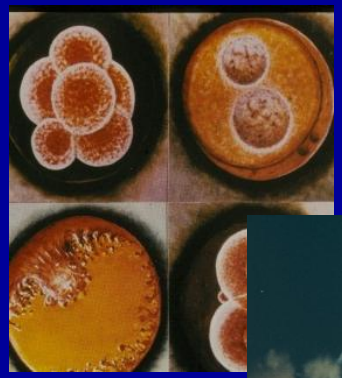
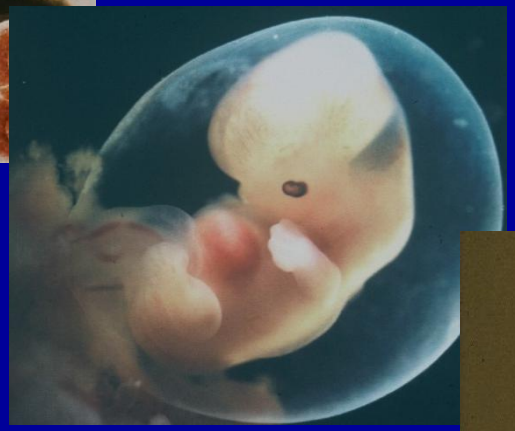
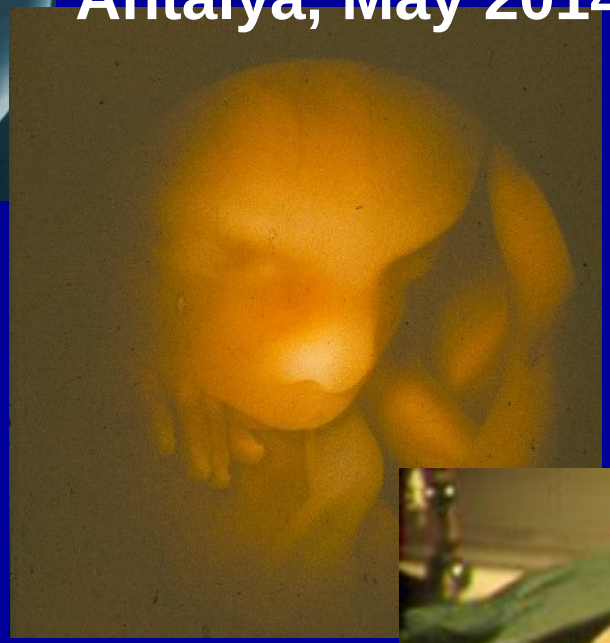
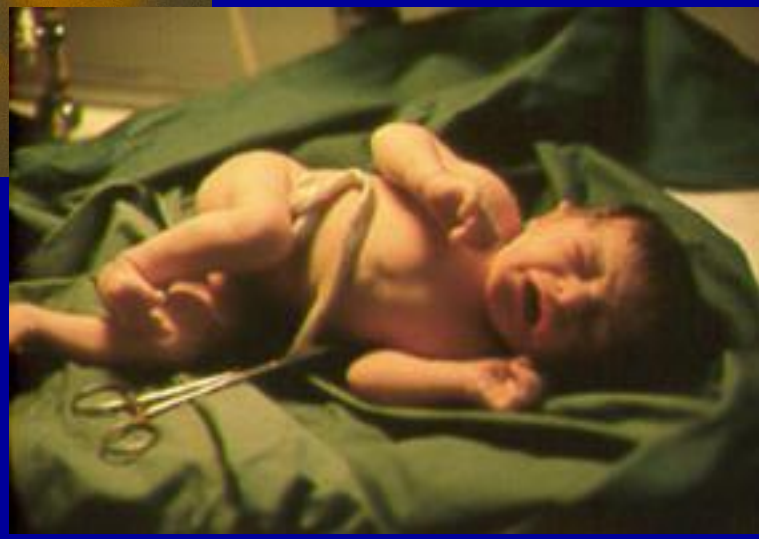




Non-invasive prenatal diagnosis from maternal blood- finally after 20 years of research available in clinical practice

Antalya, May 2014





Prof. Dr. med. Dr. h. c.mult.

