Reproductive genetics, Carrier Testing and Teratology

Genetics 2012

STANFORD
UNIVERSITY

October 15th, 2015

Learning Goals

- Gain familiarity with indications for and limitations of carrier testing
  - Ethnicity based carrier testing / Universal carrier testing
- Gain familiarity with the concepts of prenatal screening and diagnostic testing
  - Screening
    - First trimester nuchal translucency (NT) ultrasound
    - First and second trimester biochemical screening
    - Second trimester ultrasound
  - Diagnostic
    - Preimplanation genetic diagnosis
    - Chorionic villus sampling
    - Amniocentesis
  - NIPT – Noninvasive prenatal testing

Learning Goals

- Describe the embryologic and epidemiologic underpinnings of teratology

Lecture Outline

- Clinical Case
  - Carrier testing / Universal carrier testing
  - Prenatal screening testing for aneuploidy
  - Prenatal diagnostic testing / Preimplantation genetic diagnosis
  - Non-invasive prenatal testing / NIPT
  - Prenatal ultrasound screening for congenital anomalies
  - Introduction to teratology

What if I were interested in learning about my genetic risks?

- Visit a physician's office
  - Personal genomics
  - Carrier screening
  - Prenatal screening testing

What risks would you want to know about?

- Relatively high probability of occurring
- Would have important consequences
- Can plan to prepare for or avoid the event
  - Actionable/Not actionable
- Could affect other family members

- The risks of obtaining the risk information are appropriately small in relation to the risks being assessed

How would such risk estimates be determined?

- Prior probability
- Conditional probability
- Joint probability
- Final probability

- Updating a risk estimate based upon additional information

Clinical case – Hannah and Jacques

- A 25 year old woman and her partner are of Northern European descent (Hannah and Jacques). They are seen for their first prenatal obstetric visit at 8 weeks of gestation.

Carrier Screening

- Testing, genetic or otherwise, performed to assess the possibility that an asymptomatic individual could be a carrier for a genetic disorder
  - Autosomal recessive
  - X-linked
  - Balanced chromosomal rearrangements

The pre- and post-test probability

- Could carrier screening be helpful for Hannah and Jacques?

- There are thousands of Mendelian disorders.
  - For which of these is there a significant pre-test probability?
  - Is there a test capable of significantly modifying the pretest probability?

Carrier Screening circa 2015

- **Cystic Fibrosis (CF)**
- **Spinal Muscular Atrophy**
- Hemoglobinopathies
  - Thalassemias
  - Sickle cell disease
- Ashkenazi Jewish Disorders
  - Tay-Sachs disease, cystic fibrosis, Canavan disease, familial dysautonomia
- Tay-Sachs disease in French Canadians

Founder Effect and how it changes allele frequencies

USMLE

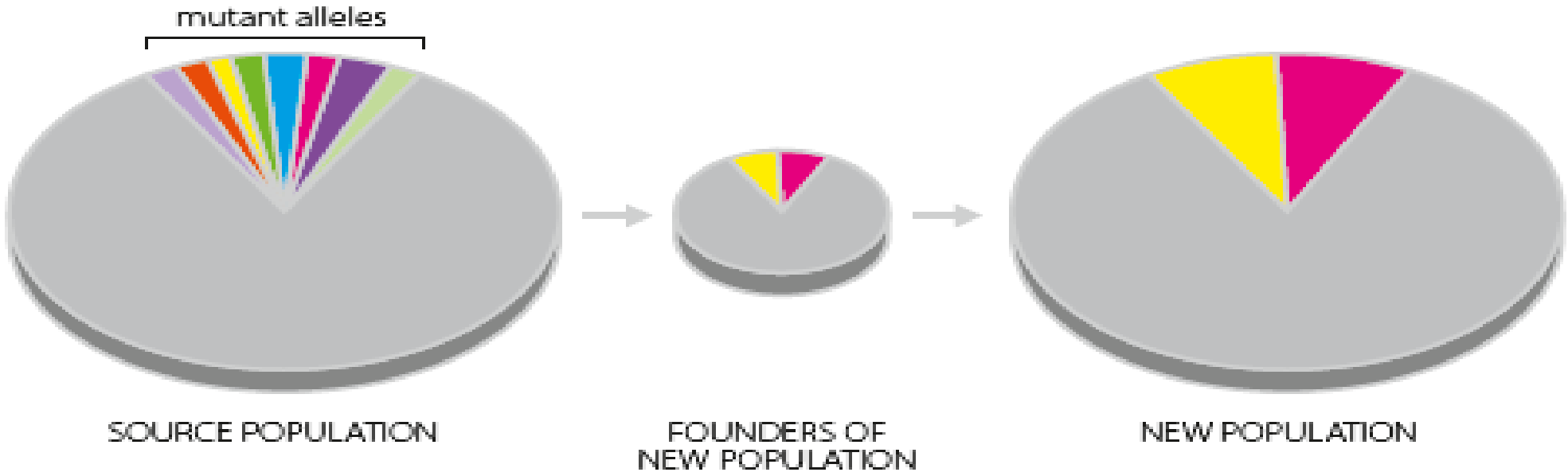
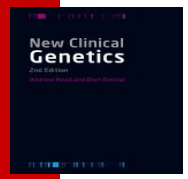


Figure 10.2 – **Founder effects.**



New Clinical Genetics 2e
Andrew Read and Dian Donnai
ISBN: 9781904842804

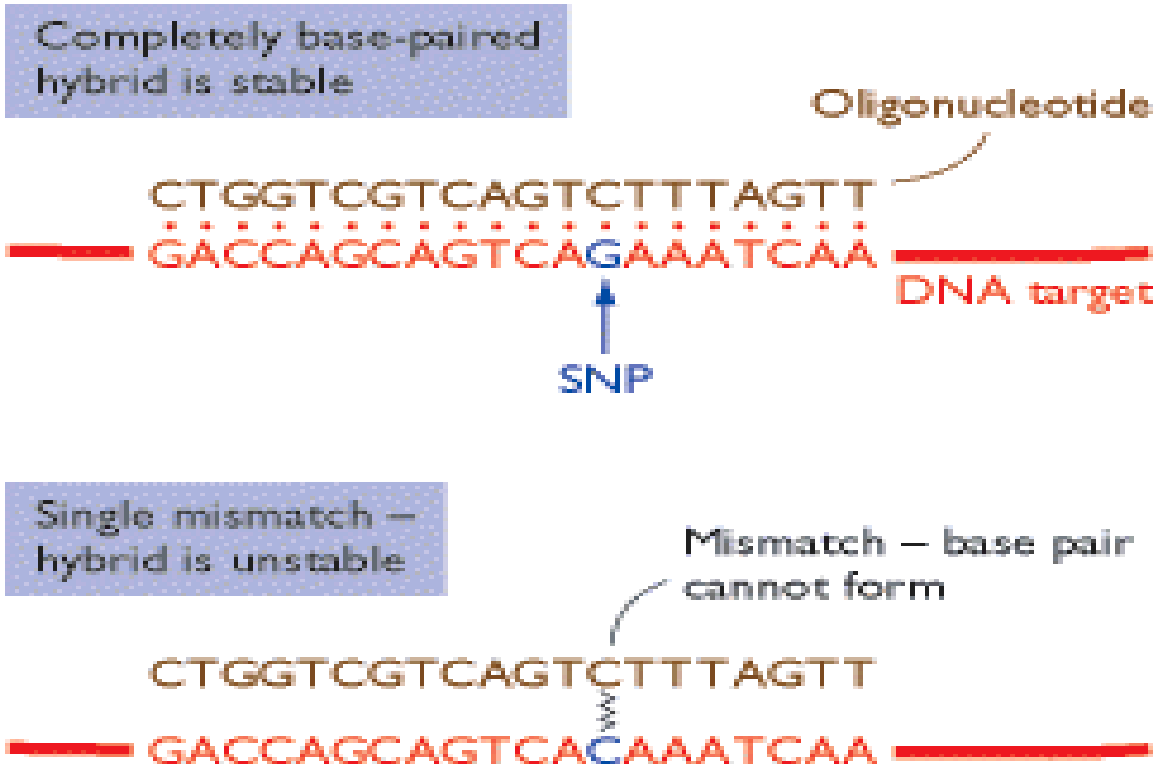
Mutation screening

- You are planning program to screen for mutations associated with cystic fibrosis in a large population.
- You would like to test each person for 23 common CF mutations, but your resources are limited.
- You cannot afford with regard to time or money to perform full gene sequencing on each individual.

