. of embryo-transfer procedures	1.1±0.4	1.3±0.5	−0.1 (−0.2 to −0.1)	
Interval since randomization — mo	12.5±2.0	12.4±2.3	0.1 (−0.2 to 0.4)	
Frozen embryos				
No. of unused embryos	5.2±3.2	5.5±2.9	−0.3 (−0.6 to 0.1)	
No. of unused embryos in women without a live birth	4.4±2.8	4.9±2.9	−0.4 (−1.2 to 0.3)	

Yan, et al N Engl J Med 2021;385:2047-58

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Table S3. The rates of pregnancy, pregnancy loss and live birth after the first embryo transfer between PGT-A and IVF.

Outcome	PGT-A group (N=576)	IVF group (N=594)	Absolute Difference (95%CI)	Rate Ratio for PGT-A vs. IVF (95%CI)
Primary outcome: live birth-no. (%)	382/576 (66.3)	369/594 (62.1)	4.2 (-1.3, 9.7)	1.07 (0.98, 1.16)
Singleton	376/576 (65.3)	357/594 (60.1)	5.2 (-0.4, 10.7)	1.09 (0.99, 1.19)
Twin	6/576 (1.0)	12/594 (2.0)	-1.0 (-2.4, 0.4)	0.52 (0.19, 1.36)
Secondary outcomes				
Biochemical pregnancy-no. (%)	451/576 (78.3)	462/594 (77.8)	0.5 (-4.2, 5.3)	1.01 (0.95, 1.07)
Clinical pregnancy-no. (%)	422/576 (73.3)	427/594(71.9)	1.4 (-3.7, 6.5)	1.02 (0.95, 1.09)
Ongoing pregnancy-no. (%)	393/576 (68.2)	384/594 (64.6)	3.6 (-1.8, 9.0)	1.06 (0.97, 1.15)
Pregnancy loss-no./total no. (%)				
Biochemical pregnancy loss	26/451 (5.8)	33/462 (7.1)	-1.4 (-4.6, 1.8)	0.81 (0.49, 1.33)
Clinical pregnancy loss	39/451 (8.7)	55/462 (11.9)	-3.3 (-7.2, 0.7)	0.73 (0.49, 1.07)
First trimester	30/451 (6.7)	44/462 (9.5)	-2.9 (-6.4, 0.7)	0.70 (0.45, 1.09)
Second trimester	9/451 (2.0)	11/462 (2.4)	-0.4 (-2.3, 1.5)	0.84 (0.35, 2.00)

No adjustment was made for multiplicity of secondary outcomes. 95% CIs should not be used to infer definitive treatment outcomes.

- *No difference even after the first IVF cycle*

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Table S2. Live birth rate after each embryo transfer cycle.

Outcome	PGT-A group (N=606)	IVF group (N=606)	Absolute Difference (95% CI)	Rate Ratio for PGT-A vs. IVF (95%CI)
Live birth after 1st embryo transfer-no. (%)	382/576 (66.3)	369/594 (62.1)	4.2 (-1.3, 9.7)	1.07 (0.98, 1.16)
Live birth after 2nd embryo transfer-no. (%)	74/119 (62.2)	106/192 (55.2)	7.0 (-4.2, 18.2)	1.13 (0.93, 1.36)
Live birth after 3rd embryo transfer-no. (%)	2/5 (40.0)	19/49 (38.8)	1.2 (-43.8, 46.3)	1.03 (0.33, 3.19)
Live birth conceived naturally-no.	10	2	-	-

No adjustment was made for multiplicity of secondary outcomes. 95% CIs should not be used to infer definitive treatment outcomes.

- *More women in the conventional-IVF group underwent a second or third embryo-transfer cycle:*
  - *Second Cycle -192 women in the conventional-IVF group and 119 in the PGT-A group*
  - *Third Cycle - 49 women in the conventional-IVF group and 5 in the PGT-A group*

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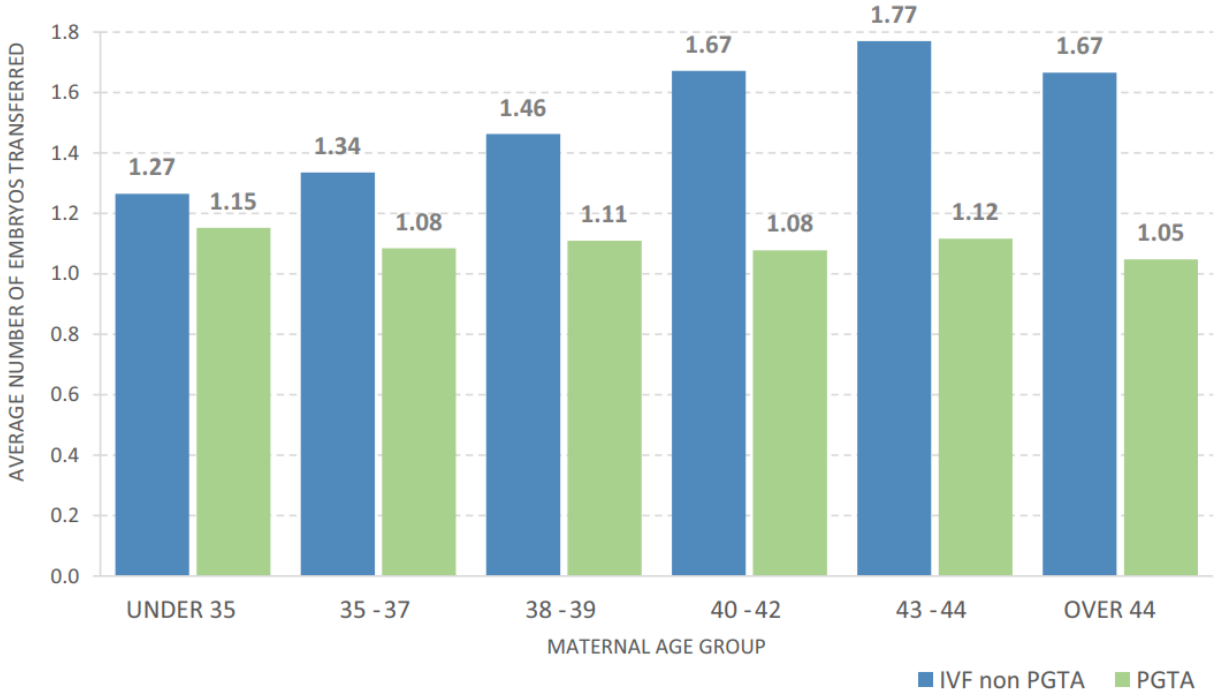
Yan, et al N Engl J Med 2021;385:2047-58

PGT-A Retrospective Cohort Study
2464 PGT-A, 190,010 cycles



- *Fewer embryos are required to achieve a pregnancy following PGT-A compared to regular IVF*

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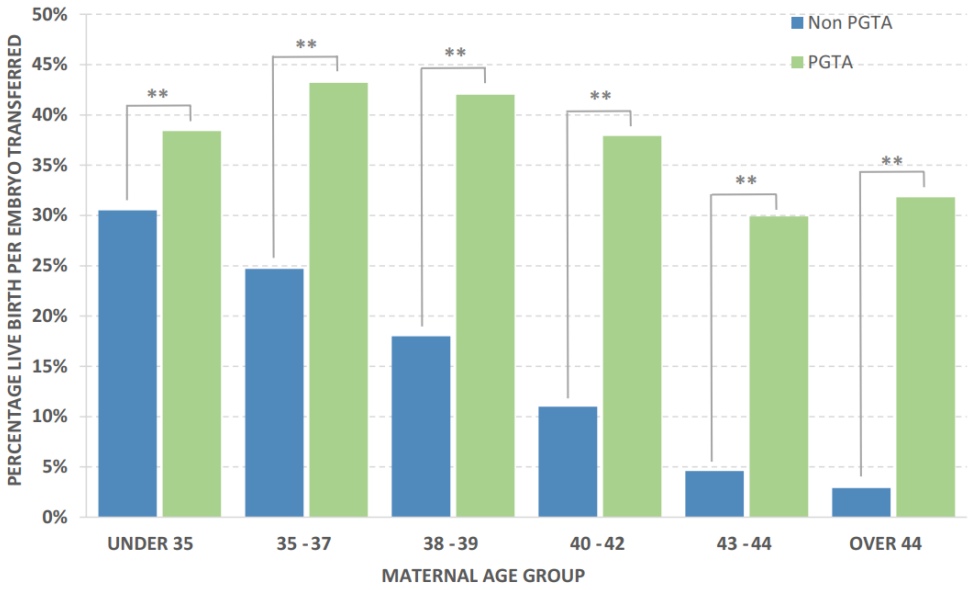
Sanders et al Journal of Assisted Reproduction and Genetics (2021) 38:3277–3285