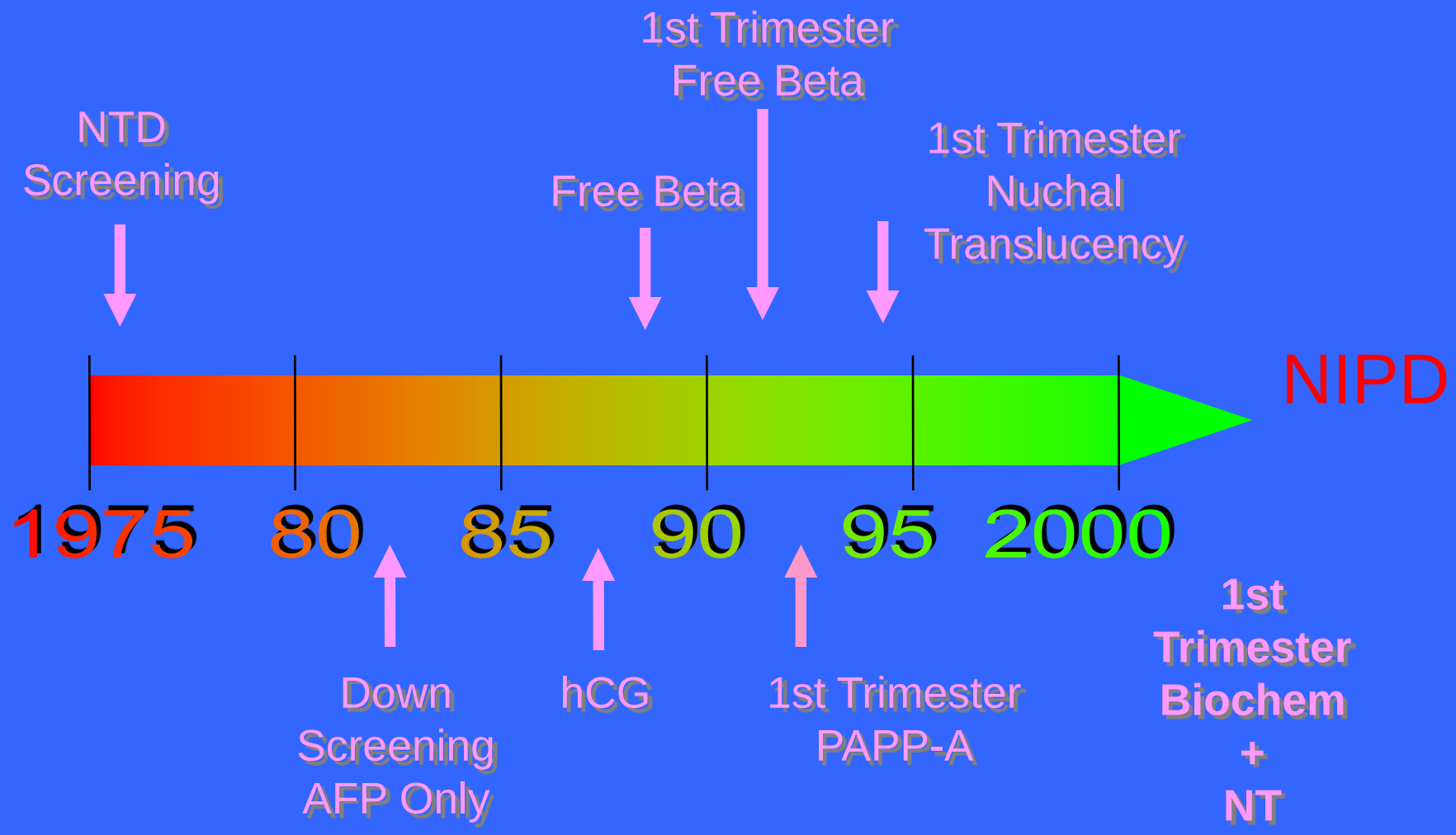
Wolfgang Holzgreve, MBA

Ärztlicher Direktor/ Vorstandsvorsitzender

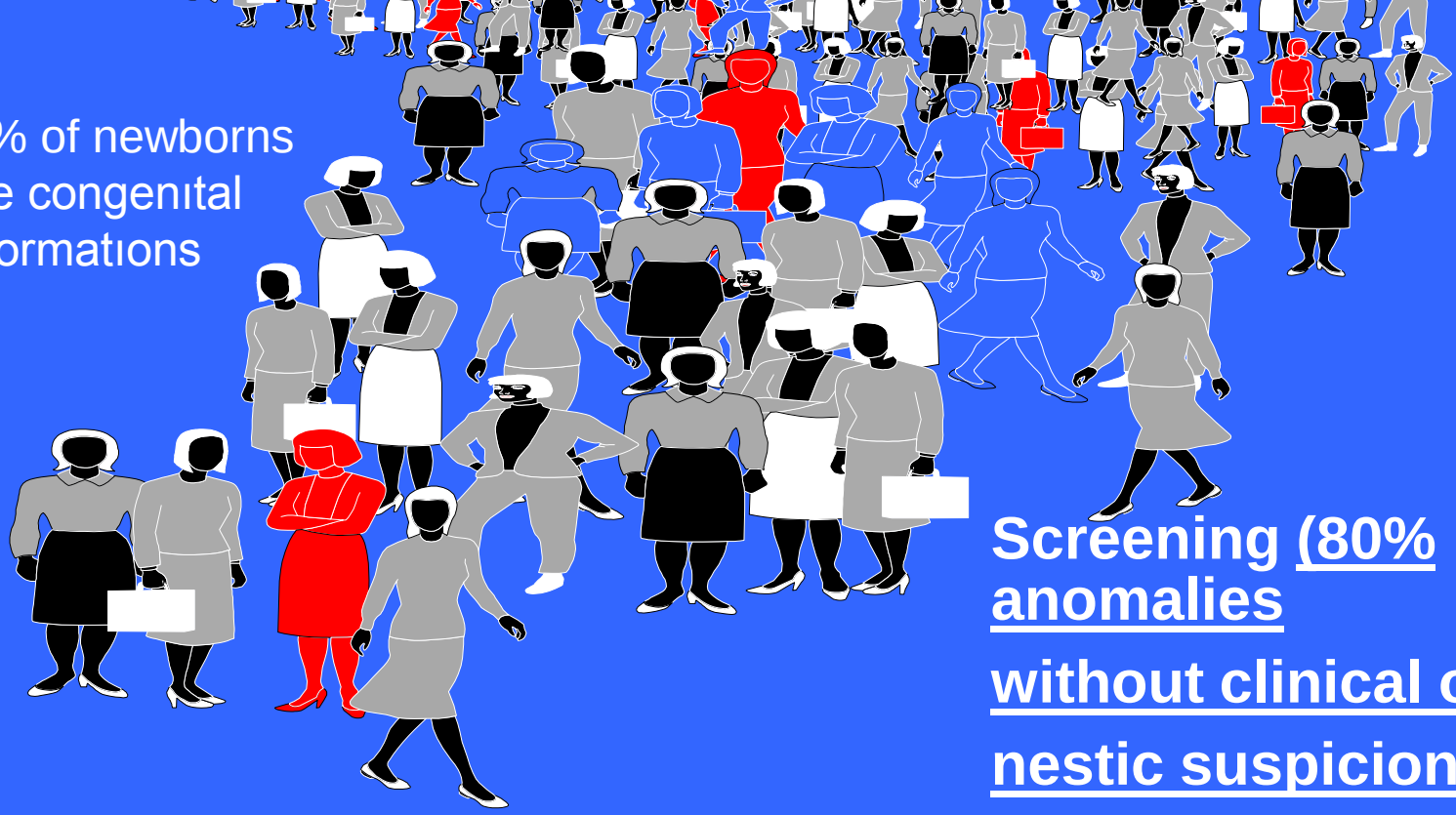
Universitätsklinikum Bonn

PRENATAL SCREENING

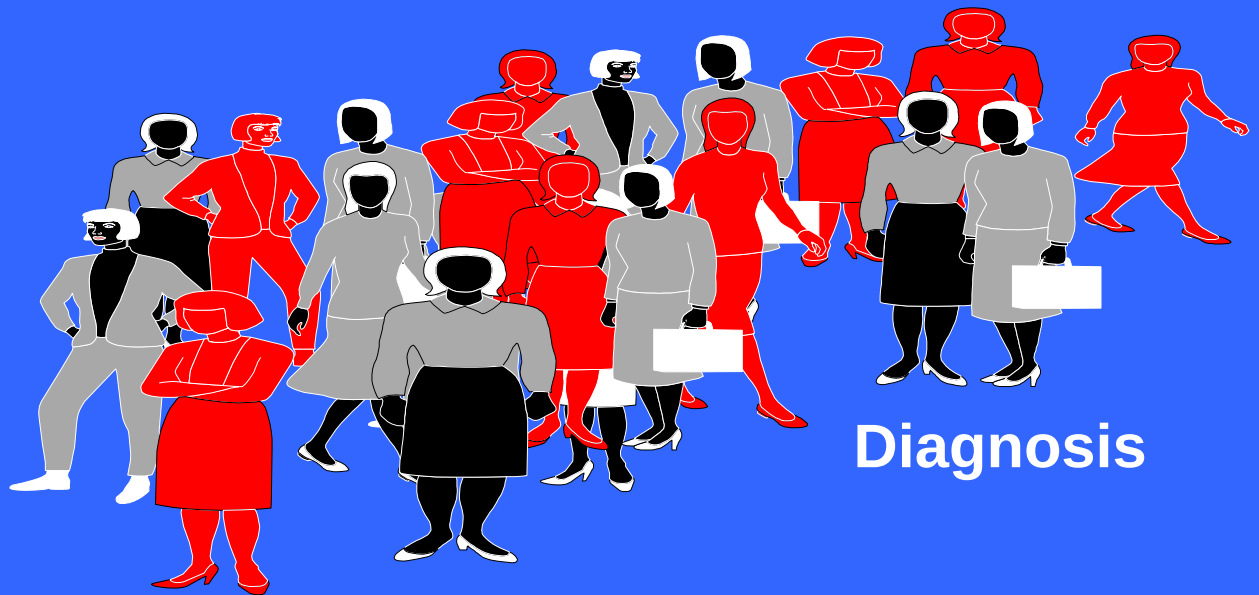
25 YEAR HISTORY



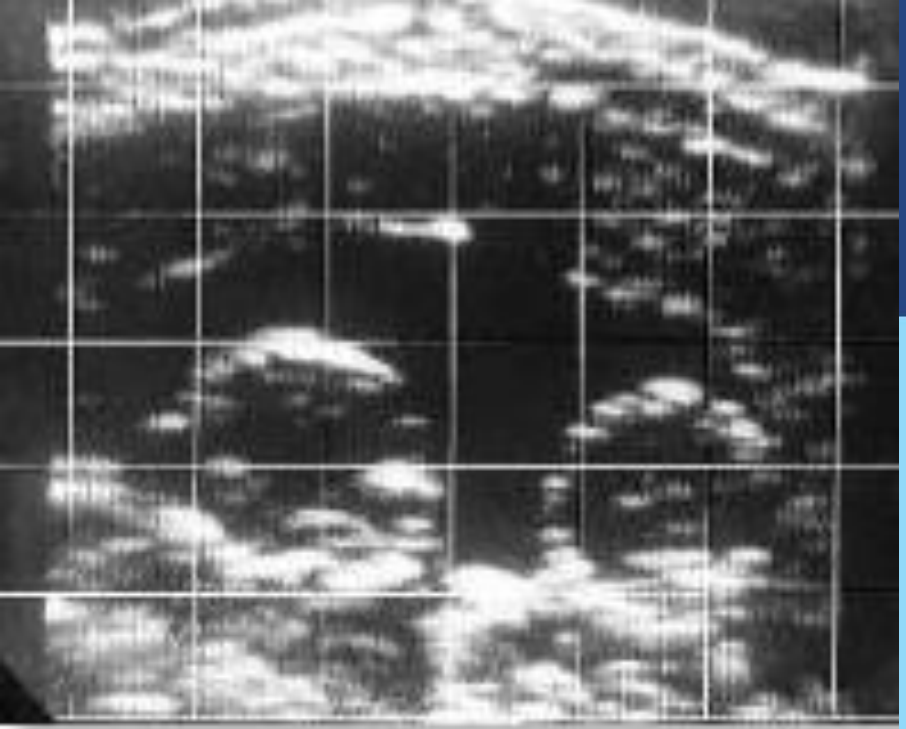
2-4 % of newborns
Have congenital
malformations



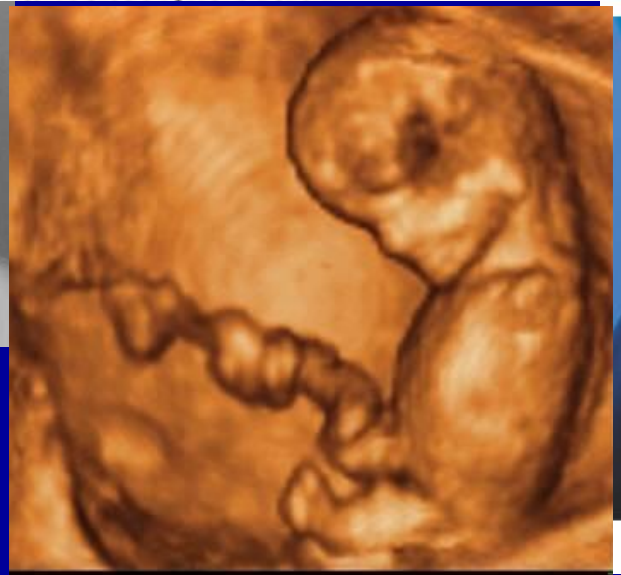
Screening (80% anomalies without clinical or anamnestic suspicion)



Diagnosis



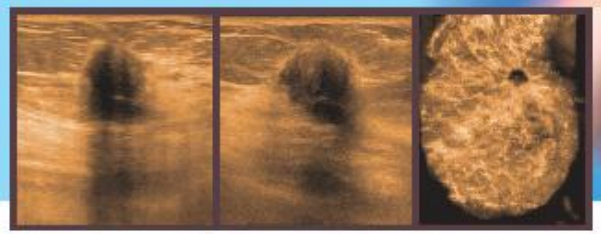
Twin pregnancy depicted on the Vidoson, 1965