Allele specific oligonucleotides – mutation screening

Only tests for specific set of point mutations.

Detection by fluorescence



Brown, 2002, Genomes 2

Pretest Probability

- Carrier frequency in Northern European ancestry populations for CF is approximately 1 in 30
  - ½ x 1/30 x ½ x 1/30 = 1/3600

- Carrier testing is commonly performed using a screening panel of 23 mutations
  - Risk after a negative carrier test is approximately 1 in 200 for Northern European ancestry

What if Hannah screened positive and Jacques did not?

- Estimated risk to have an affected child
  - 1 x ½ x 1/200 x ½ = 1/800

- What other carrier testing options are potentially available to refine this risk?

Universal Carrier Screening

- Simultaneous carrier screening for tens to hundreds of conditions. Could in theory be for thousands of conditions.

- Methods include
  - Array based genotyping or other mutation screening methodology
  - Next-generation capture sequencing

www.counsyl.com

Universal carrier testing vs traditional carrier testing

- A 25 year old woman of European descent elects to undergo universal carrier screening.
  - She is found to be a carrier for VLCADD and Hereditary Hemochromatosis.

Very long-chain acyl-CoA dehydrogenase (VLCAD)	ACADVL	20%	1 in 89	1 in 111

Next steps after universal carrier testing

- Risk to have an affected child
  - ½ x 1 x 1/111 x ½ = 1/444 VLCADD
- Carrier testing for father
  - Full gene sequencing
  - ? Deletion-Duplication testing
- Hemochromatosis
  - Adult onset, low penetrance, treatable
  - Patient is still worried -> Carrier testing for father

Baseline risk of a disorder recognizable at birth

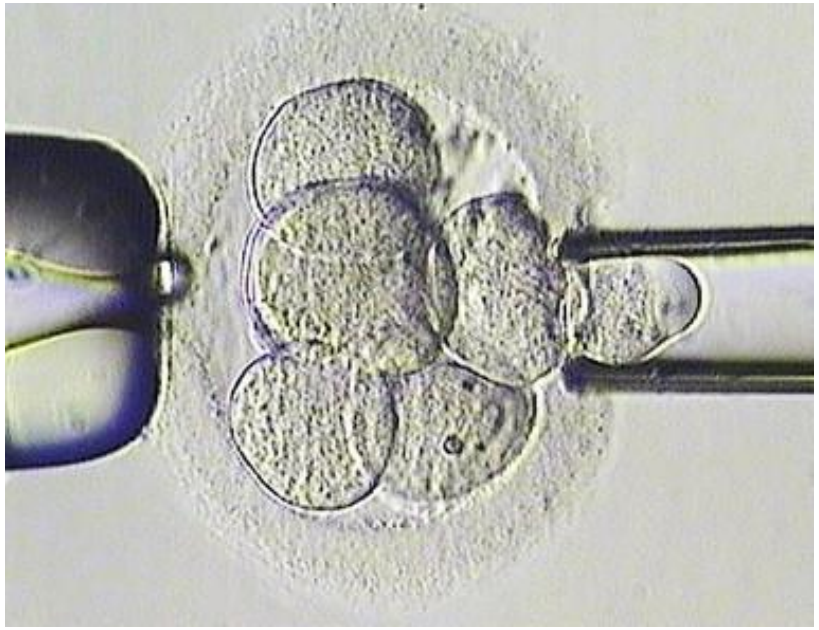
- Three to four percent
  - 1/33 to 1/25
  - Risk identified in this case is possibly 1/444
- What is the optimal use of Universal Carrier testing?
  - With extensive use of limited pre-test counseling?
  - With unlimited resources or limited resources?

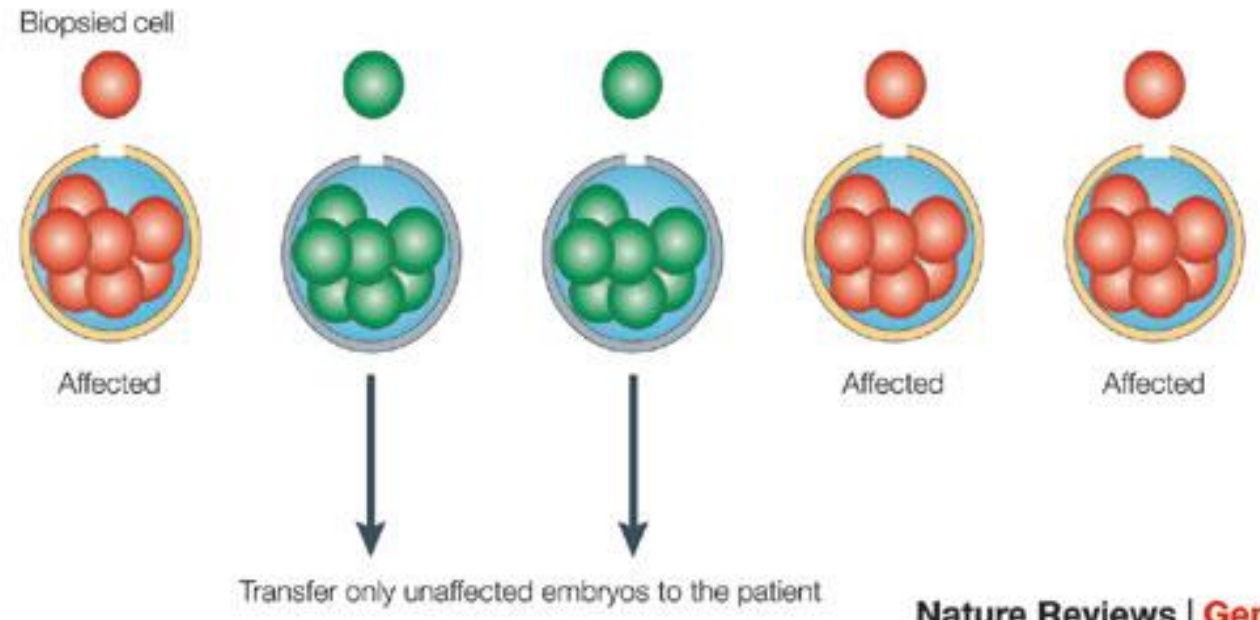
Options available to at risk couples

- Adoption
- Accept the risk and conceive
- Gamete donor
- Preimplantation genetic diagnosis
- Chorionic villus sampling
- Amniocentesis

Preimplantation Genetic Diagnosis (PGD)

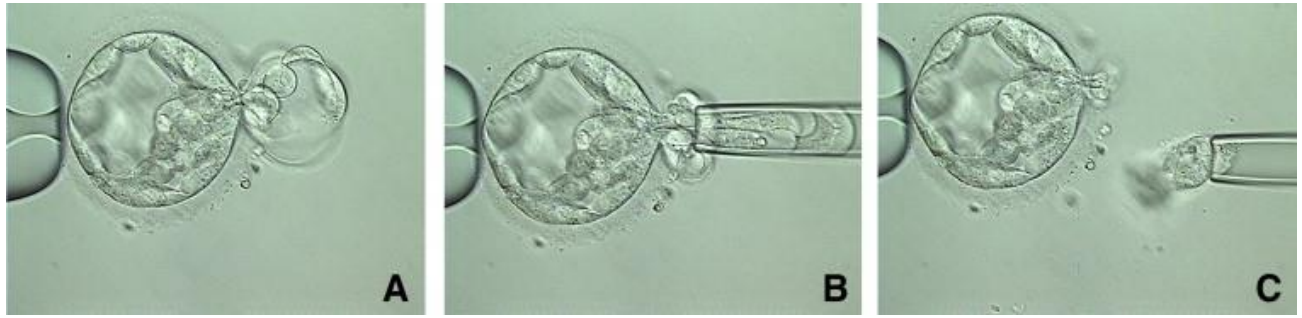
- In vitro fertilization is required
- One cell is removed from 6-8 cell stage embryo





Iwarsson E, et al., Semin Fetal Neonatal Med. 2011 Apr;16(2):74-80. PMID: 21176890