PGT-A Retrospective Cohort Study
2464 PGT-A 190,010 cycles



- *PGT-A versus non PGT-A*
  - *Live birth rates were significantly higher in all age groups*
  - *Mostly single embryo transfers (SET)*
  - *Less number of transfers per live birth , particularly if over 40 years*

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Sanders et al Journal of Assisted Reproduction and Genetics (2021) 38:3277–3285

PGT-A: Recommendations

- ***Recommendations***
  - *Shortened time to pregnancy and increased success for women over 37 yo*
  - *Potential benefit in select populations of younger reproductive age women*
  - *Selection of embryo for elective single embryo transfer – Decrease risk of multiple gestations*
  - *Beneficial if proceeding with PGT-M or PGT-SR*
  - *Potential benefit for long term fertility preservation*
  - *Cost benefit – minimize number of frozen embryo transfer cycles?*
- ***Considerations***
  - *Would embryos that don't survive to the stage of biopsy for genetic testing lead to successful pregnancies*
  - *Are false positive test results possible (mosaicism 3-20%) that could lead to a healthy genetically normal pregnancy?*
- ***Counseling is necessary for shared decision making for PGT***

Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology Fertil Steril 2018;109:429–36. 2018; Practice Committee and Genetic Counseling Professional Group (GCPG) of the American Society for Reproductive Medicine. Fertil Steril 2020;114:246-54

Genetics and Pregnancy – Purpose

- *Prenatal testing for chromosomal abnormalities are designed to provide an accurate assessment of a patient's risk of carrying a fetus with a chromosomal disorder.*
- *Testing for chromosomal abnormalities should be an informed patient choice based on adequate and accurate information.*
- *All patients should be offered both screening and diagnostic tests, and all patients have the right to accept or decline testing after counseling.*

ACOG Practice Bulleting Screening for Fetal Chromosomal Abnormalities. 2018
Photo: https://www.theguardian.com/world/2021/aug/03/hopes-uk-trial-will-allay-pregnant-womens-covid-vaccine-concerns

Pregnancy – Genomic testing capabilities

- *Recommendations*

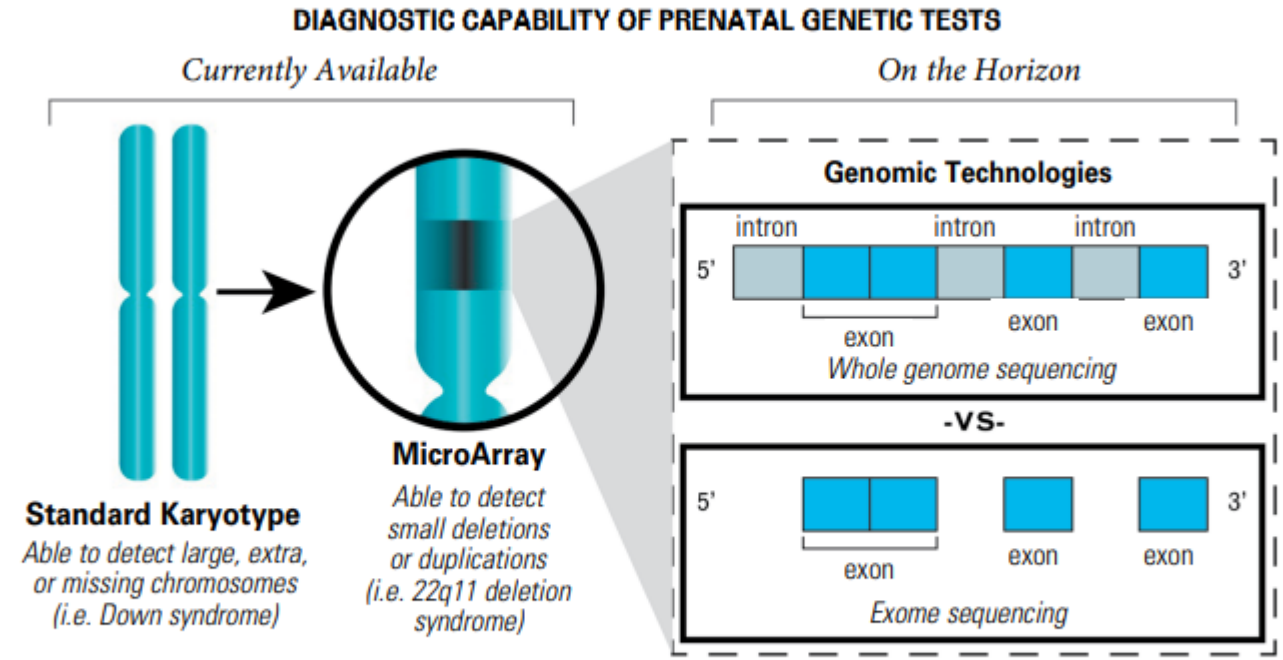


Figure 1. Diagnostic capability of prenatal genetic tests. (Reprinted from Hardisty EE, Vora NL. Advances in genetic prenatal diagnosis and screening. Curr Opin Pediatr 2014;26:634–8.) ⇦

COMMITTEE OPINION Number 682 (Reaffirmed 2020) Committee on Genetics Society for Maternal–Fetal Medicine Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology

Genetics and Pregnancy – Chromosomal Abnormalities

Table 1. Chromosomal Abnormalities in Second-Trimester Pregnancies Based on Maternal Age at Term

	Trisomy 21	Trisomy 18	Trisomy 13	Sex Chromosome Aneuploidy (XXX, XY, XYY, 45, X)	Microarray or Rare Chromosomal Abnormality	All Chromosomal Abnormalities
Age 20	8 per 10,000 1 in 1,250	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	82 per 10,000 1 in 122
Age 25	10 per 10,000 1 in 1,000	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	84 per 10,000 1 in 119
Age 30	14 per 10,000 1 in 714	4 per 10,000 1 in 2,500	2 per 10,000 1 in 5,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	91 per 10,000 1 in 110
Age 35	34 per 10,000 1 in 294	9 per 10,000 1 in 1,111	4 per 10,000 1 in 2,500	35 per 10,000 1 in 285	37 per 10,000 1 in 270	119 per 10,000 1 in 84
Age 40	116 per 10,000 1 in 86	30 per 10,000 1 in 333	14 per 10,000 1 in 714	51 per 10,000 1 in 196	37 per 10,000 1 in 270	248 per 10,000 1 in 40