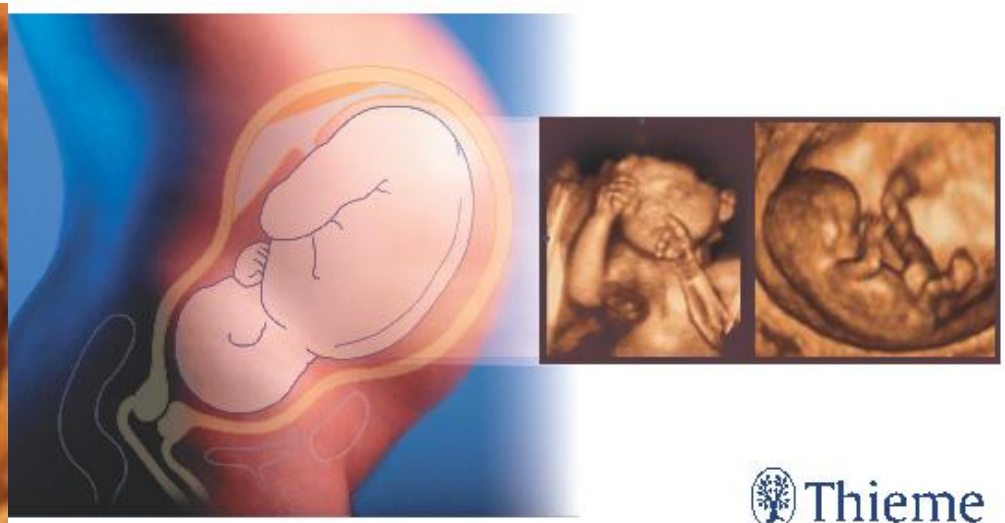


Ultraschall in Gynäkologie und Geburtshilfe

Christof Sohn
Wolfgang Holzgreve

3., vollständig überarbeitete Auflage





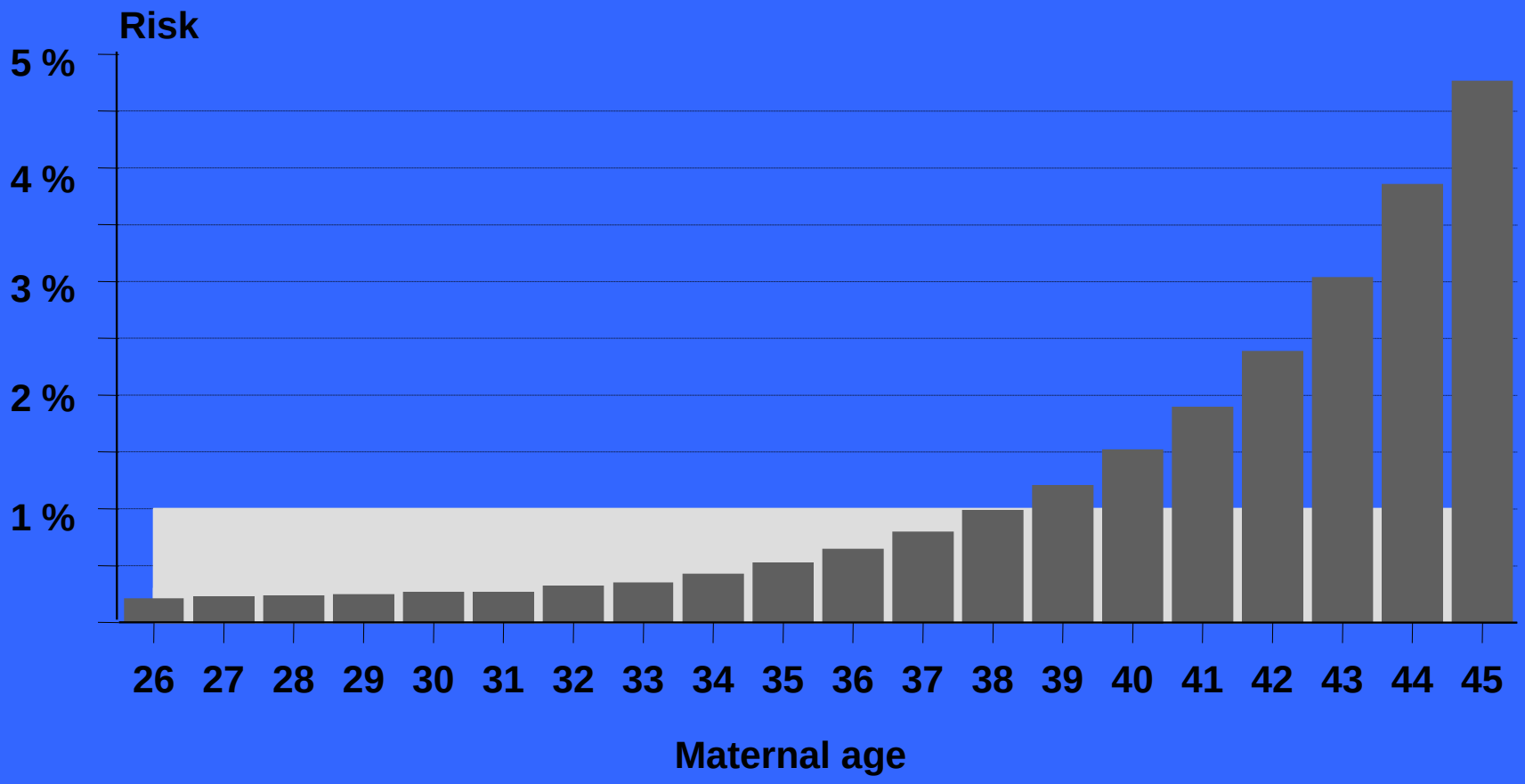
Thieme

Tjio JH, Levan A (1956)
The chromosome numbers of man. Hereditas 42:1-6

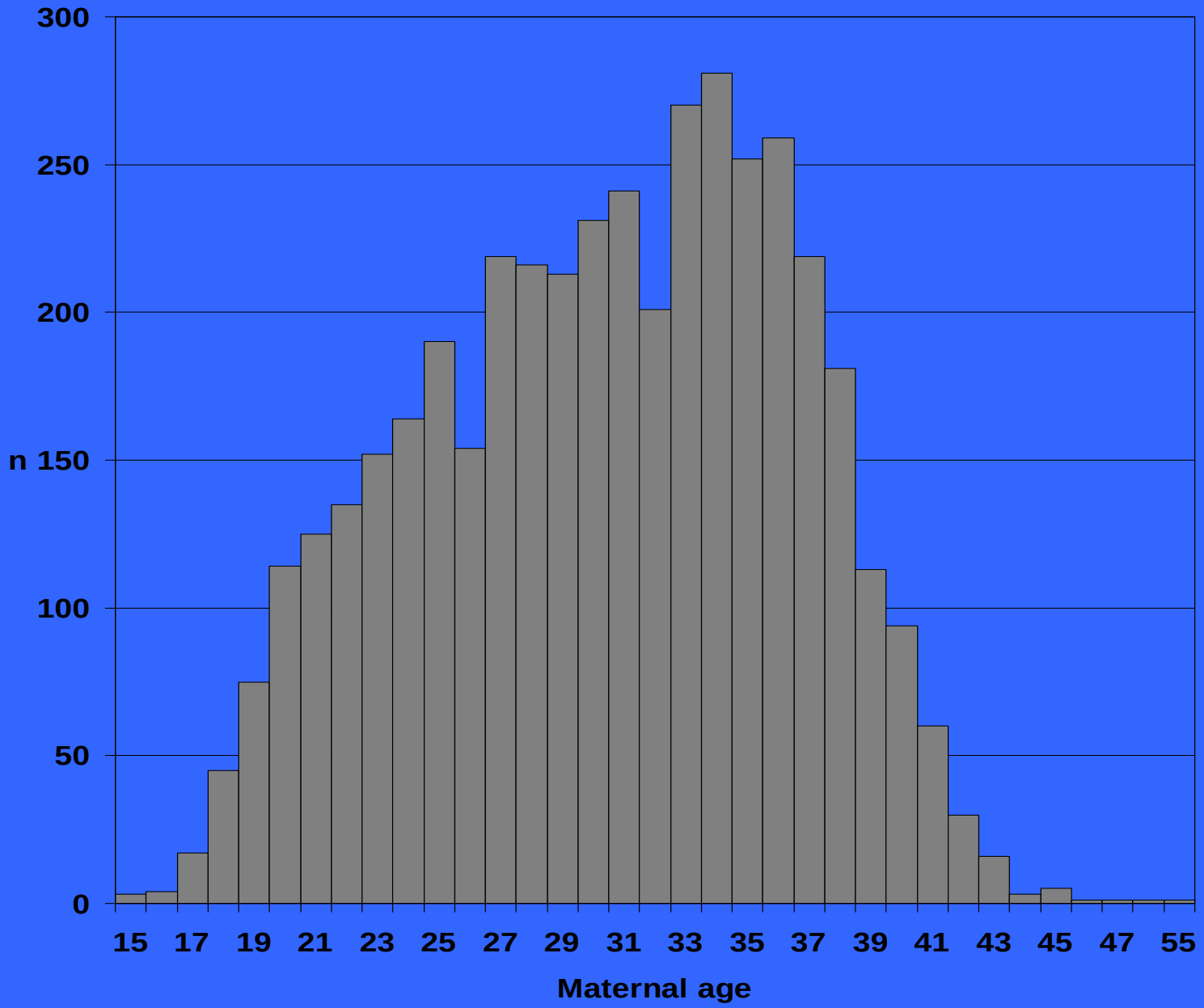
... we do not wish to generalize our present findings into a statement that the chromosome number of man is 2n = 46, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations.

Lejeune J, Gautier M, Turpin R (1959)
Etude des chromosomes somatiques de neuf enfants mongoliens. Compt Rend Acad Sci 248:1721-1722

Aufklärung, dass Down-Syndrom durch Trisomie 21 verursacht ist


Risk
5 %
4 %
3 %
2 %
1 %
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Maternal age

Maternal age distribution in Switzerland; mean = 30.4 yrs



Invasive Prenat. Dg.: Steady decline

Jahr	ACs
2006	80 684
2007	77 042
2008	71 092
2009	60 936
2010	52 690

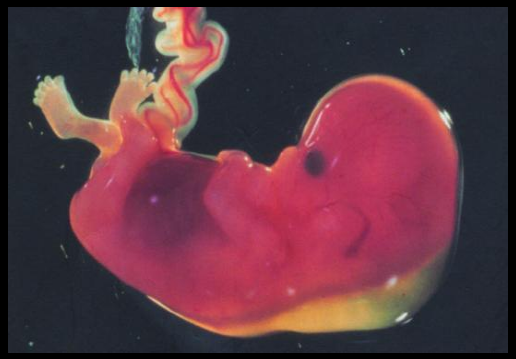
Jahr	CVS
2006	8814
2007	9278
2008	9418
2009	9534
2010	9378

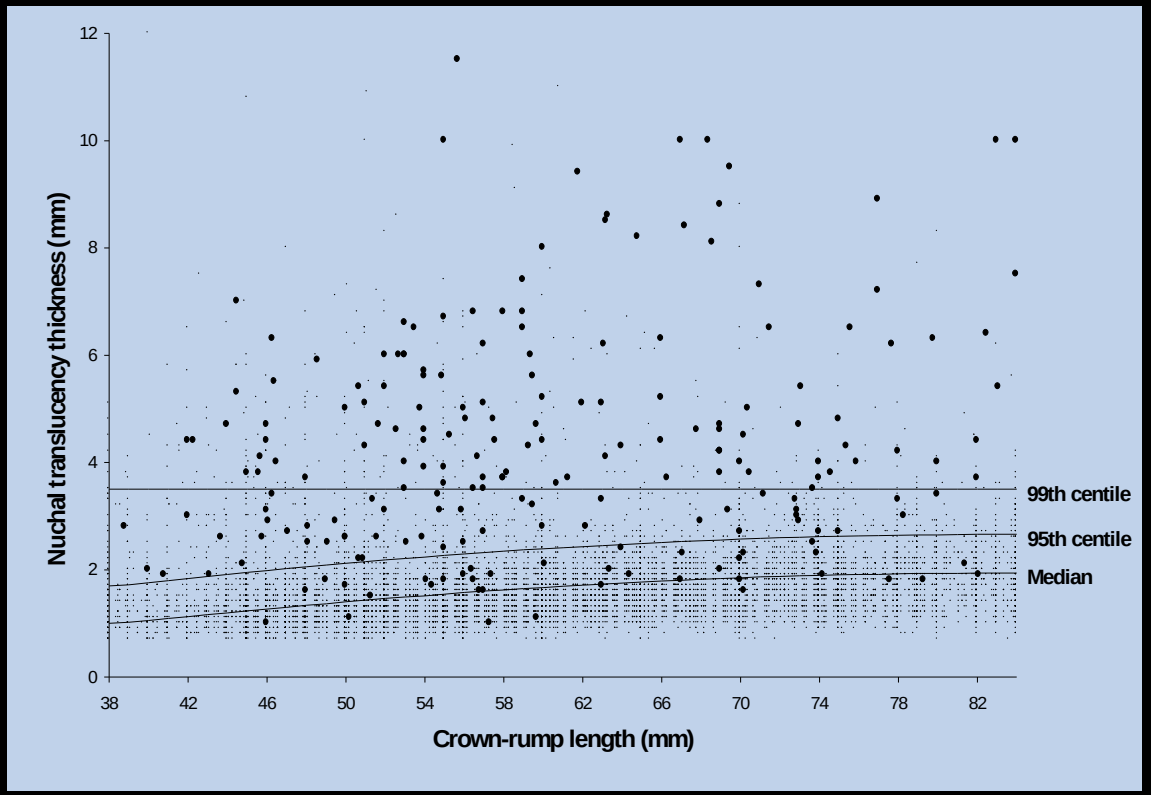
NT screening in Austria, Germany and Switzerland

n= 23'805

Basel, Berlin, Dresden, Düsseldorf, Frankfurt, Hamburg, Heidelberg, Innsbruck, Kiel, Lübeck, Ludwigsburg, Mainz, München, Numberg, Peine, Schaffhausen,