PGD – Trophoectoderm Biopsy



Fertil Steril. 2010 Oct;94(5):1700-6. PMID: 19939370

PGD: Diagnosis

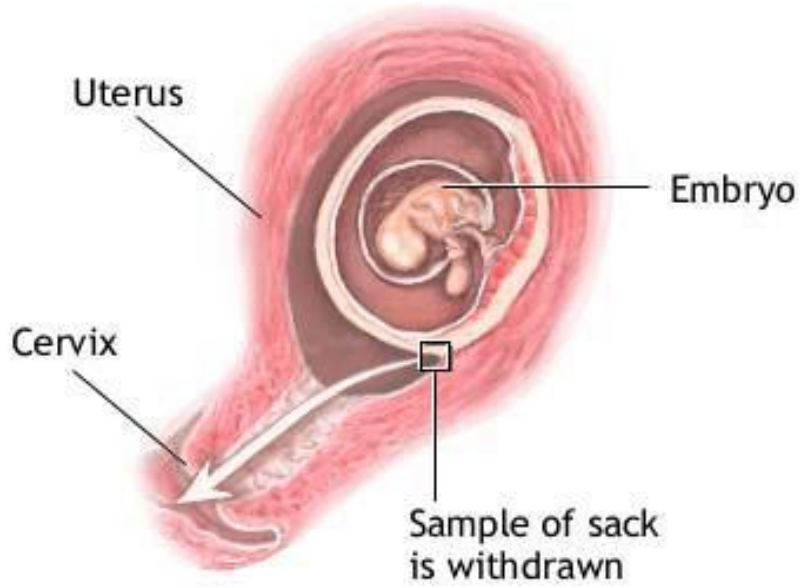
- Diagnosis is typically performed via FISH or PCR, therefore, 5-10% false results
- Confirmation by chorionic villus sampling (CVS) or amniocentesis is offered

PGD: Cost

- In vitro fertilization
  - $5000-$10,000 per cycle
- Genetic diagnosis
  - Can be as much as $5000
- Often not be covered by insurance

Chorionic Villus Sampling (CVS)

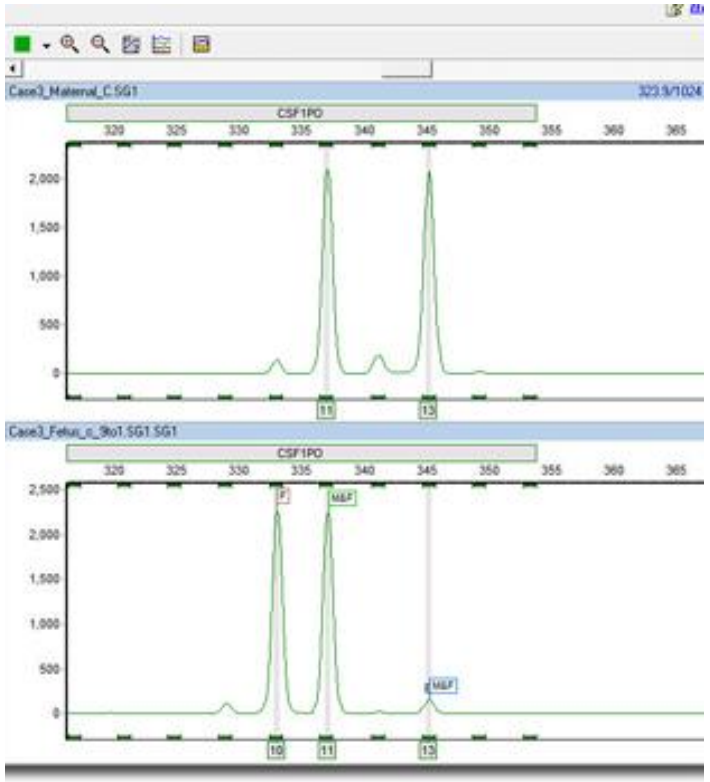
- Performed between 10-12 weeks gestation
- Risk for miscarriage: 1 in 200-300



http://www.nlm.nih.gov/medlineplus/ency/imagepages/9181.htm

The placenta has maternal and fetal components

- What sort of genetic test can be used to evaluate for the possibility that the tissue being tested is of maternal origin?
  - Maternal cell contamination (MCC) studies
  - Karyotype



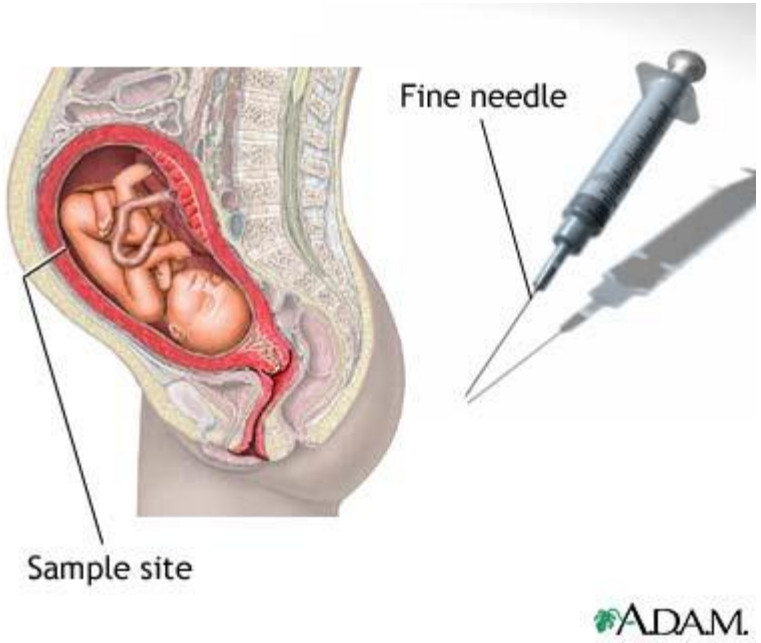
http://www.softgenetics.com

Mosaicism and Prenatal Diagnosis

- Does the genetic make-up of the placenta represent that of the fetus?
  - What if a mixture of trisomic and euploid cells are seen on CVS
    - Mitotic error in cells forming placenta
      - Fetus generally unaffected
    - Trisomic rescue of meiotic error
      - Fetus more likely to be affected

Amniocentesis

- Ideally performed between 16-18 weeks but can be performed anytime there is enough amniotic fluid
  - <15 weeks: too little fluid
  - >24 weeks: may induce labor of premature infant
- Risk for miscarriage
  - 1 in 300

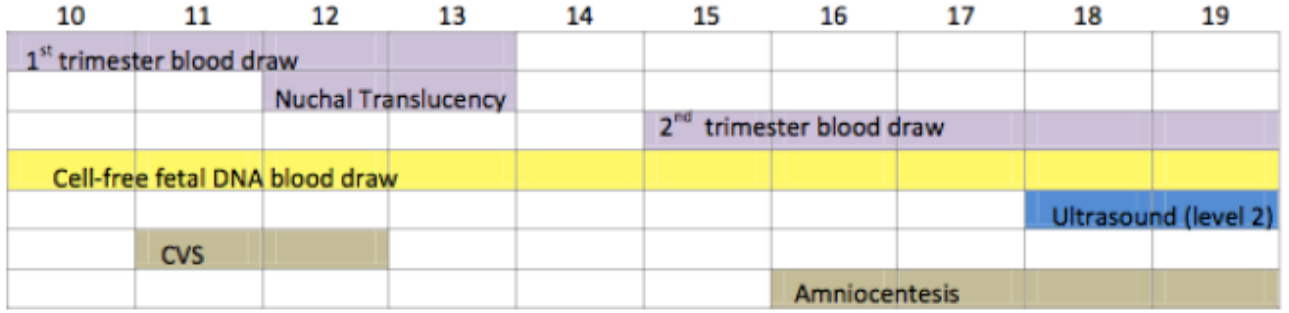


http://www.nlm.nih.gov/medlineplus/ency/imagepages/9631.htm

What else might be offered to Hannah and Jacque as Prenatal Screening Tests?