ACOG Practice Bulleting Screening for Fetal Chromosomal Abnormalities. 2018

Microdeletions, Duplications and other Variants

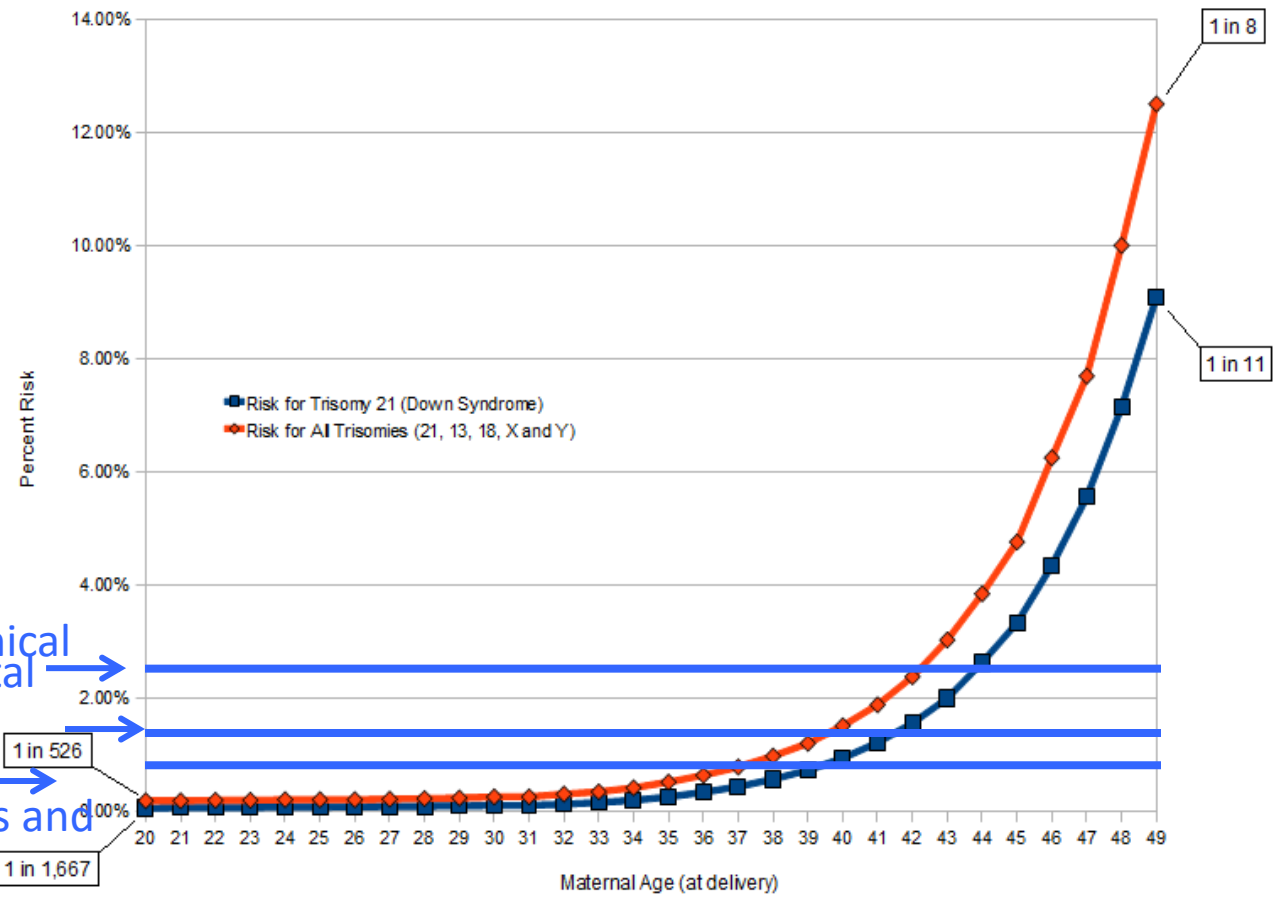
Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Indication for Prenatal Diagnosis	Normal Karyotype	Common Benign	Pathogenic	Uncertain Clinical Significance (N=130)		Total Known Pathogenic and Potential for Clinical Significance*
				Likely to Be Benign	Potential for Clinical Significance	
	no.		*no. (%)*			*no. (%) [95% CI]†*
Any	3822	1234 (32.3)	35 (0.9)	69 (1.8)‡	61 (1.6)	96 (2.5) [2.1–3.1]
Advanced maternal age	1966	628 (31.9)	9 (0.5)	37 (1.9)	25 (1.3)	34 (1.7) [1.2–2.4]
Positive on Down's syndrome screening	729	247 (33.9)	3 (0.4)	13 (1.8)	9 (1.2)	12 (1.6) [0.9–2.9]
Anomaly on ultrasonography	755	247 (32.7)	21 (2.8)	16 (2.1)	24 (3.2)	45 (6.0) [4.5–7.9]
Other§	372	112 (30.1)	2 (0.5)	3 (0.8)	3 (0.8)	5 (1.3) [0.6–3.1]

* Total includes those predetermined as known to be pathogenic and those classified by the clinical advisory committee as clinically relevant.
† CI denotes confidence interval.
‡ Includes 36 samples determined likely to be benign by the study geneticist and 33 determined by the independent clinical advisory committee on the basis of size, gene content, inheritance, the literature, and ultrasonography findings.
§ Other indications include family history, previous pregnancy with chromosomal abnormalities, and elective decision.

Wapner, et al. December 6, 2012N Engl J Med 2012; 367:2175-2184 DOI: 10.1056/NEJMoa1203382

Risk for pathogenic and potential clinically significant microdeletions and duplications



Variants of clinical significance

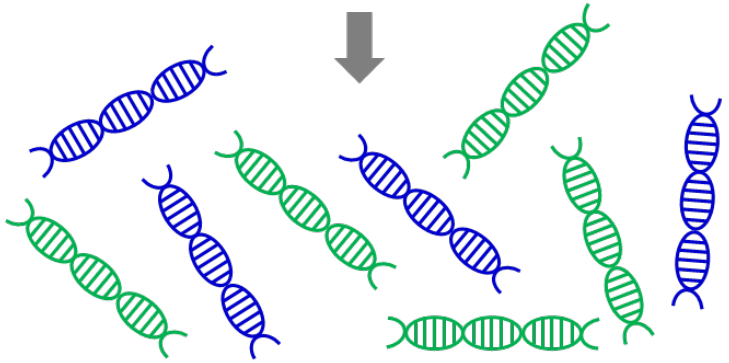
Total

Pathogenic microdeletions and duplications

NIPT

Maternal blood sample

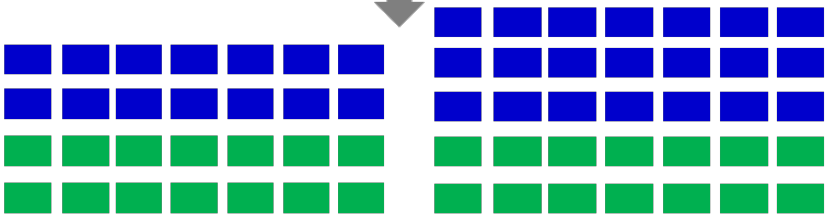
Maternal and fetal cell-free DNA



Cell-free DNA sequenced via massively parallel sequencing (MPS)

CCTAGCTCGAACCTAGCCAAGGTTAACTTAATTCCCCATCATCATATTCC
GGCCTTTAAAATTCCAATCATGTCTCATGGCCATCGTGGAAACTCTAAGGT
CCCATCATCATATTCCATGGCCATCGTGGAAACTCTAAGGTTTGACGTTAA
AGGTCCCTAGCTCGAACCTAGCCAAGGTTAACTTAATTCCCCATCTATTCC

Alignment and counting



Chromosome 21
No Aneuploidy

Chromosome 21
Aneuploidy

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Modified from Drury, et al Cell Free Fetal DNA Testing for Prenatal Diagnosis. Advances in Clinical Chemistry Vol 76, 2016

Pregnancy – NIPT high risk populations

Chromosomal Abnormality	Sensitivity (%)	95% CI (%)	Specificity (%)	95% CI (%)
Trisomy 21	**99.5**	**96.3-99.9**	**100**	**99.87-100**
Trisomy 18	**97.7**	**87.9-99.6**	**99.97**	**99.81-99.99**
Trisomy 13	**100**	**83.2-100**	**99.97**	**99.81- 99.99**

- *High sensitivity and high specificity*
- *Not reportable or no call results – increased risk of chromosomal abnormality – diagnostic testing is recommended*

Geppert, et al Prenatal Diagnosis. 2020;40:454–462.
ACOG Practice Bulleting Screening for Fetal Chromosomal Abnormalities. 2018