	N	NT> 95 %
Normal	21479	8 %
Trisomy 21	210	83 %
Trisomiy 18	112	88 %
Trisomiy 13	29	79 %
Turner	55	94 %
Triploidiy	17	65 %
Others	30	49 %





Gasiorek-Wiens A, Tercanli S, Kozlowski P, Kossakiewicz A, Minderer S, Meyberg H, Kamin G, Germer U, Bielicki M, Hackelör BJ, Sarlay D, Kuhn P, Klapp J, Bahlmann F, Pruggmayer M, Schneider KTM, Seefried W, Fritzer E, von Kaisenberg CS

7th problem: There are more and more good alternatives

Table 1. False-Positive Rate and Odds of Being Affected Given a Positive Result to Achieve an 85% Down Syndrome Detection Rate*

Screening Test (All With Maternal Age)	Trimester of Pregnancy	FPR, %	OAPR
NT	1st	18	1:132
Double (AFP, free ß-hCG)	2nd	17	1:125
Triple (AFP uE_3, hCG)	2nd	14	1:100
Quadruple (AFP, uE_3, hCG, inhibin-A)	2nd	8.4	1:61
Combined (NT, free ß-hCG, PAPP-A)	1st	4.8	1:35
Serum integrated (PAPP-A [1st] and quadruple test [2nd])	1st and 2nd	4.3	1:33
Integrated (NT, PAPP-A [1st] and quadruple test [2nd])	1st and 2nd	0.8	1:6

AFP indicates α-fetoprotein; FPR, false-positive rate; hCG, human chorionic gonadotropin; OAPR, odds of being affected given a positive result; PAPP-A, pregnancy-associated plasma protein A; and uE_3, unconjugated estriol.

*These estimates are based on screening parameters previously used[2](derived from 3 large data sets[3–5]), except for AFP6 and the age distribution of maternities in England and Wales in 1996 through 1998.

Wald, J Ultrasound Med 2002 Apr;21(4):481; discussion 483-7

The NEW ENGLAND JOURNAL of MEDICINE

EDITORIALS

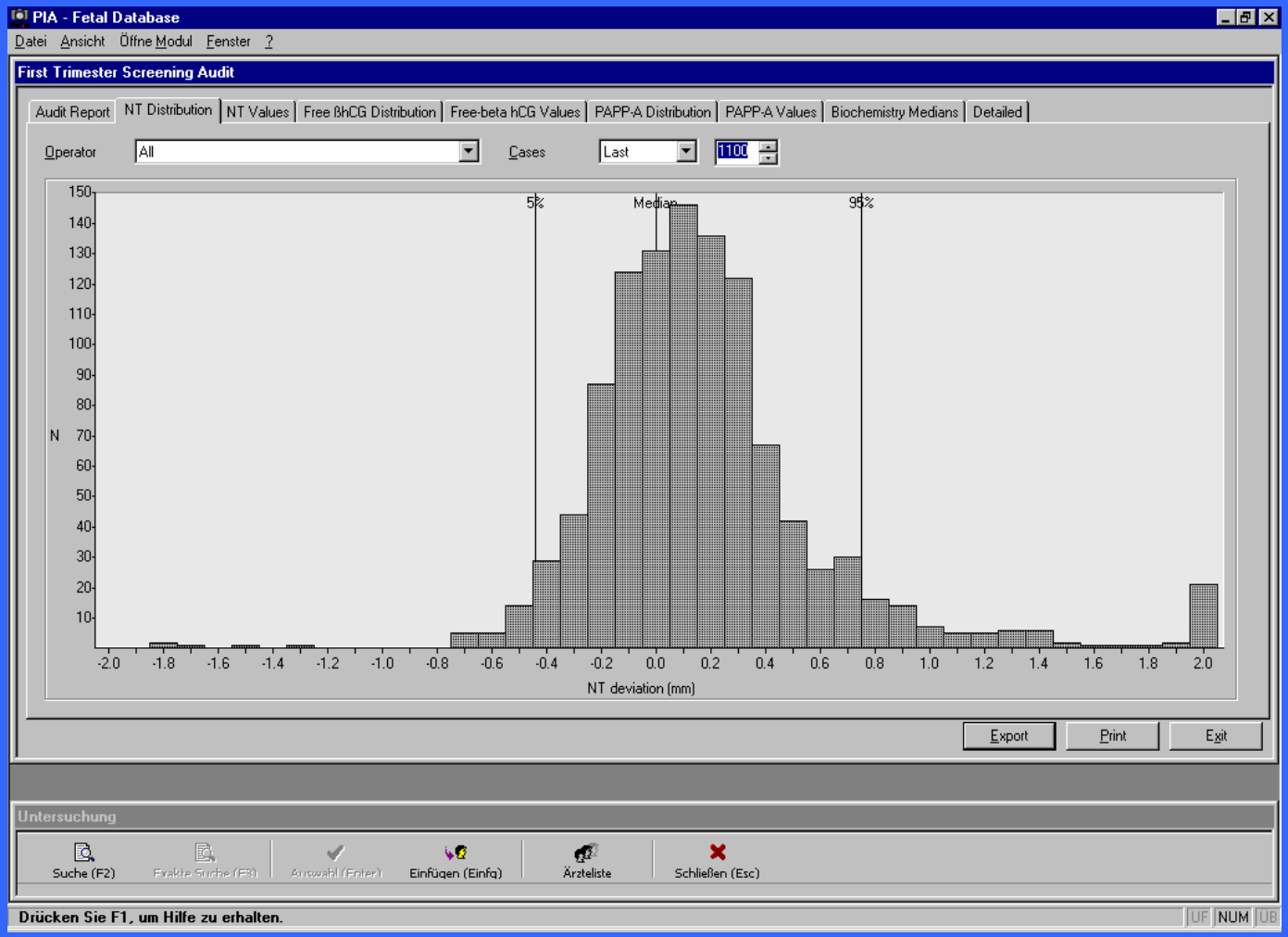
Screening for Down's Syndrome — Too Many Choices?

Michael T. Mennuti, M.D., and Deborah A. Driscoll, M.D.

Teaching in councelling

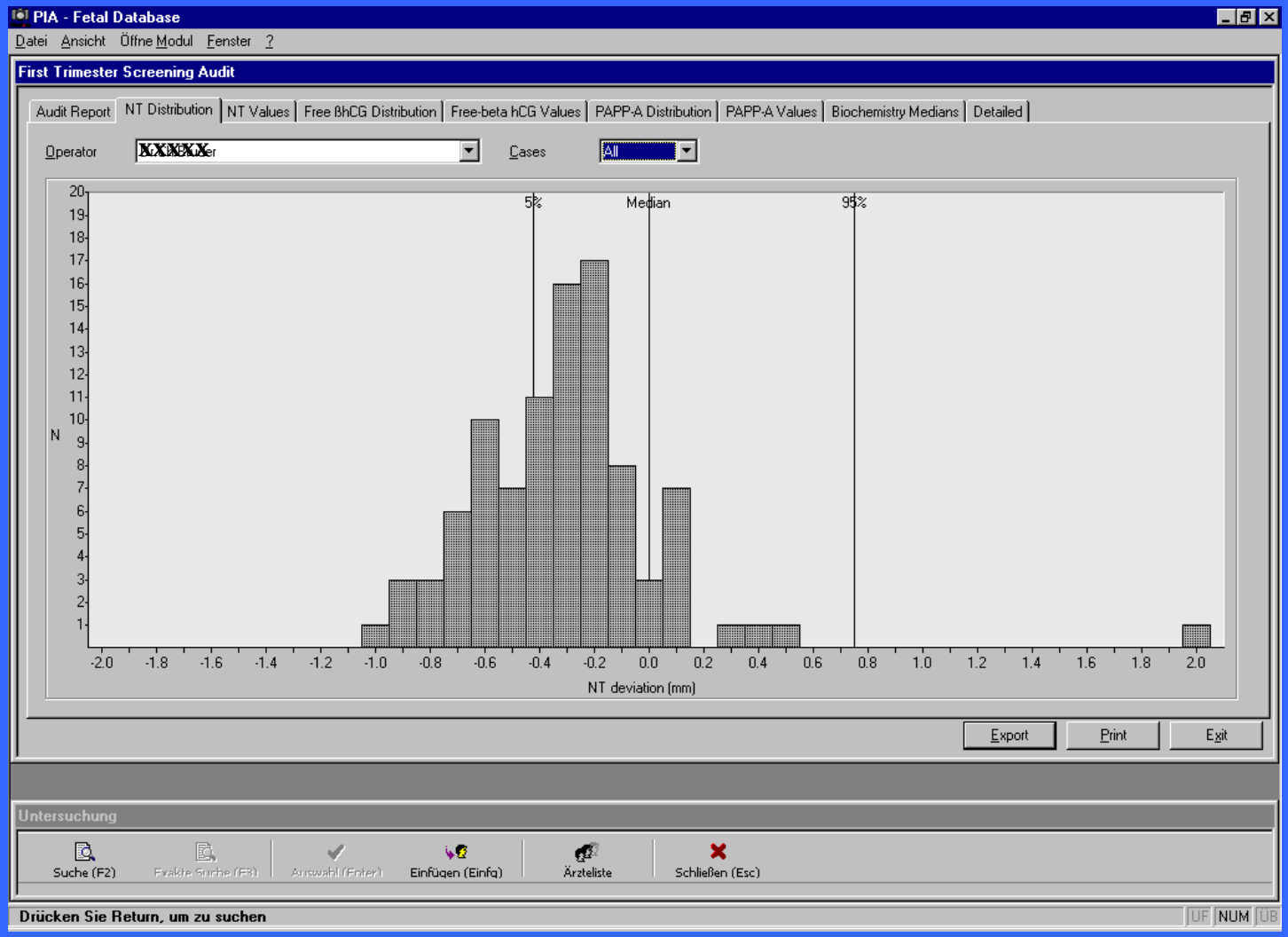
- Accuracy of information
- *Understandable language*
- Feedback about councelling