- Screening for aneuploidy
  - Maternal serum screening with nuchal translucency ultrasound
  - Non-invasive prenatal testing (NIPT)

Testing and Screening Timeline by Gestational Week:



(LPCH 2012)

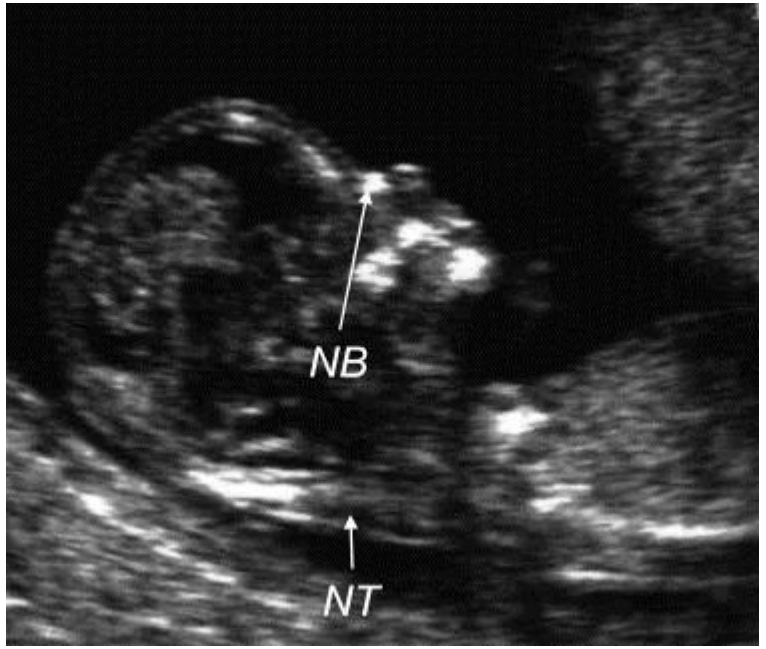
- Screening for congenital anomalies
  - Second trimester ultrasound

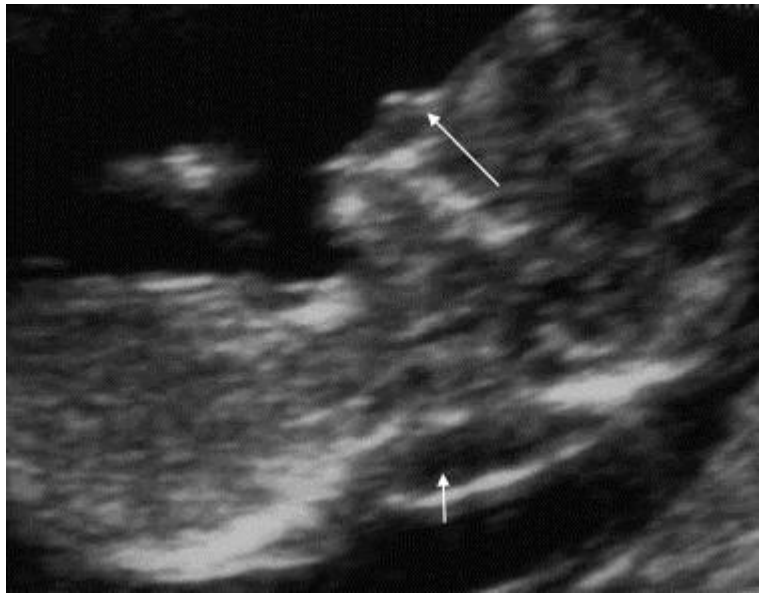
Maternal serum screening

- 1st trimester (10-14 weeks)
  - +/- nuchal translucency ultrasound
  - Human chorionic gonadotropin (hCG)
  - Pregnancy associated plasma protein A (PAPP-A)
- 2nd trimester (15-20 weeks)
  - Alpha fetoprotein (AFP)
  - hCG
  - Unconjugated estriol (UE3)
  - Inhibin A (Inh)
- Integrated first and trimester results

Nuchal Translucency

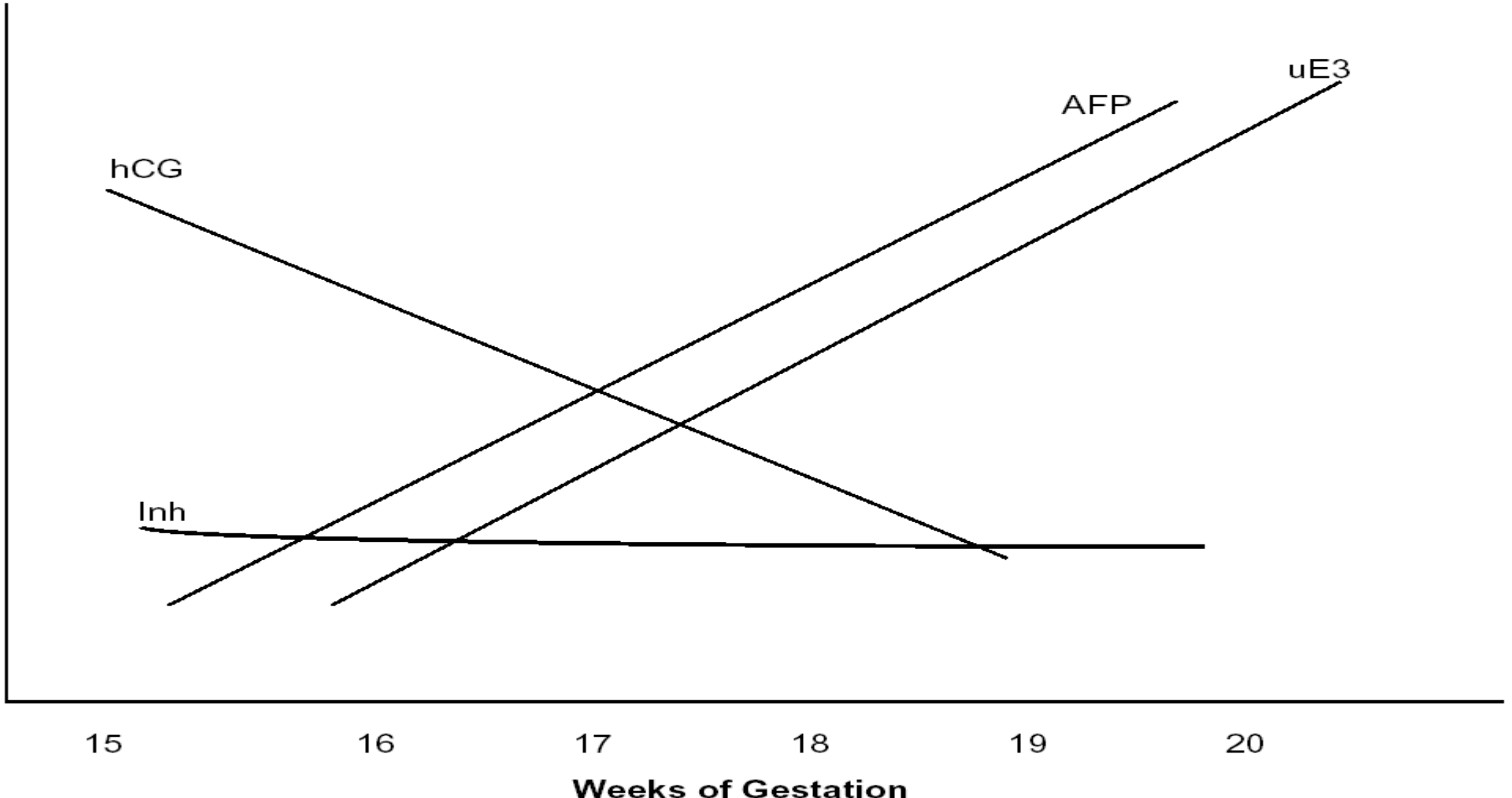
Measurement of the thickness of the posterior fetal neck which appears translucent on ultrasound.





Nicolaides KH. Am J Obstet Gynecol. 2004 Jul;191(1):45-67. PMID: 15295343

Simplified Diagram of Quad Marker Screening Analyte Level
Between 15 – 20 gestational weeks



Prenatal Screening Patterns

USMLE

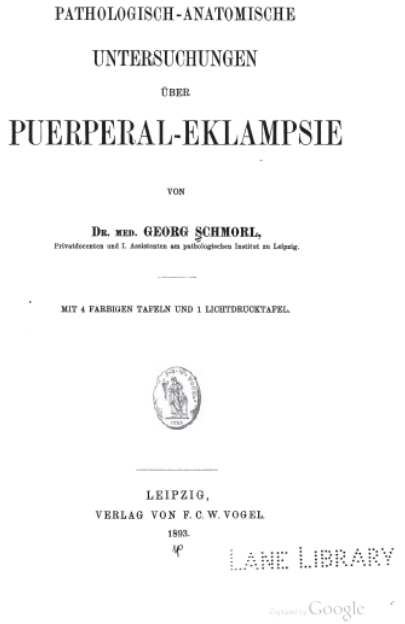
Condition	HCG1	PAPP-A	Nuchal Translucency	AFP	HCG	UE3	INH
Trisomy 21	↑	↓	↑	↓	↑	↓	↑
Trisomy 18	↓	↓	↑	↓	↓	↓	Not Used
Neural Tube Defect/ Abdominal Wall Defect	Not Used	Not Used	Not Used	↑	Not Used	Not Used	Not Used

Hannah screened positive for Down syndrome

- Her estimated risk based on integrated maternal serum screening was 1 in 140; was previously 1 in 1,250
  - 139/140 chance pregnancy is unaffected
  - A POSITIVE test result
  - Might elect to perform a diagnostic test such as chorionic villus sampling (CVS) or amniocentesis

Is there a non-invasive way to perform prenatal testing?