Pregnancy – NIPT low risk population

- *Low risk population*
- 13,043 (73.1%) were considered low-risk for aneuploidy < 35
- 3,873 that were ≥35 but had a low-risk result on a blood screening test

Chromosomal abnormality	Sensitivity % (n)	Specificity % (n)	PPV % (n)	NPV % (n)
Trisomy 21	**100 (18/18)**	**99.98 (12,815/12,818)**	**85.71 (18/21)**	**100 (12,815/12,815)**
Trisomy 18	**75 (3/4)**	**99.98 (12,829/12,832)**	**50 (3/6)**	**99.99 (12,829/12,830)**
Trisomy 13	**100 (5/5)**	**99.98 (12,828/12,831)**	**62.50 (5/8)**	**100 (12,828/12,828)**

Dar, et al American Journal of Obstetrics and Gynecology (2022), doi: https://doi.org/10.1016/j.ajog.2022.01.019

Pregnancy – NIPT

Table 3. The Effect of Maternal Age on the Positive Predictive Value of Cell-Free DNA Screening for Trisomy 21, 18, and 13 at 10 Weeks Gestation*

	Maternal Age	Age Related Risk†	Positive Predictive Value‡
Trisomy 21	20	1:804 or 12 per 10,000	38–80%
	35	1:187 or 53 per 10,000	73–95%
	40	1:51 or 196 per 10,000	91–99%
Trisomy 18	20	1:1,993 or 5 per 10,000	11–41%
	35	1:465 or 22 per 10,000	34–75%
	40	1:126 or 79 per 10,000	66–92%
Trisomy 13	20	1:6,347 or 1.6 per 10,000	5–13%
	35	1:1,481 or 7 per 10,000	17–40%
	40	1:401 or 24 per 10,000	43–71%

*Sensitivity and specificity approximately 99%

†Age related risk of aneuploidy per 10,000 pregnancies at 10 weeks gestation based on maternal age at term

‡Percent varies by laboratory

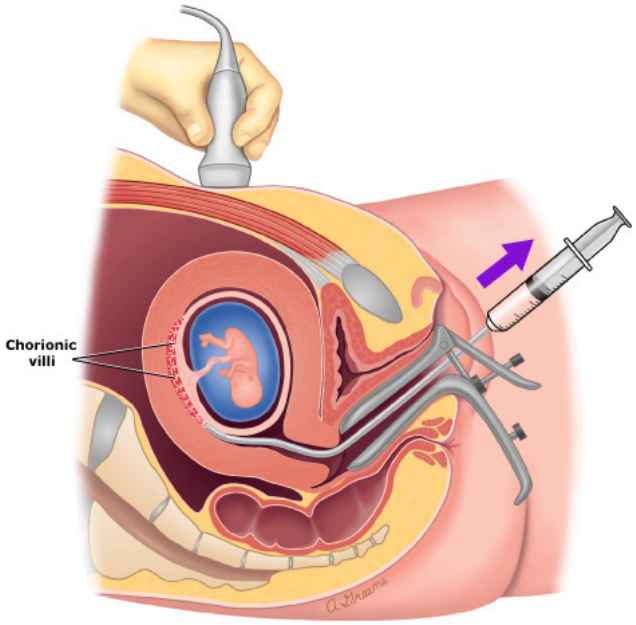
Adapted from University of North Carolina at Chapel Hill. Positive predictive value of cell free DNA calculator. Available at: https://www.med.unc.edu/mfm/nips-calc. Retrieved February 24, 2020.

- *Low positive predictive value means many false positive test results*

ACOG Practice Bulletin Screening for fetal Chromosomal Abnormalities 2018

Pregnancy – Diagnostic Testing

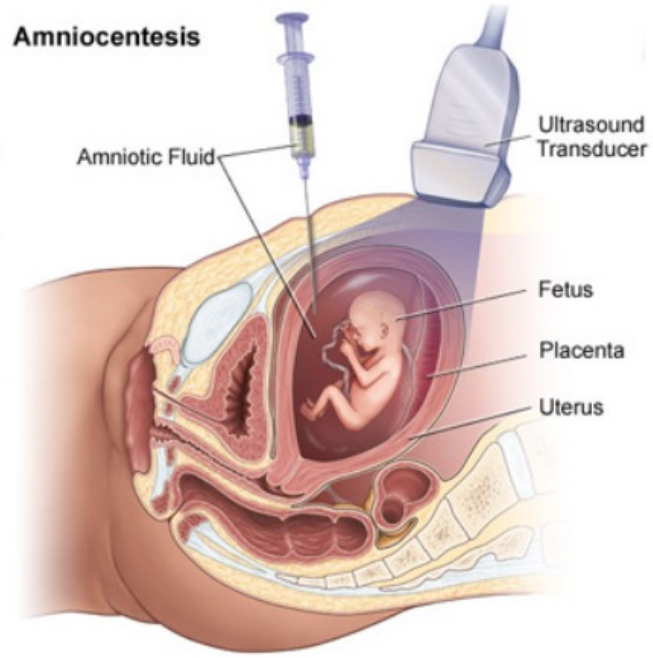
- *Chorionic villus sampling*
- *Karyotype and microarray*
- *Detects 99.8% of trisomies, pathogenic microdeletions/duplications, clinically significant variants, point mutations, chromosomal rearrangements and de novo mutations*
- *Performed between 10-13 weeks*
- *Miscarriage rate overall - 0.5-3.0%*
- *Procedure-related risk of miscarriage 0.22% =1/500*



ACOG Committee Opinion Preimplantation Genetic Testing 2020
Akolekar, et al.Ultrasound Obstet Gynecol 2015; 45: 16–26

Pregnancy – Diagnostic Testing

- *Amniocentesis*
- *Karyotype preferred for balanced translocations and triploidy*
- *Performed between 15-20 weeks*
- *Miscarriage rate overall - 0.5-1.0%*
- *Procedure-related risk of miscarriage 0.11% =1/900*



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ACOG Committee Opinion Preimplantation Genetic Testing 2020
Akolekar, et al.Ultrasound Obstet Gynecol 2015; 45: 16–26
Image courtesy -UCLA MFM website

Pregnancy – Prenatal testing

- *Testing for chromosomal abnormalities should be an informed patient choice based on adequate and accurate information*
- *All patients should be offered both screening and diagnostic tests, and all patients have the right to accept or decline testing after counseling*
- *Due to the background rate of pathogenic microdeletions/duplications and clinically significant variants (2.5%) - chromosomal microarray analysis through diagnostic testing should be offered to all women regardless of age*
- *Diagnostic testing/chromosomal microarray is recommended for a fetus with a structural abnormality on ultrasound*
- *Procedure related risk of loss (0.11-0.22%) should be addressed with the patient*
- *At this time, NIPT is a screening test best suited ONLY for identification of aneuploidies (Trisomy 21, 18. and 13?) in high- risk populations*

COMMITTEE OPINION Number 682 Committee on Genetics Society for Maternal–Fetal Medicine Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology 2020

Preimplantation Genetic Testing – Now I am pregnant, what's next?