NT-measurement: The teaching problem

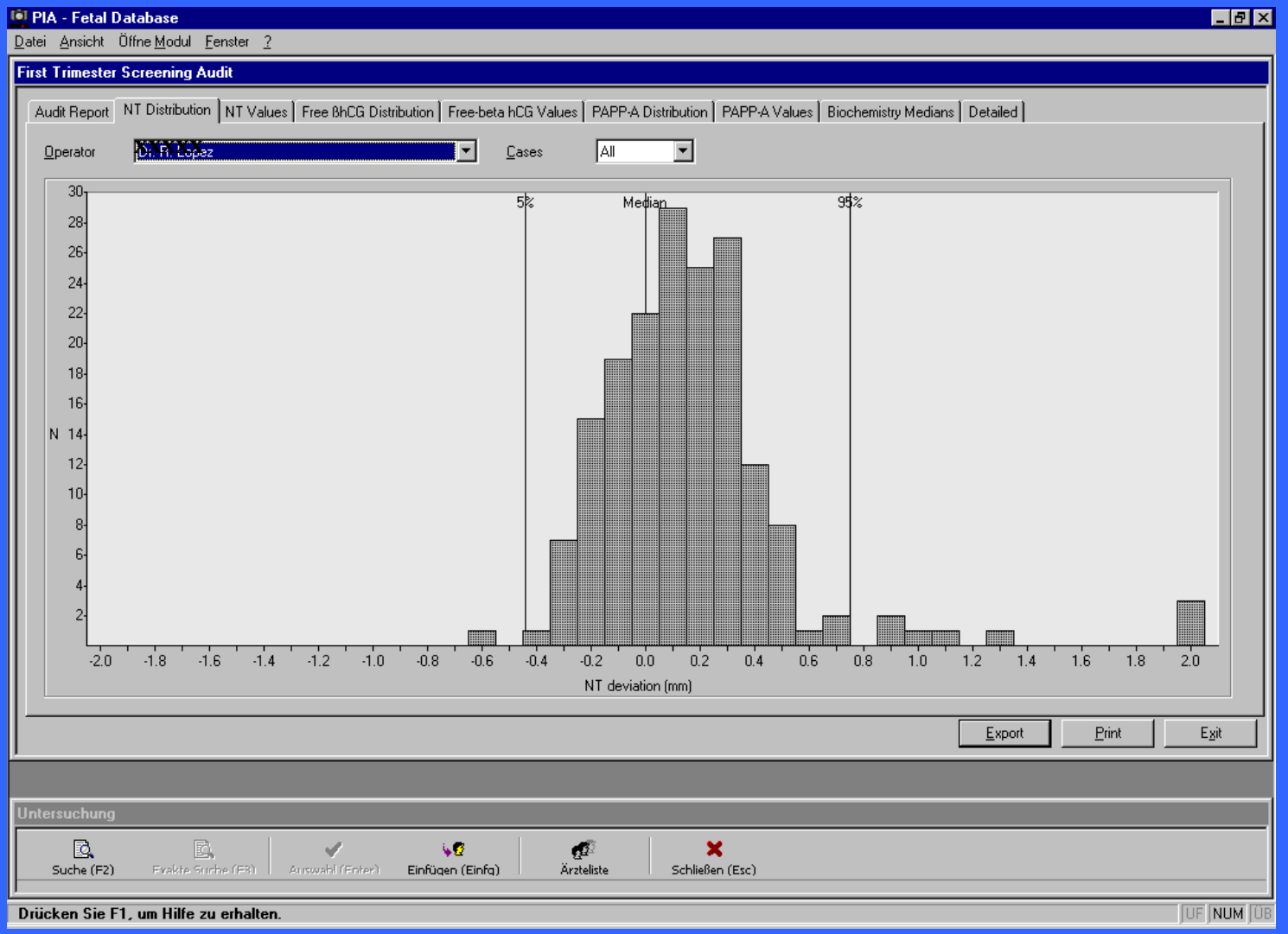


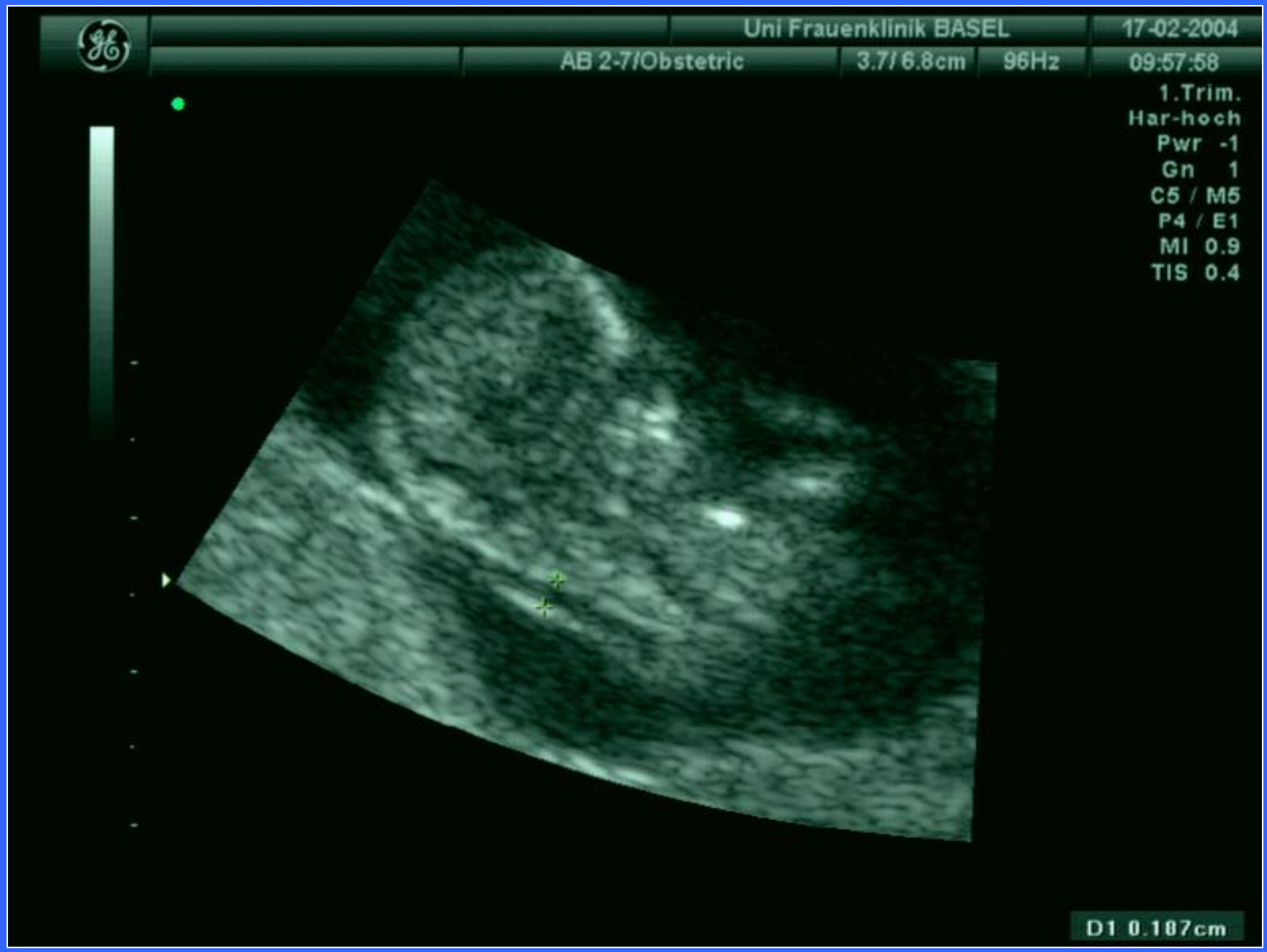
Lerning curve: min. n=100 examinations
9/14 reached normal distribution after 80 examinations
12/14 reached normal distribution after 100 exams
Most frequent problem: measurements too small

NT-measurement: the teaching problem



NT-measurement: the teaching problem




Uni Frauenklinik BASEL
17-02-2004
AB 2-7/Obstetric
3.7/ 6.8cm
96Hz
09:57:58
1.Trim.
Har-hoch
Pwr -1
Gn 1
C5 / M5
P4 / E1
MI 0.9
TIS 0.4
D1 0.187cm

Datei Öffne Modul Bearbeiten Fenster ?