- 19th Century, trophoblast cells identified in maternal lungs at autopsy
  - Schmorl G. Pathogisch-anatomische untersuchungen uber puerperaleklampsie. Leipzig: Vogel, 1893


PATHOLOGISCH-ANATOMISCHE

UNTERSUCHUNGEN

ÜBER

PUERPERAL-EKLAMPSIE

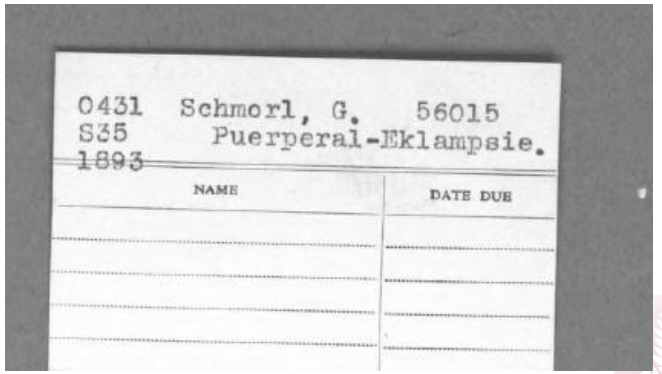
VON

DR. MED. GEORG SCHMORL,

Privatdocenten und I. Assistenten am pathologischen Institut zu Leipzig.

MIT 4 FARBIGEN TAFELN UND 1 LICHTDRUCKTAFEL.

LEIPZIG,
VERLAG VON F. C. W. VOGEL.
1893.



The long road to non-invasive prenatal testing

- 1969 fetal lymphocytes identified in maternal circulation
  - Walknowska J, Conte FA, Grumbach MM. Lancet 1969; 1: 1119–1122
  - But fetal lymphocytes can persist for years in the maternal circulation
    - Ongoing work on nucleated red blood cells
- 1997, cell free fetal DNA is present in maternal plasma and has a relatively short half-life
  - Lo YM, Corbetta N, Chamberlain PF, et al. Lancet 1997;350:485– 487
  - Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Am J Hum Genet 1999; 64: 218–224.

How to assay cell free fetal DNA

- Presence or absence of genetic material by PCR
  - Fetal sex
    - Y chromosome markers
      - Lo YM, Tein MS, Lau TK et al. Am J Hum Genet 1998; 62: 768– 775.
    - Paternal X-chromosome markers
      - Tang NL, Leung TN, Zhang J, Lau TK, Lo YM. Clin Chem 1999; 45: 2033–2035.
  - Dominant single gene disorders for which mutation is not present in maternal genome
    - Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Lancet 2000; 356: 1170

How about aneuploidy (trisomies)

- More difficult
  - Fetal DNA is only a fraction of total DNA in maternal circulation
  - Trying to find a quantitative difference rather than a qualitative difference

- How about next generation sequencing (read depth analysis)?

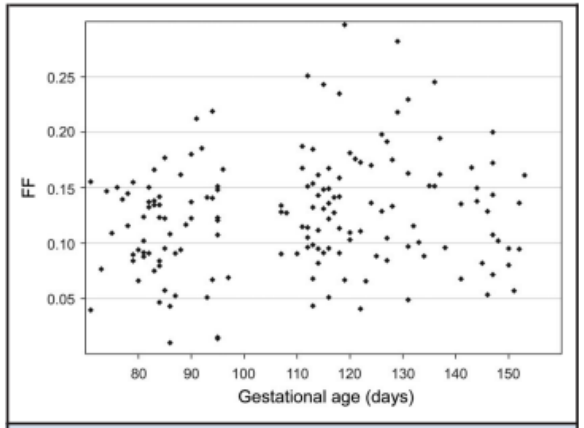


Fig. 3. FFs in the plasma of pregnant women carrying euploid male fetuses vs. gestational age in days.

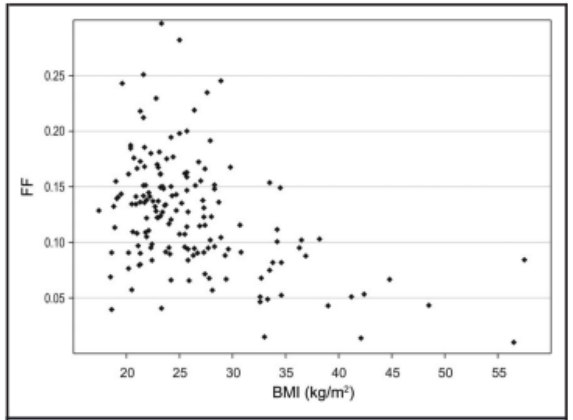
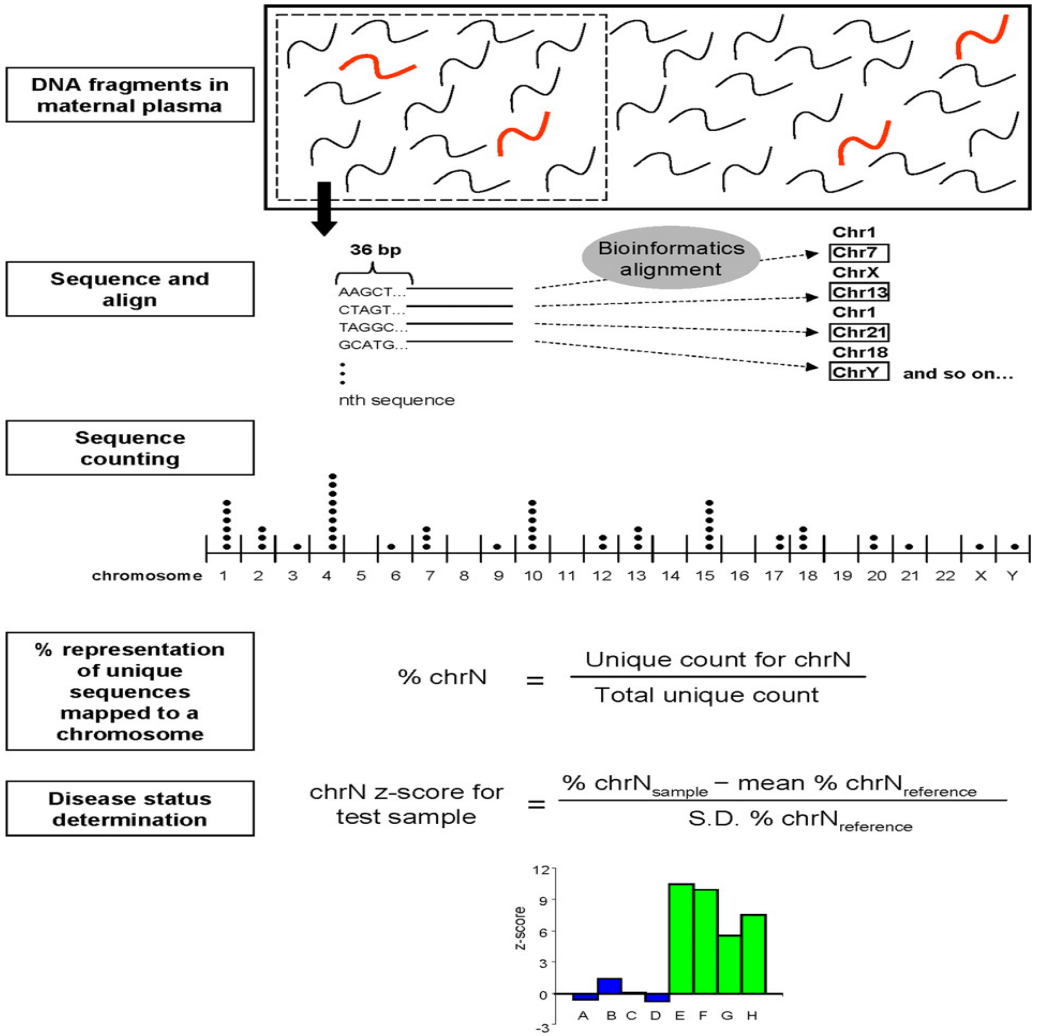


Fig. 4. FFs in the plasma of pregnant women carrying euploid male fetuses vs. maternal BMI.