- *A normal or negative PGT result is not a guarantee of a newborn without genetic abnormalities.*
- *Traditional diagnostic testing or screening for aneuploidy should be offered to all patients who have PGT-A, in accordance to recommendations for all pregnant patients*
- *Confirmation of preimplantation genetic testing – monogenic results with CVS or amniocentesis should be offered*
- *PGT-SR to detect structural chromosomal abnormalities such as translocations - Confirmation of preimplantation genetic testing – and confirmation of unaffected or balanced translocation in offspring via CVS or amniocentesis should be offered,*
- *Limitations of PGT – do not detect microdeletions and microduplications, de novo variants, and imprinting disorders*
- ***PGT and NIPT remain only as screening tests!***

Genetic testing - Beyond

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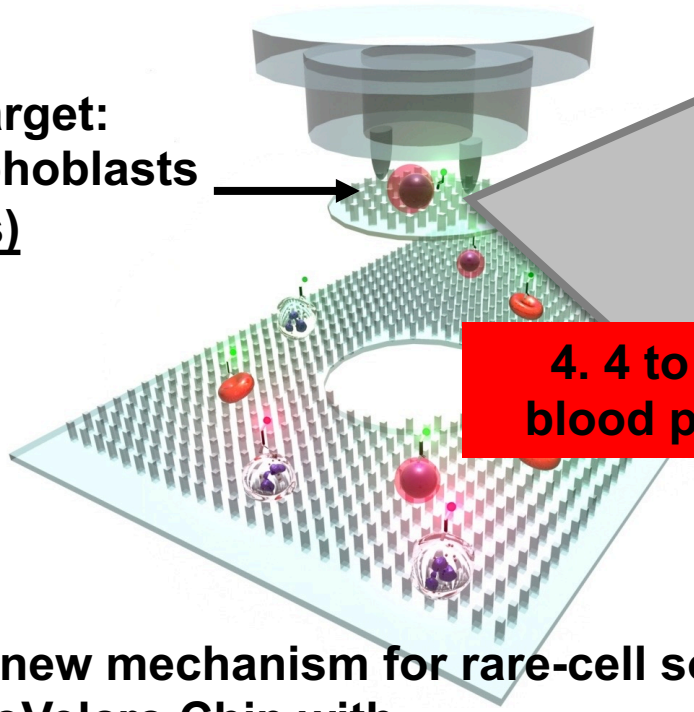
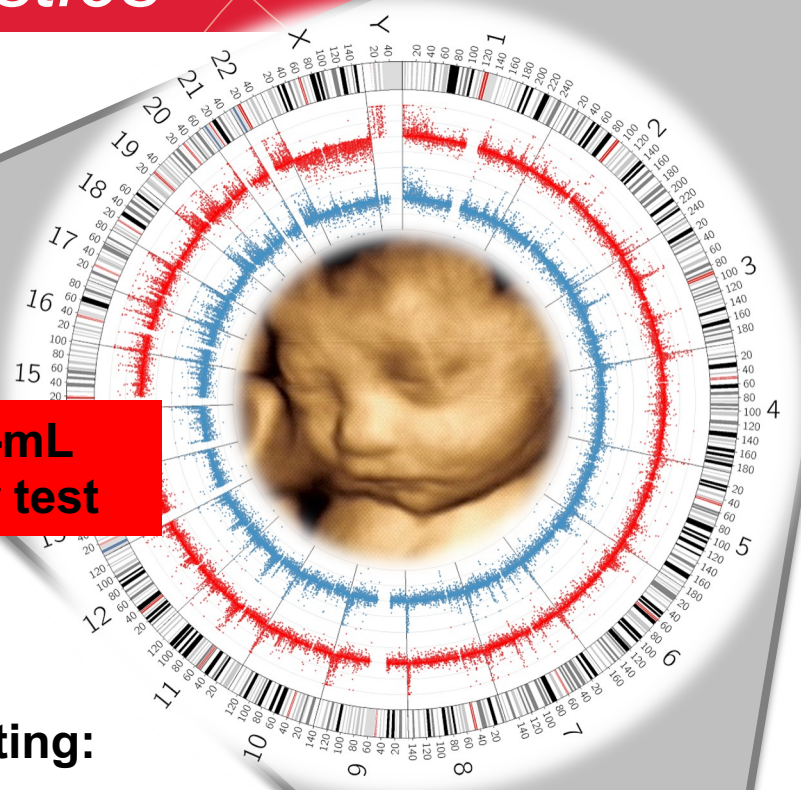
Gavriil P, et al 1993 Pediatr Pathol 13:453-462
Zhang Y, et al 2008 Proteomics 8:4344-4356
Collier AC, et al 2009 J Steroid Biochem Mol Biol 116:21-28
Delle Piane L, et al 2008 Reproductive Sciences 15:81A-81A

Next-Generation Prenatal Diagnostics

1. Target: Trophoblasts (TBs)





4. 4 to 8-mL blood per test

2. A new mechanism for rare-cell sorting: NanoVelcro Chip with imprinted PLGA nanostructures

3. Three types of downstream analyses: FISH, microarray, and WGS

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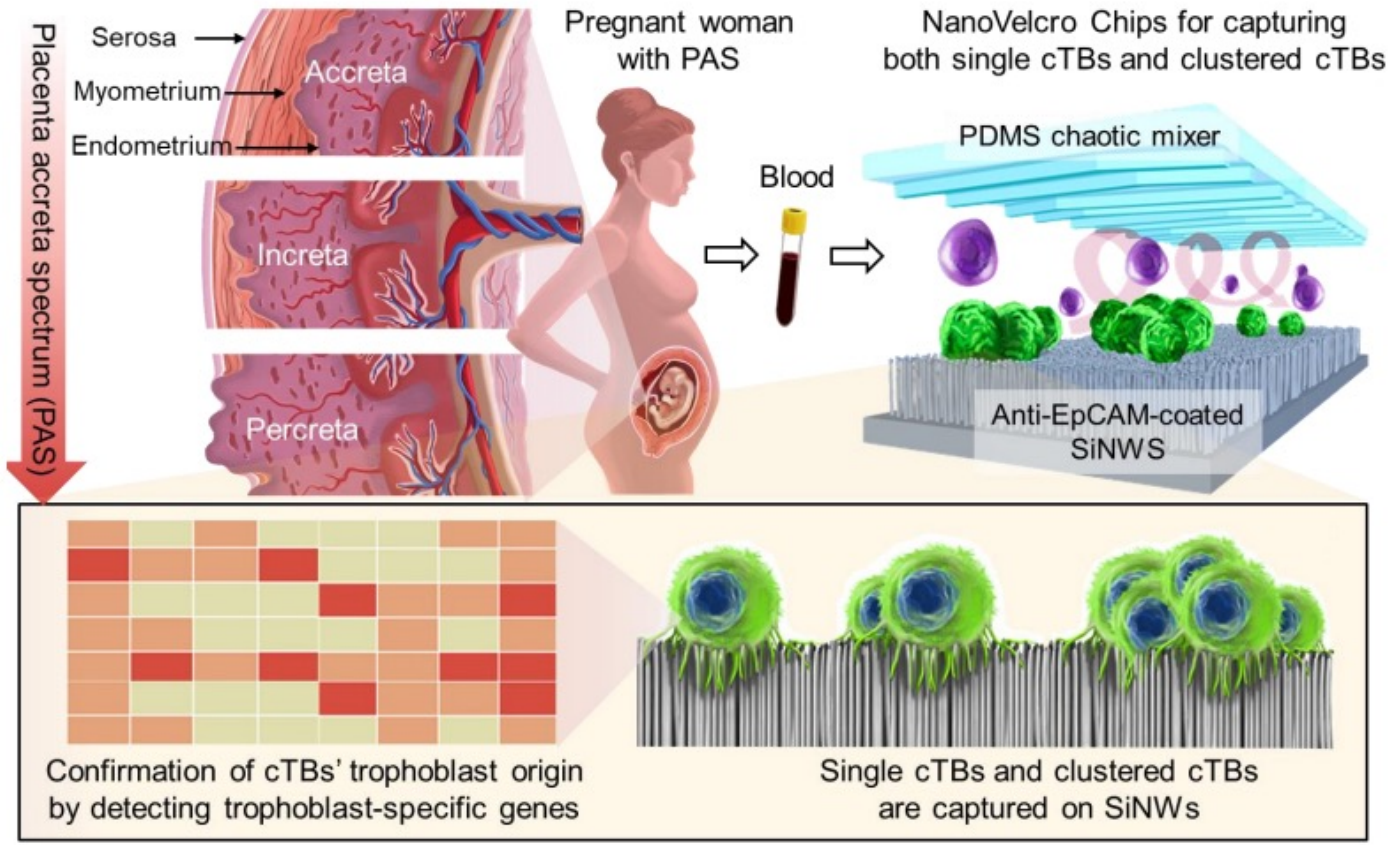
Hou S...Pisarska, Zhu, Tseng. ACS Nano. 2017 Aug 22;11(8)