Befund F2

Geburt Strg+F2

Bild F3

Drucken F4

Archivieren F12

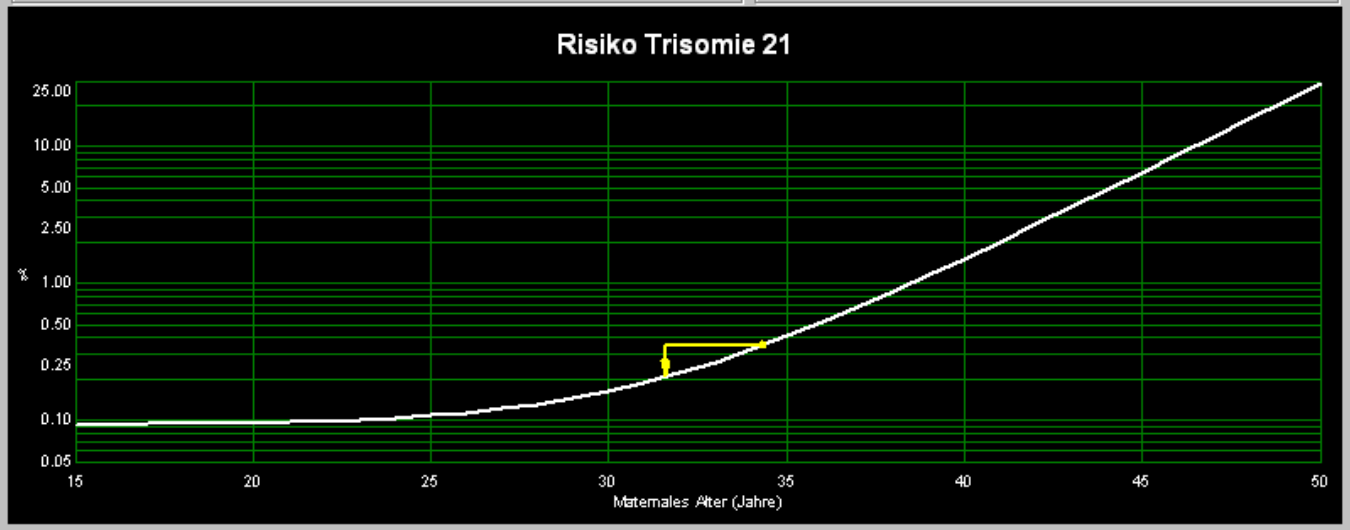
Hilfe F1

Risiko Trisomie 21 im 1.Trimenon

Maternales Alter: 34 Jahre, Gestationsalter: 11W + 3T, Fetus 1

Chromosomenaberration, frühere Schwangerschaft: ☐ Tr 21 ☐ Tr 18 ☐ Tr 13

Hintergrundrisiko Trisomie 21	1 :	284	Hintergrundrisiko Trisomie 13+18	1 :	480
Einschätzung enthält: ☑ 1. Trimenon NT					
Adjustiertes Risiko Trisomie 21	1 :	479	Adjustiertes Risiko Trisomie 13+18	1 :	811

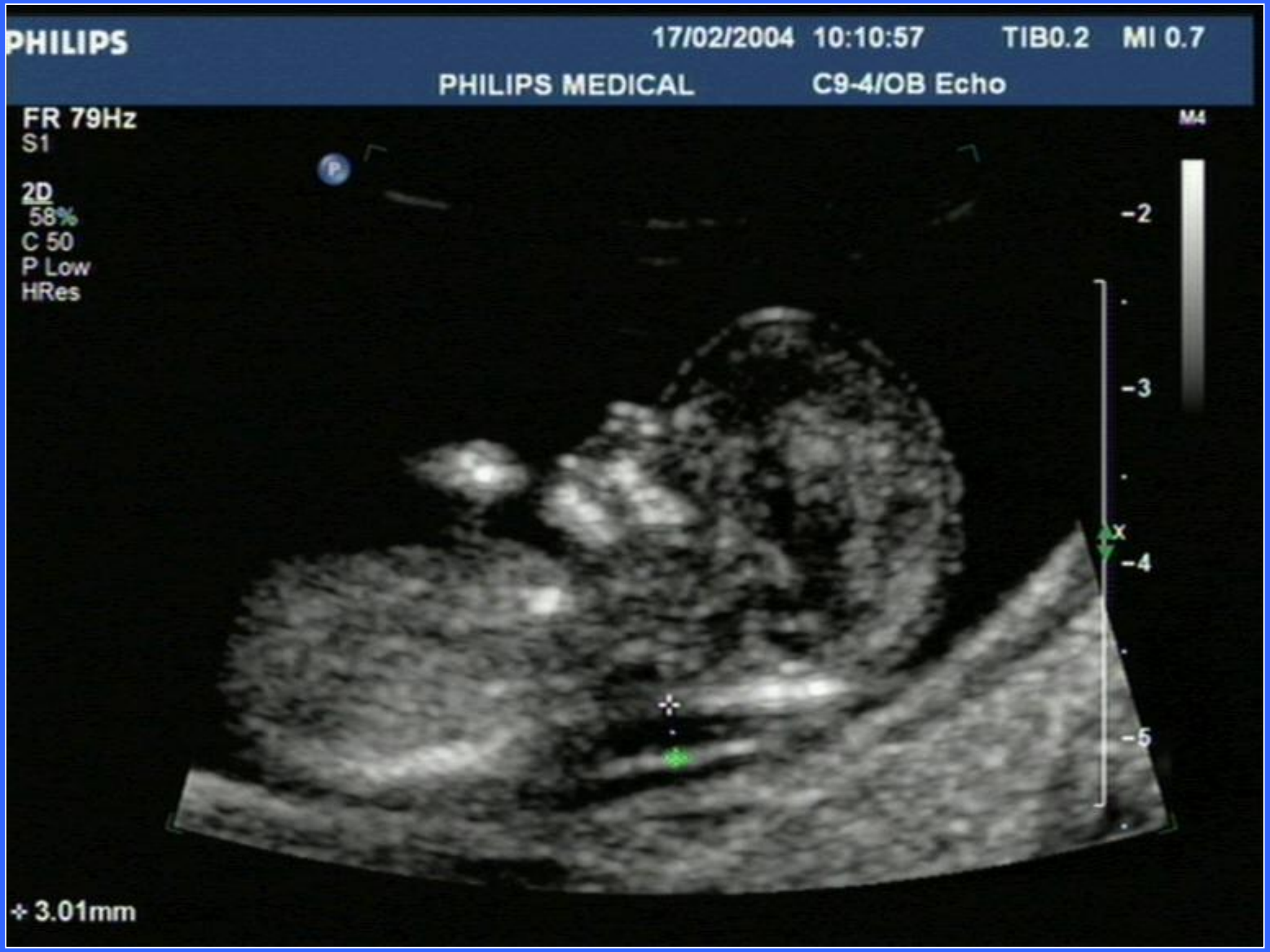


Referenzen:

1. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency at 10-14 weeks of gestation
Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH for the Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;352:343-56

2. A screening program for trisomy 21, at 10-14 weeks using fetal nuchal translucency, maternal serum free ß-human chorionic gonadotropin and pregnancy-associated plasma protein-A, Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. Ultrasound Obstet Gynecol 1999;13:231-237

Drücken Sie F10 um diese Maske abzuschließen | Drücken Sie Esc um Ihre Eingaben zu verwerfen | Benutzer: pia | UF | NUM | ÜB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17/02/2004 10:10:57
TIB0.2
MI 0.7
PHILIPS MEDICAL
C9-4/OB Echo
FR 79Hz
S1
2D
58%
C 50
P Low
HRes
M4
-2
-3
-4
-5
+ 3.01mm

Datei Öffne Modul Bearbeiten Fenster ?

Befund F2

Geburt Strg+F2

Bild F3

Drucken F4

Archivieren F12

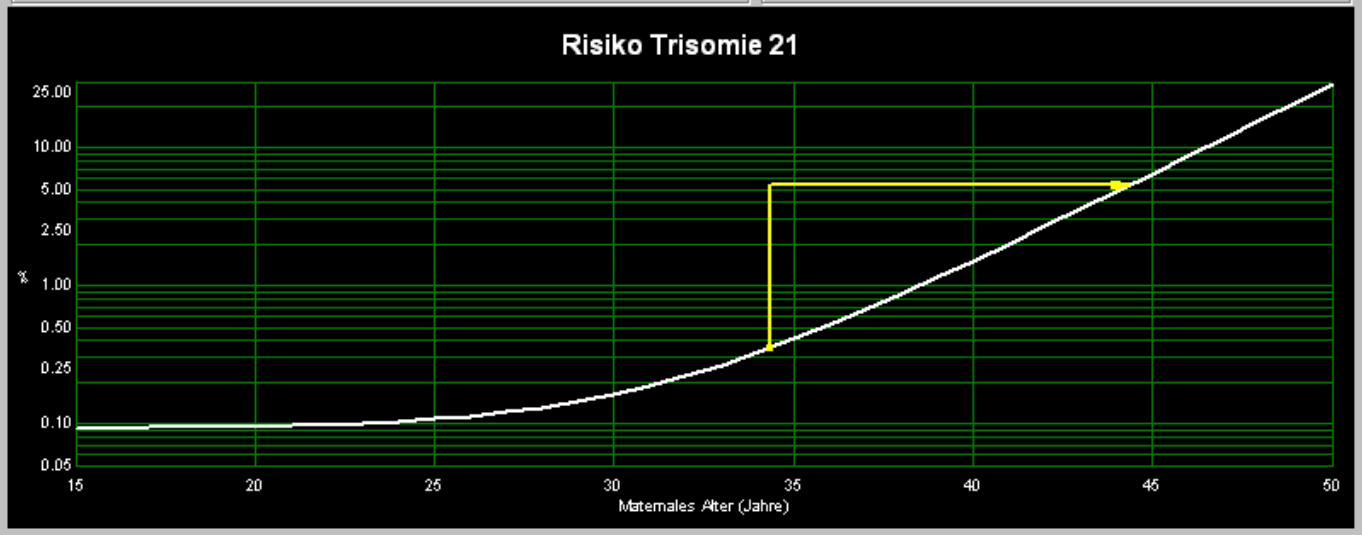
Hilfe F1

Risiko Trisomie 21 im 1.Trimenon

Maternales Alter: 34 Jahre, Gestationsalter: 11W + 3T, Fetus 1

Chromosomenaberration, frühere Schwangerschaft: ☐ Tr 21 ☐ Tr 18 ☐ Tr 13

Hintergrundrisiko Trisomie 21	1 :	284	Hintergrundrisiko Trisomie 13+18	1 :	480
Einschätzung enthält:	☑ 1. Trimenon NT				
Adjustiertes Risiko Trisomie 21	1 :	19	Adjustiertes Risiko Trisomie 13+18	1 :	31



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Drücken Sie F10 um diese Maske abzuschließen | Drücken Sie Esc um Ihre Eingaben zu verwerfen | Benutzer: pia | UF NUM ÜB