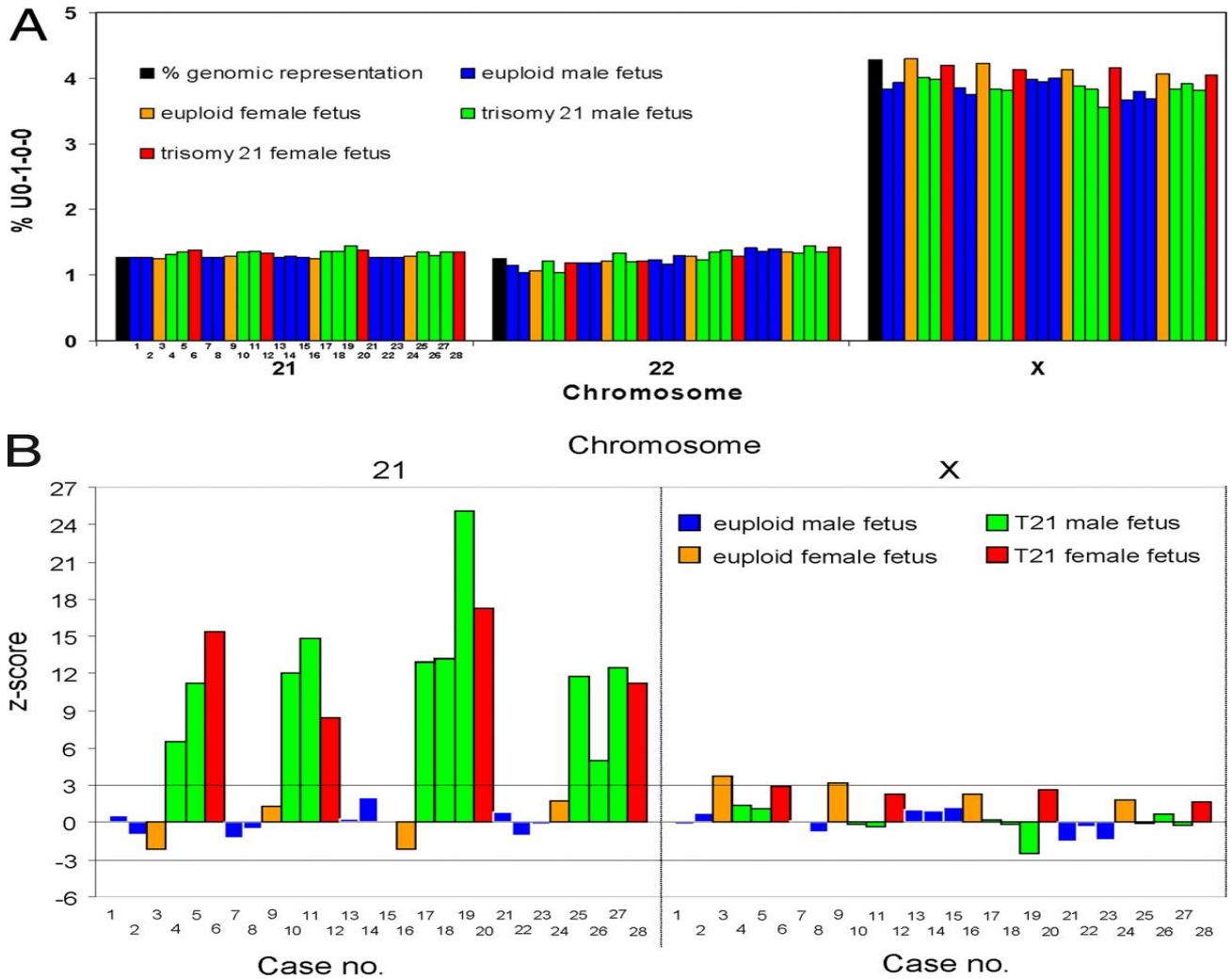
Rava et al., Clinical Chemistry 60:1 (2014), PMID: 24046201

Schematic illustration of the procedural framework for using massively parallel genomic sequencing for the noninvasive prenatal detection of fetal chromosomal aneuploidy.



Chiu R W K et al. PNAS 2008;105:20458-20463

Plot of (A) % U0–1–0–0 counts and (B) z-scores for chromosome 21 and chromosome X for 28 maternal plasma samples.



Chiu R W K et al. PNAS 2008;105:20458-20463

NIPT in Clinical Trials

Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. BMJ. 2011 Jan 11.
PMID: 21224326

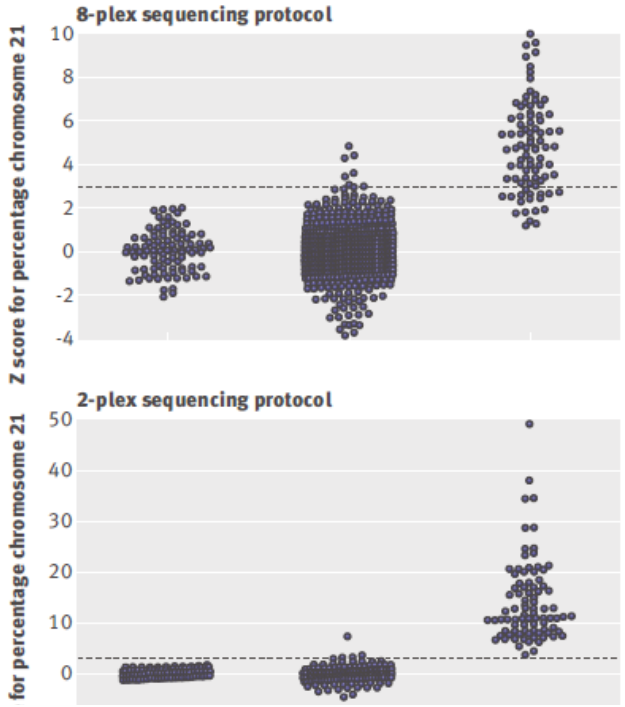


Table 2 | Diagnostic performance of maternal plasma DNA sequencing for detecting fetal trisomy 21 and fetal sex

	8-plex sequencing protocol		2-plex sequencing protocol	
	True detection rate	False positive rate	True detection rate	False positive rate
Trisomy 21 detection*	Among 86 trisomy 21 cases	Among 571 non-trisomy 21 cases	Among 86 trisomy 21 cases	Among 146 non-trisomy 21 cases
	79.1% (68/86)	1.1% (6/571)	100% (86/86)	2.1% (3/146)
Fetal sex (male) detection†	Among 386 male fetuses	Among 365 female fetuses	Among 196 male fetuses	Among 117 female fetuses
	99.5% (384/386) (95% CI 98.1% to 99.9)	0.8% (3/365) (95% CI 0.2% to 2.4%)	99.5% (195/196) (95% CI 97.2% to 99.9%)	0.8% (1/117) (95% CI 0.1% to 4.7%)

*Z score for percentage chromosome 21 >3.
†Cut-off values for percentage chromosome Y identified by ROC analysis.

Table 3 | Probabilities for a trisomy 21 fetus in women by age alone and according to result of maternal plasma DNA sequencing test

Maternal age (years)	Pretest probability*	Post-test probability			
		8-plex sequencing		2-plex sequencing	
		Positive test result†	Negative test result	Positive test result†	Negative test result
20	1 in 1068	1 in 16	1 in 5082	1 in 23	1 in infinity
25	1 in 946	1 in 14	1 in 4501	1 in 21	1 in infinity
30	1 in 626	1 in 10	1 in 2977	1 in 14	1 in infinity
31	1 in 543	1 in 9	1 in 2582	1 in 12	1 in infinity
32	1 in 461	1 in 7	1 in 2191	1 in 11	1 in infinity
33	1 in 383	1 in 6	1 in 1820	1 in 9	1 in infinity
34	1 in 312	1 in 5	1 in 1482	1 in 8	1 in infinity
35	1 in 249	1 in 4	1 in 1182	1 in 6	1 in infinity
36	1 in 196	1 in 4	1 in 930	1 in 5	1 in infinity
37	1 in 152	1 in 3	1 in 720	1 in 4	1 in infinity
38	1 in 117	1 in 3	1 in 553	1 in 3	1 in infinity
39	1 in 89	1 in 2	1 in 420	1 in 3	1 in infinity
40	1 in 68	1 in 2	1 in 320	1 in 2	1 in infinity
41	1 in 51	1 in 2	1 in 239	1 in 2	1 in infinity
42	1 in 38	1 in 2	1 in 177	1 in 2	1 in infinity

*Pretest probabilities are based on prevalence of fetal trisomy 21 at 12th week of gestation.[28]
†A positive test result is a sample with a z score for percentage chromosome 21 >3.