Circulating Trophoblast Cell Clusters for Early Detection of Placenta Accreta Spectrum Disorder

NanoVelcro Chips for Detecting cTBs and cTB clusters



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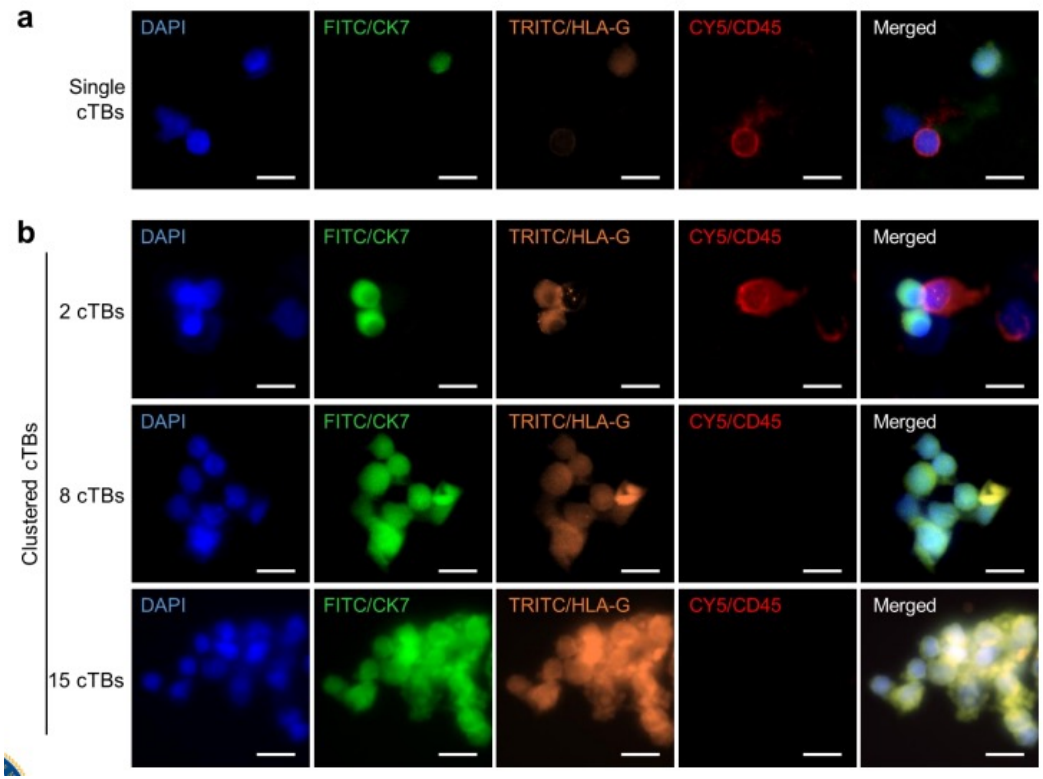
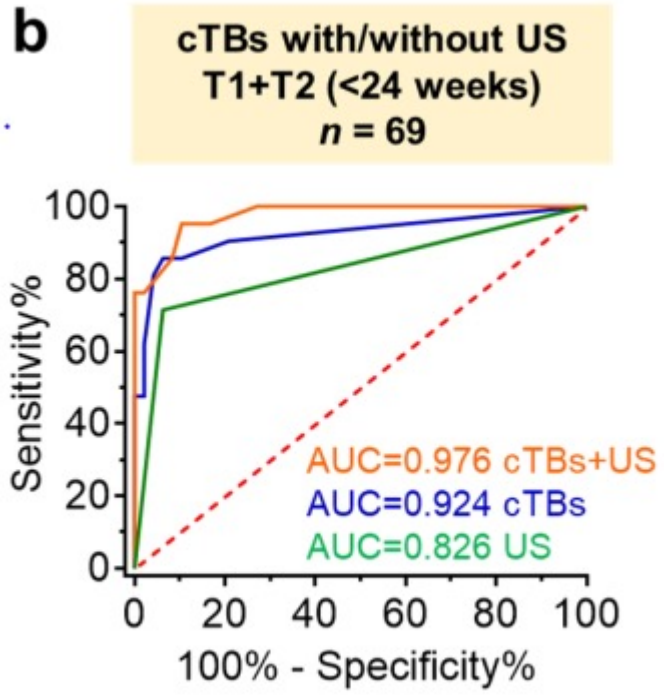
Afshar, Zhu, Pisarska, Tseng. Nat Commun 2021

Circulating Trophoblast Cell Clusters for Early Detection of Placenta Accreta Spectrum Disorder



Scale bar: 10 μm



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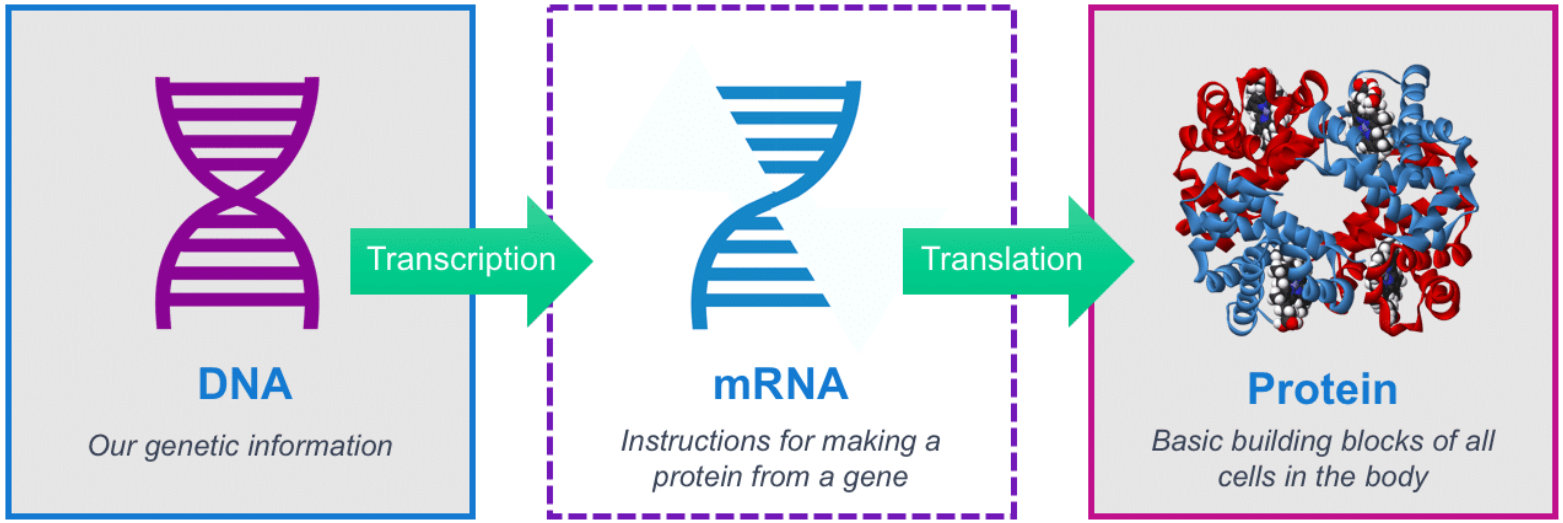
Afshar, Zhu, Pisarska, Tseng. Nat Commun 2021