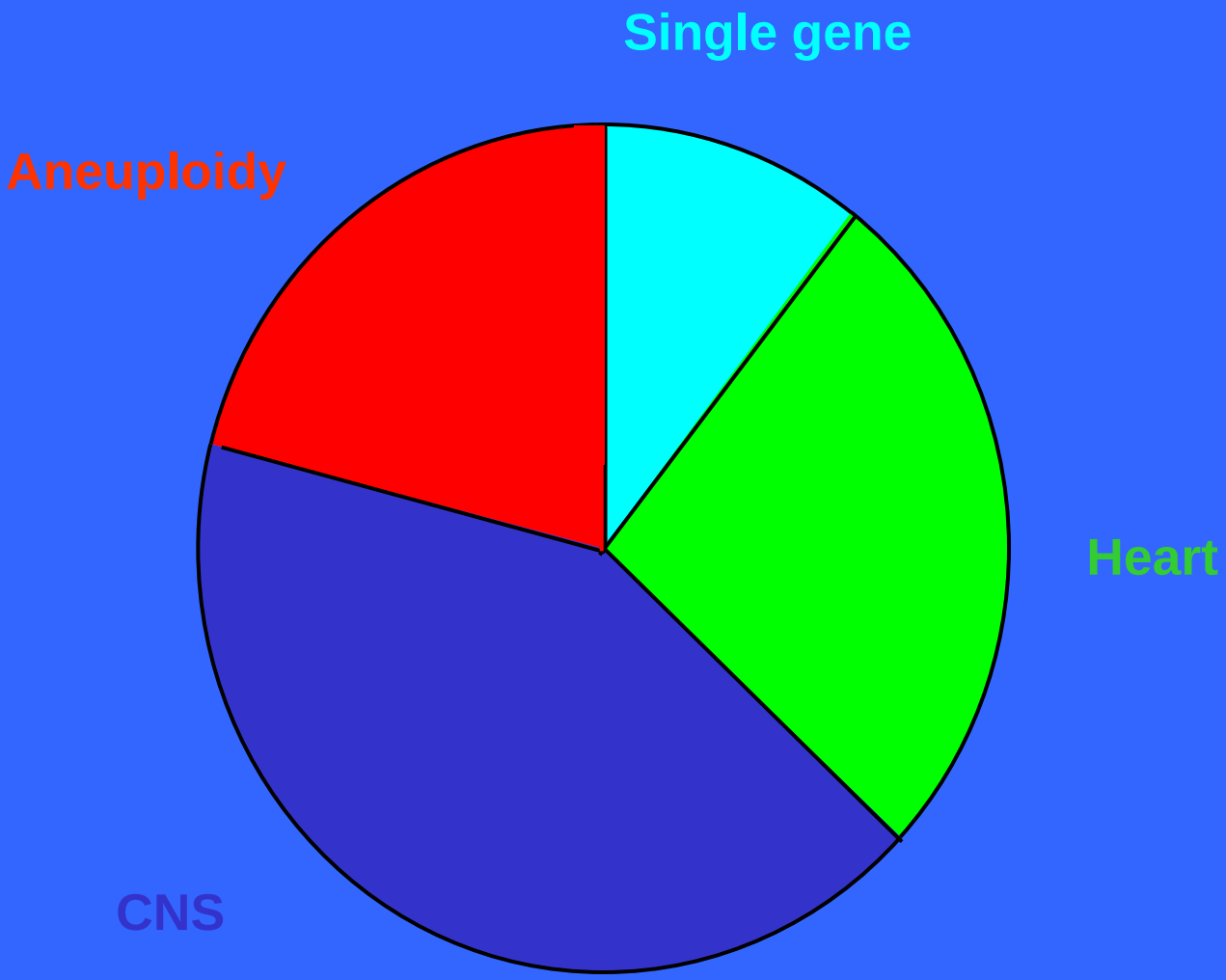

SONOFIT







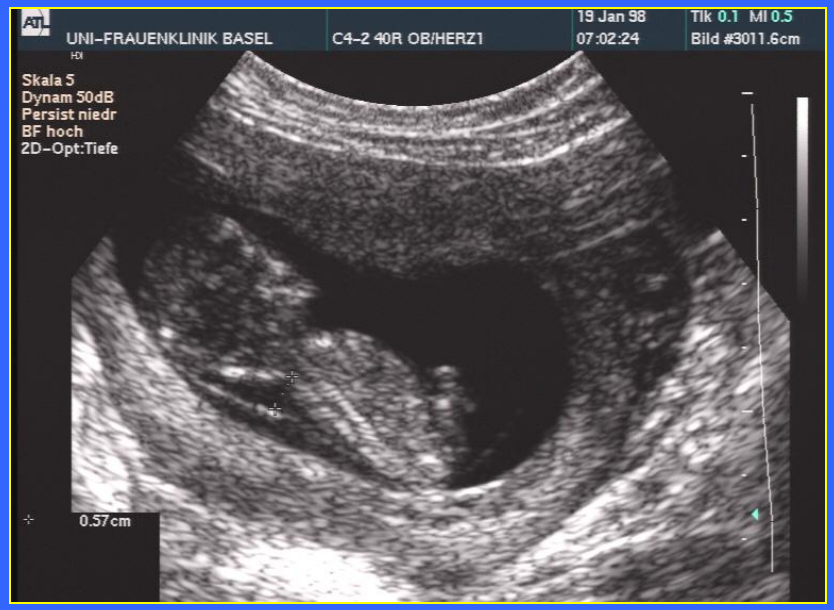
DE
14:36

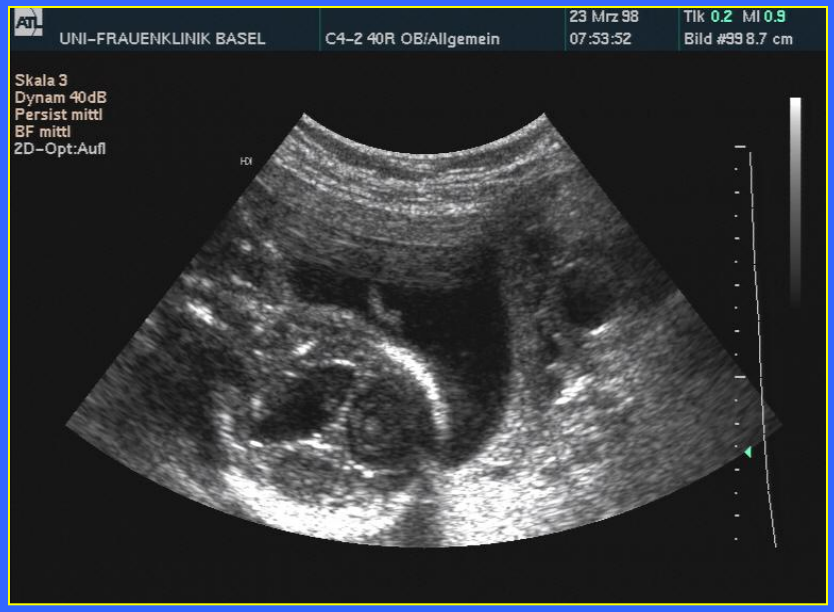

Single gene
Aneuploidy
Heart
CNS

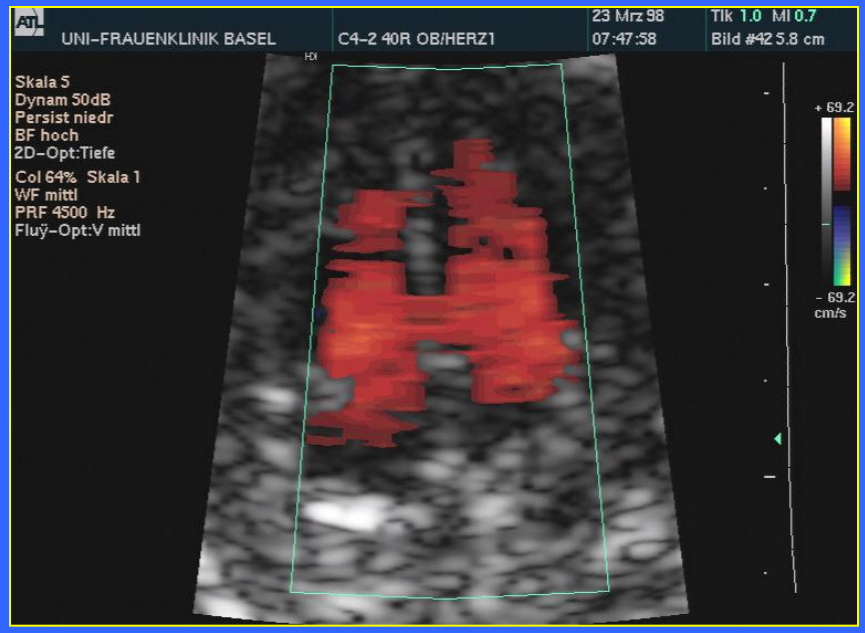
Some associations with „Nuchal Translucency“

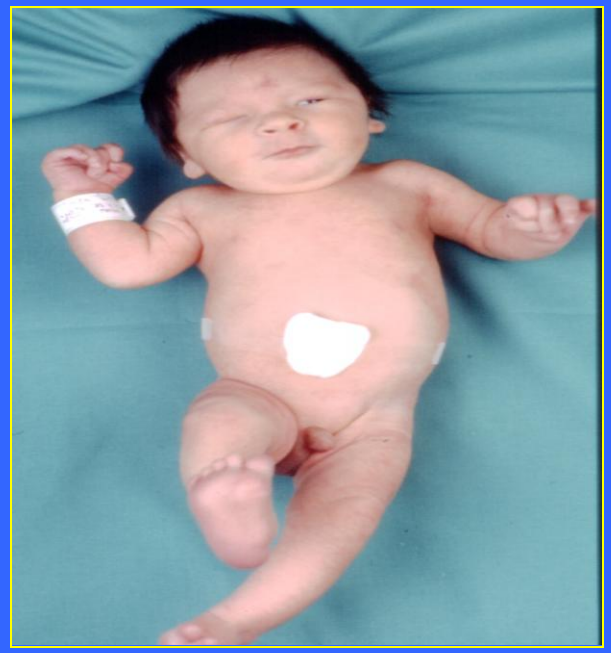
- Achondroplasia
- Beckwith-Wiedemann Syndrome
- Campomelic dysplasia
- Thanatopheric dysplasia
- Roberts syndrome
- Jeune syndrome
- Joubert syndrome
- Partial trisomy 19q
- Meckel-Gruber syndrome
- Noonan syndrome
- Dandy-Walker syndrome
- Smith-Lemli-Opitz syndrome
- VACTER association
- Zellweger syndrome
- Fryns syndrome
- Arthrogryposis
- Fanconi anemia
- Long-chain 3 Hydroxyacyl- coenzyme dehydro - genase deficiency