Comparison of Prenatal Diagnostic and Screening Options for Trisomy 21

	PGD	1st and Second Trimester Screening	Amniocentesis	CVS	NIPT (after 10 weeks GA)
Sensitivity	Varies, 90%	90%	>99%	>99%	~99%
Specificity		Low	>99%	>99%	~99%
Risk of Miscarriage	None	None	1/300	1/200-1/300	None
Part of State Program (CA)	No	Yes	If screen positive	If screen positive	If screen positive
Covered by Insurance	Generally not	Part of many state programs	If over age 35 or ultrasound abnormality	If over age 35 or ultrasound abnormality	Varies

Ultrasound at 23 weeks gestation

- Binder anomaly
  - Hypoplasia of the nasal cartilage
  - Multiple causes
    - Genetic (Mendelian)
    - Multifactorial
    - Environmental

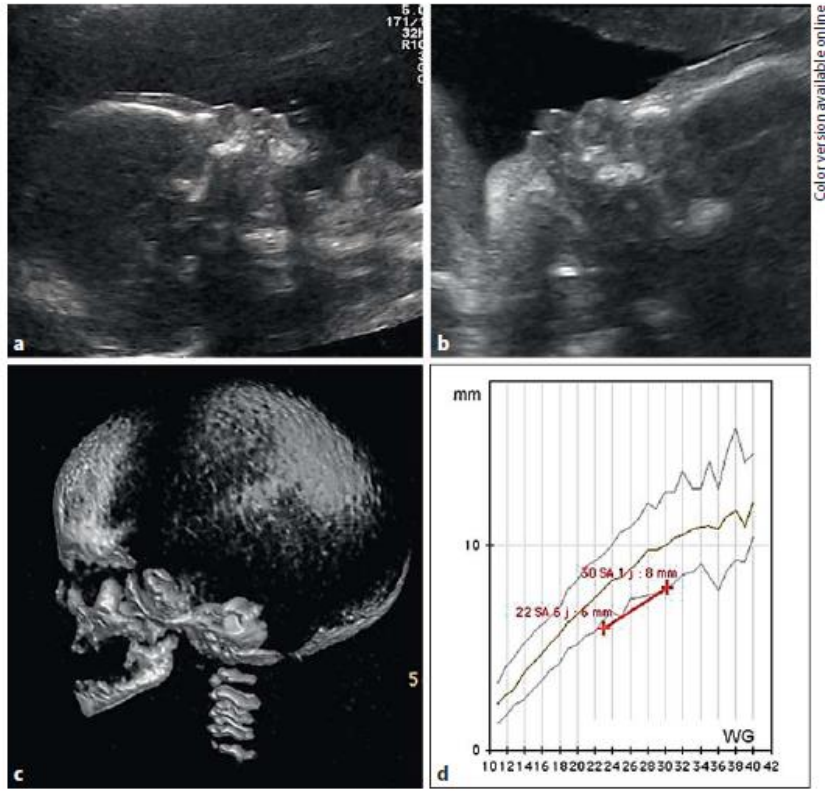


Fig. 1. Prenatal diagnosis of a Binder anomaly. The flattened profile was obvious on prenatal ultrasound at 23 WOA (**a**) and 30 WOA (**b**). Intrauterine 3D-HCT reconstruction at 30 WOA confirmed midface hypoplasia (**c**). The nasal bone was much shorter than expected (**d**).

Boulet et al., Fetal Diagn Ther 2010;28:186–190

Differential diagnosis

- Rhizomelic chondrodysplasia punctata
  - **AR**
  - Often severe, frequently lethal multisystem disorder or peroxisomal biogenesis
- Keutel syndrome
  - **AR**, vitamin K dependent Gla protein deficiency
  - Similar to X-linked CDP, but typically more severe progressive
- Warfarin Embryopathy
- Binder anomaly
  - ?Maternal vitamin K deficiency

X-linked chondrodysplasia punctata

- X-linked recessive
- Mutations in ARSE, arylsulfatase E
- Highly variable expressivity

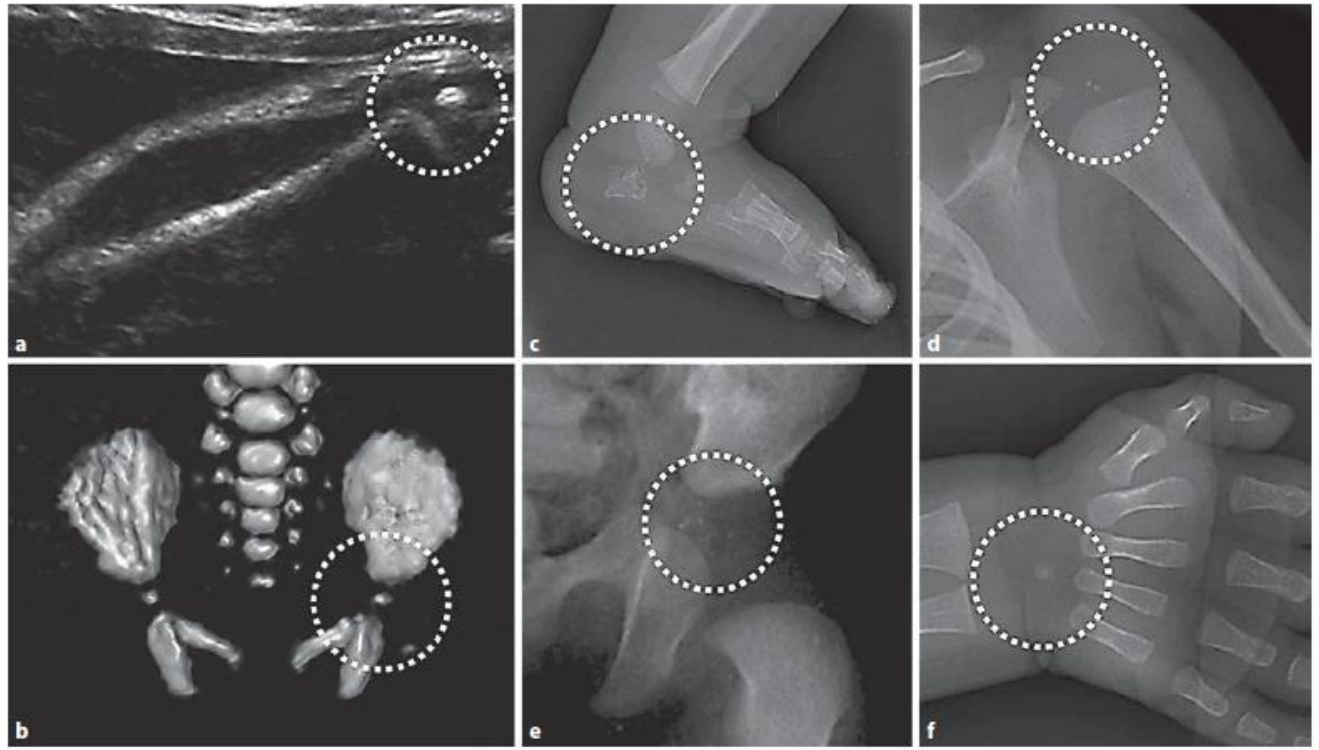
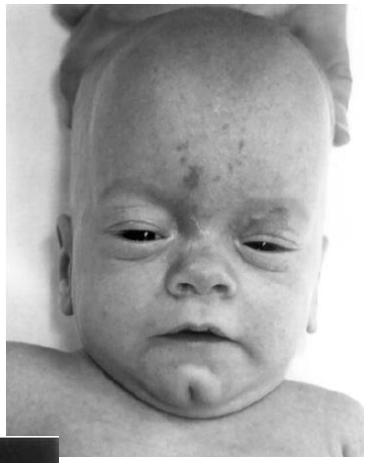
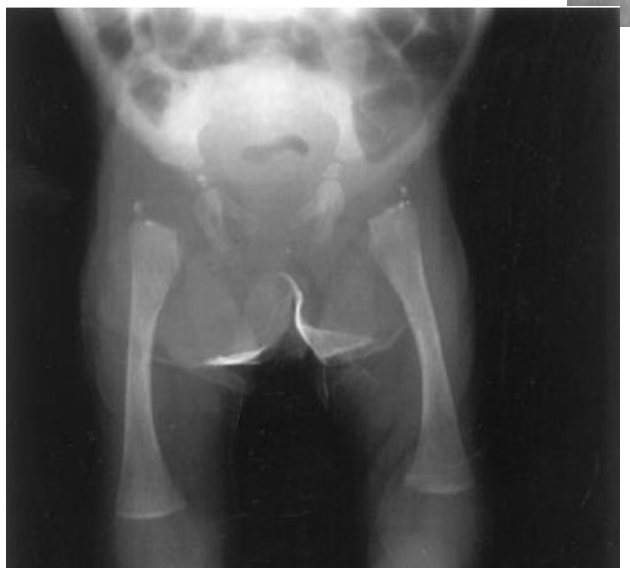