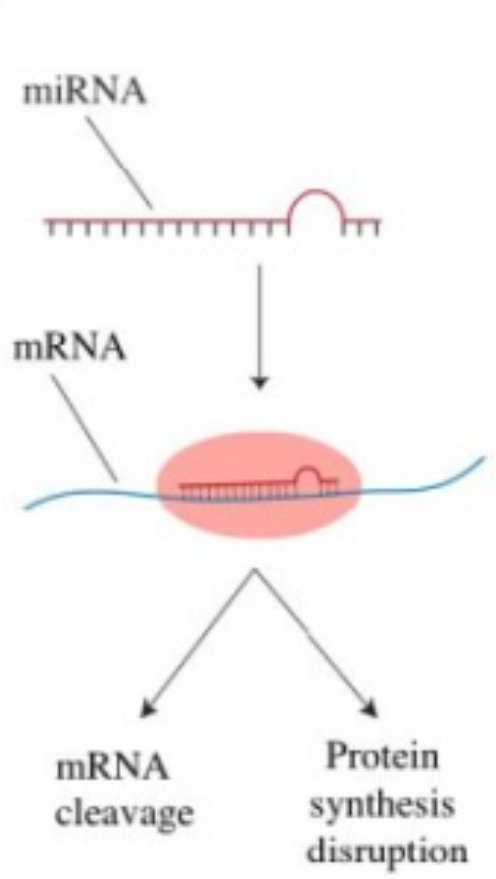
Transcriptome



- *DNA is **transcribed** to RNA which is **translated** to protein*
- *The **transcriptome** is the total messenger RNA expressed in a given tissue*
- *Transcription is regulated by epigenetics: genes can be turned on and off*
- *These epigenetic changes make up the **epigenome***

https://translate.bio/wp-content/uploads/2018/06/Central-Dogma-for-Web-4-2.png

Post-transcriptional regulation

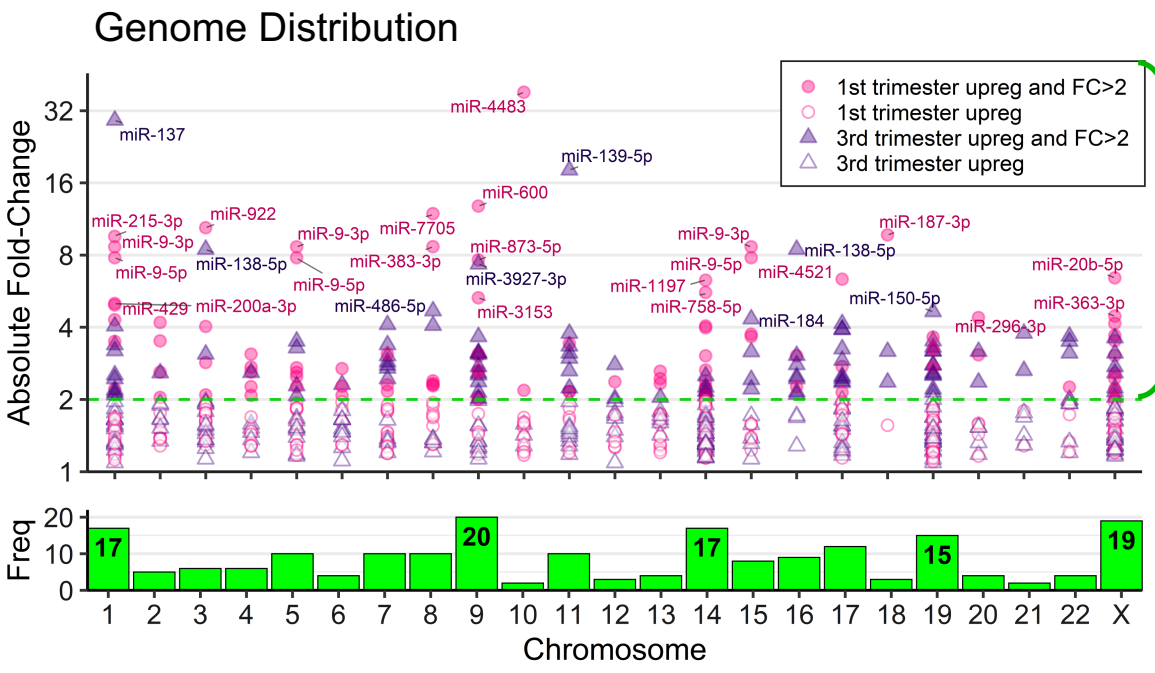


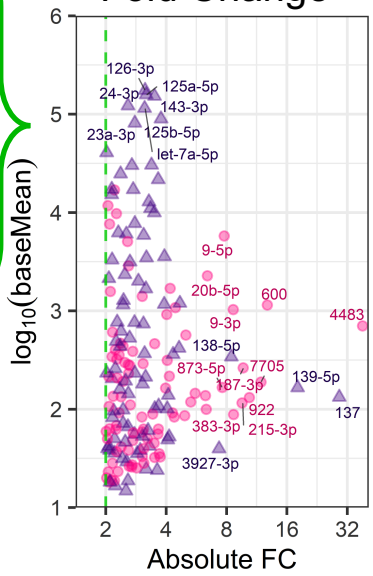
- *miRNAs are short, single-stranded RNA (22 nucleotides)*
- *They bind to RNA transcripts, preventing translation*
- *Stable in the circulation and may be used as markers to predict disease*

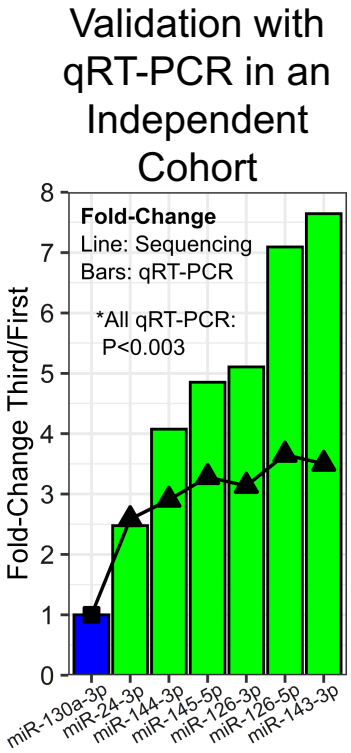
Morales-Prieto et al. (2012), *Journal of Reproductive Immunology*
Epigenetics and the human brain, *NLM Openi*

Normative Epigenome

180 differentially expressed miRNAs: FDR<0.05, FC>2, baseMean>10

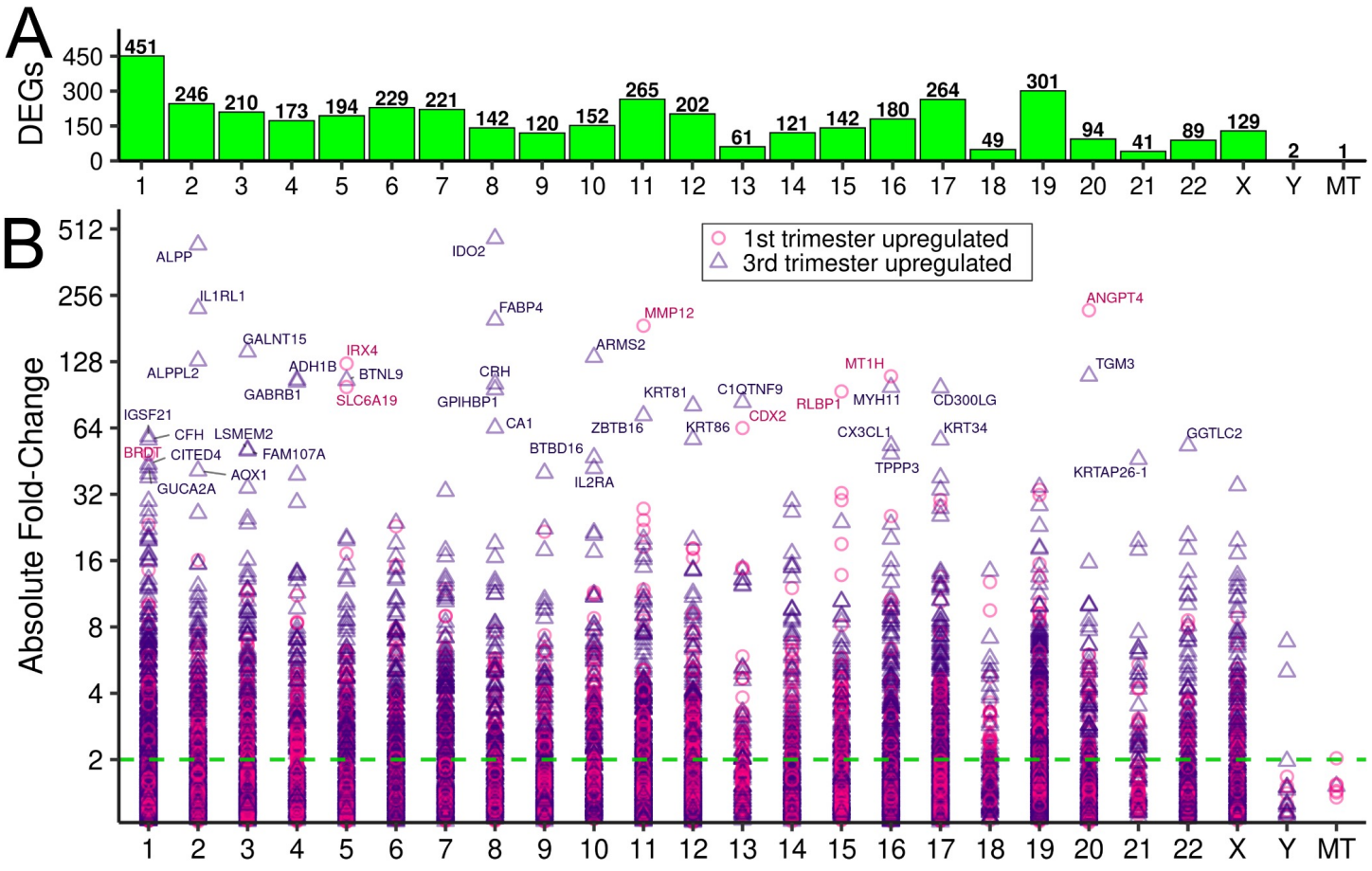


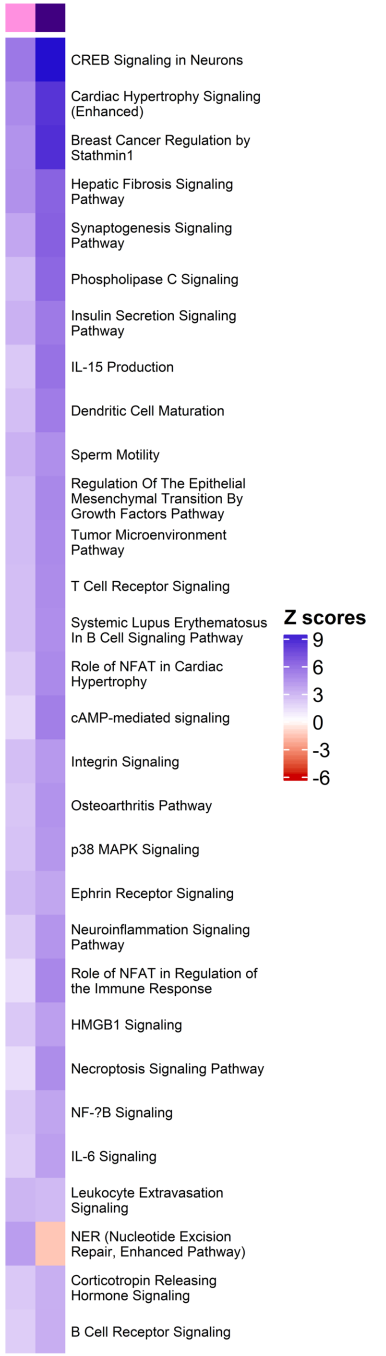




Gonzalez, Pisarska et al. (2021), *Epigenomics*

Normative transcriptome (mRNAs)





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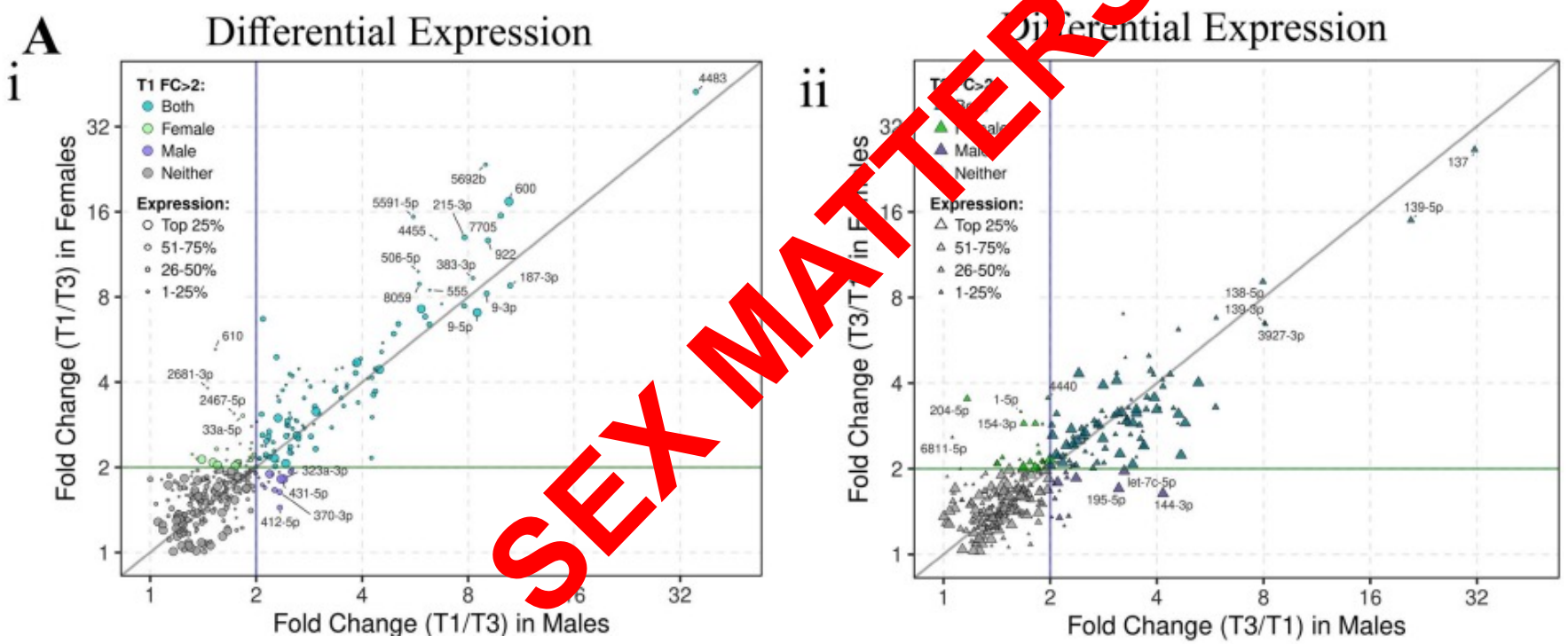
Gonzalez, et al, BOR 2023

Sex differences in miRNAs across gestation



Flowers, et al Biology of Reproduction, 2022, 1–17 https://doi.org/10.1093/biolre/ioab221

Conclusion

- ***Preconception***
  - *Current ACOG recommendations are limited – based on advances in NGS and recent recommendations by ACMG, carrier screening should screen a minimum of 74-112 genetic conditions*
  - *When utilizing commercially available genetic screening – the same panels should be performed for both genetic parents*
- ***Prenatal***
  - *IVF/PGT testing does not replace prenatal genetic counseling with genetic screening and/or diagnostic testing*
  - *NIPT is currently only recommended for high-risk populations for aneuploidy screening (Trisomy 21, 18, and ?13)*
  - *Pathogenic microdeletions/duplications and clinically significant variants affect 2.5% of pregnancies regardless of maternal age*
  - *Diagnostic testing through CVS or amniocentesis should be offered to pregnant patients regardless of age and previous genetic screening*
- ***Future***

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