Fig. 2. Epiphyseal stippling. **a–f** Prenatal ultrasound (**a**) and 3D-HCT (**b**) at 30 WOA revealed punctuation of upper femoral epiphysis (**a**) and hip bones (**b**). On postnatal X-ray at 2 months of age, additional stippling was diagnosed on calcaneums (**c**), upper humeral epiphysis (**d**), and wrists (**f**). Hip bone stippling was also visible (**e**).

What if there were a drug that inhibited arylsulfatase E?

- Warfarin (Coumadin) Embryopathy
  - Critical period 6-12 weeks gestation
  - Chondrodysplasia punctata
  - Occurs in minority of exposed pregnancies; also small risk intellectual disability

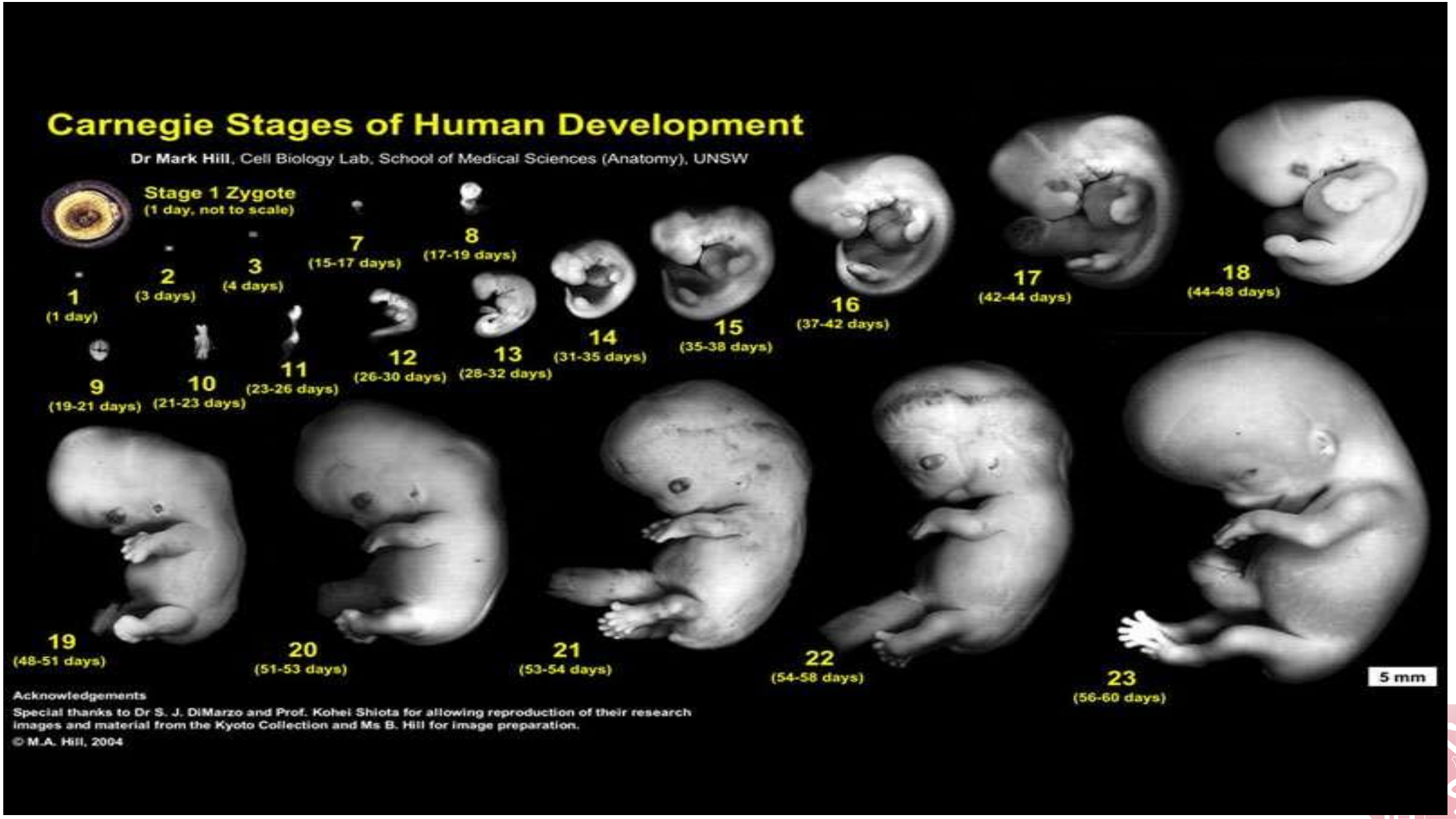




Van Driel et al., TERATOLOGY 66:127–140 (2002)

Intrauterine Exposures (Teratology)

- A variety of developmental pathways are sensitive to exposures – defining characteristics of a teratogen include:
  - Critical window for exposure
  - Typically dose dependent effects
  - Some individuals more susceptible than others
  - Epidemiologic studies support association
  - Biologically plausible mechanism


Carnegie Stages of Human Development
Dr Mark Hill, Cell Biology Lab, School of Medical Sciences (Anatomy), UNSW
Stage 1 Zygote
(1 day, not to scale)
1
(1 day)
2
(3 days)
3
(4 days)
7
(15-17 days)
8
(17-19 days)
9
(19-21 days)
10
(21-23 days)
11
(23-26 days)
12
(26-30 days)
13
(28-32 days)
14
(31-35 days)
15
(35-38 days)
16
(37-42 days)
17
(42-44 days)
18
(44-48 days)
19
(48-51 days)
20
(51-53 days)
21
(53-54 days)
22
(54-58 days)
23
(56-60 days)
5 mm
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Common Human Teratogens

- Maternal diabetes
- Maternal alcohol use
- Prescription Medications

Case Summary – Hannah and Jacques

- Offered multiple screening tests to assess risk of adverse pregnancy outcome
  - Positive carrier testing result for CF
    - Residual risk 1/800
  - Positive carrier testing results for VLCADD and Hemochromatosis
  - Positive aneuploidy screening result
    - Residual risk 1/139
  - Reassuring NIPT result
  - Positive ultrasound result
- Baby boy born with mild X-linked chondrodysplasia punctata

Lecture Summary

- Individuals planning a family are frequently interested in genetic risk assessment
  - Carrier testing
  - Prenatal screening testing
- A variety of options are potentially available to those at risk

Lecture Summary

- Screening tests can reduce, but not eliminate the estimated risk of an event
- Post-test risk estimates depend on the magnitude of the pre-test risk and the properties of the test
- Teratology is the field of medicine focused on understanding the cause of and preventing congenital anomalies or birth defects
  - Relies on knowledge of developmental genetics and biology more broadly

Review Question

- Screening tests typically have
  - A) High sensitivity
  - B) Acceptable specificity
  - C) Low risk
  - D) Low cost
  - E) All of the above

Review Question

- Exposure to Warfarin in the third trimester may result in?
  - A) Chondrodysplasia punctata
  - B) Some risk of bleeding
  - C) Abdominal wall defects
  - D) Neural tube defects