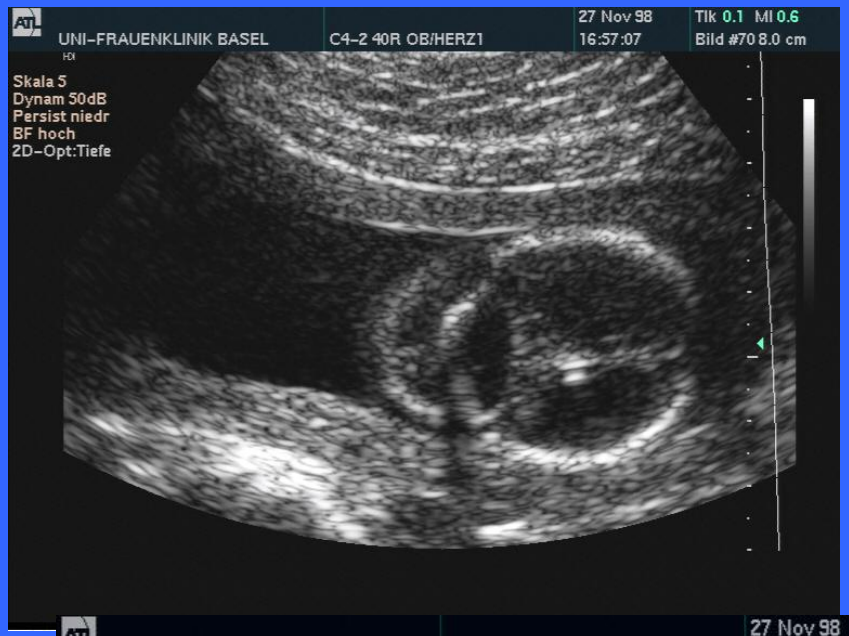
FANCONI ANEMIA

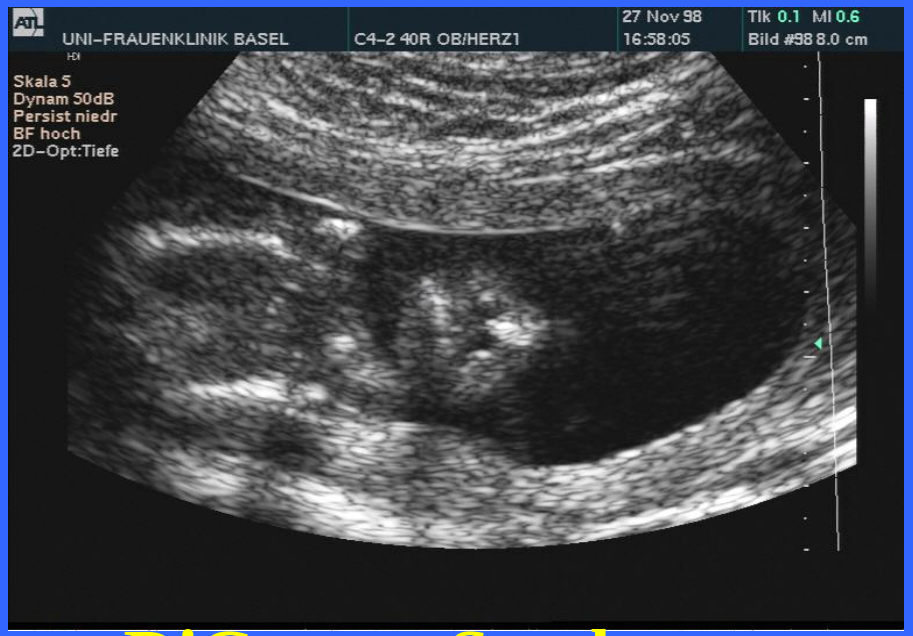


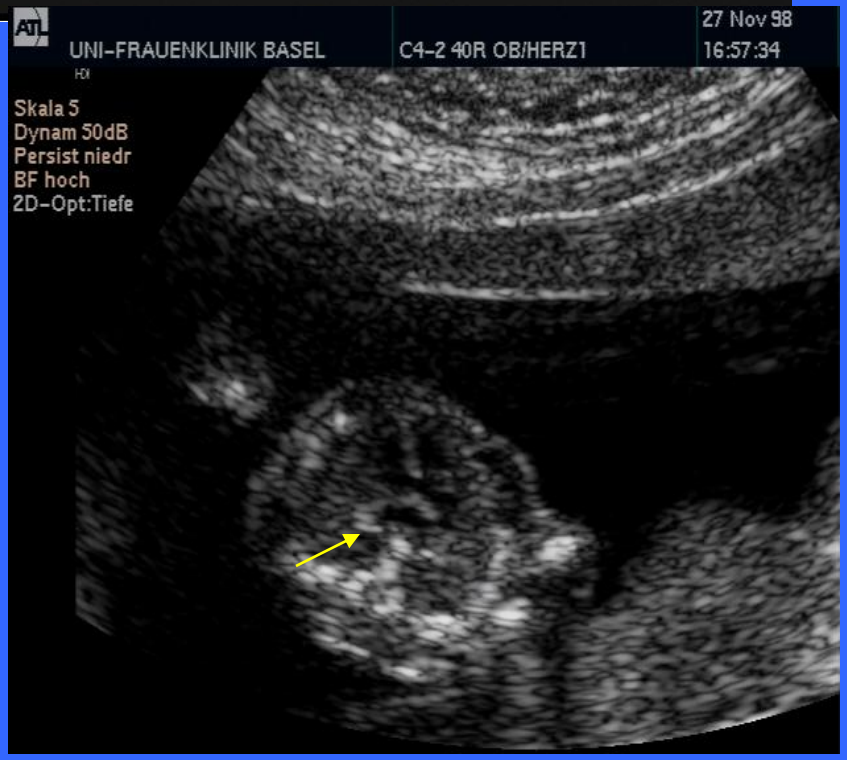




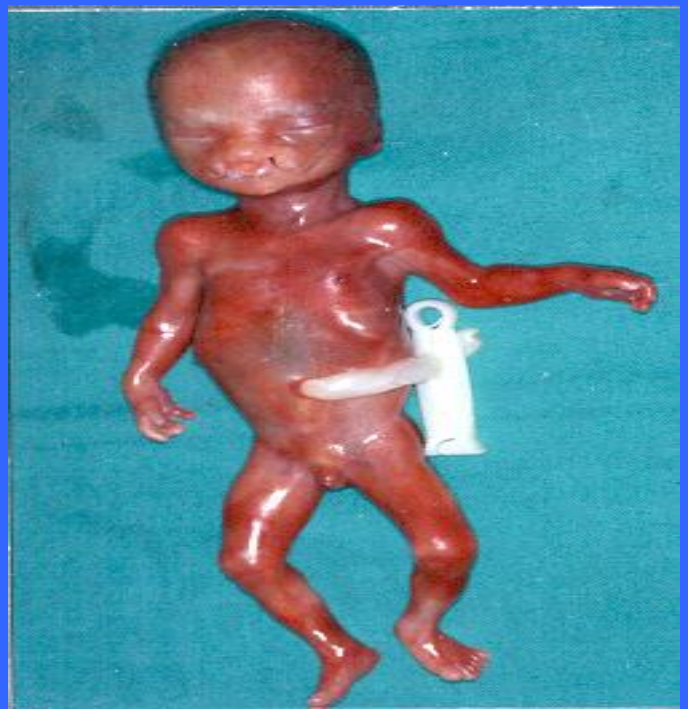




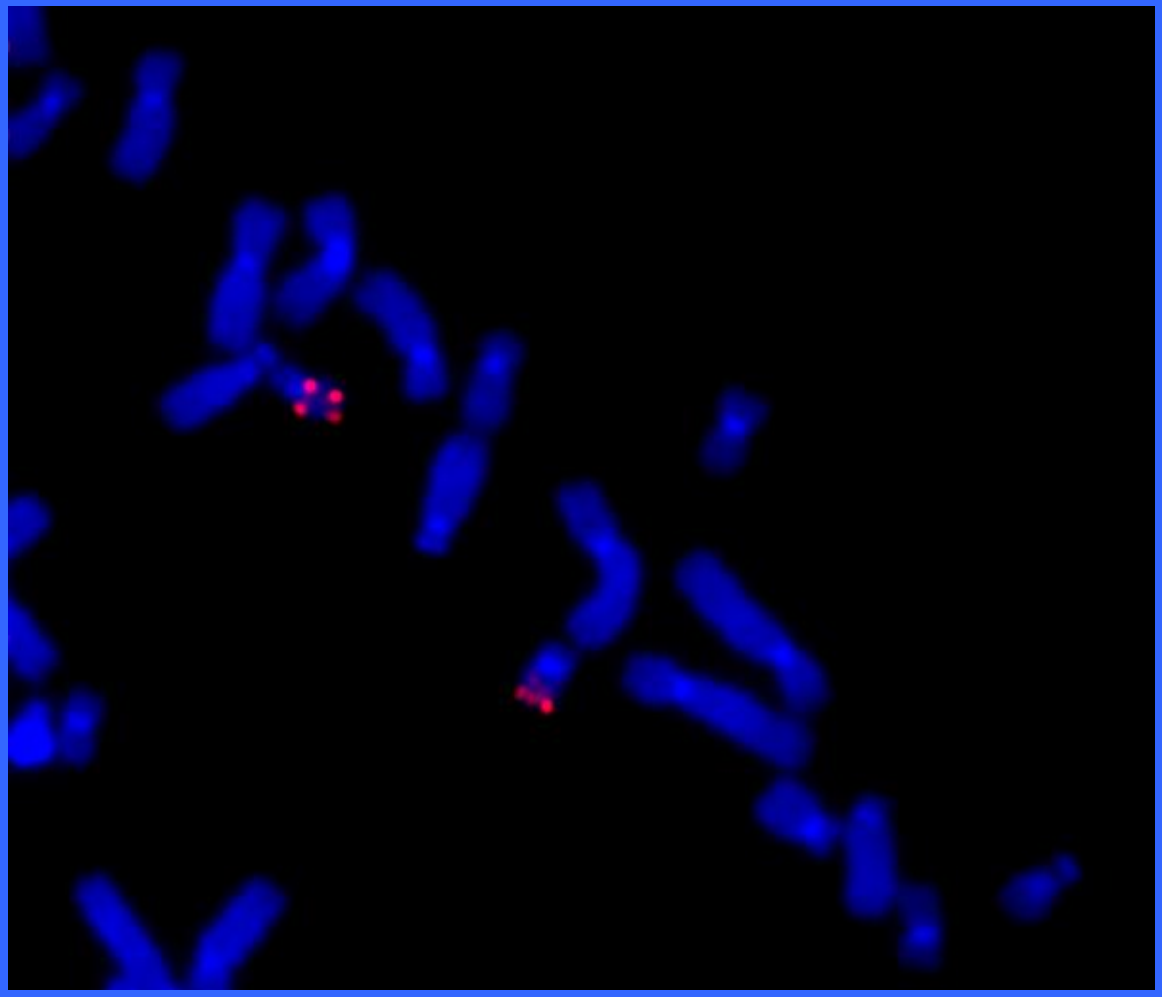




DiGeorge Syndrome

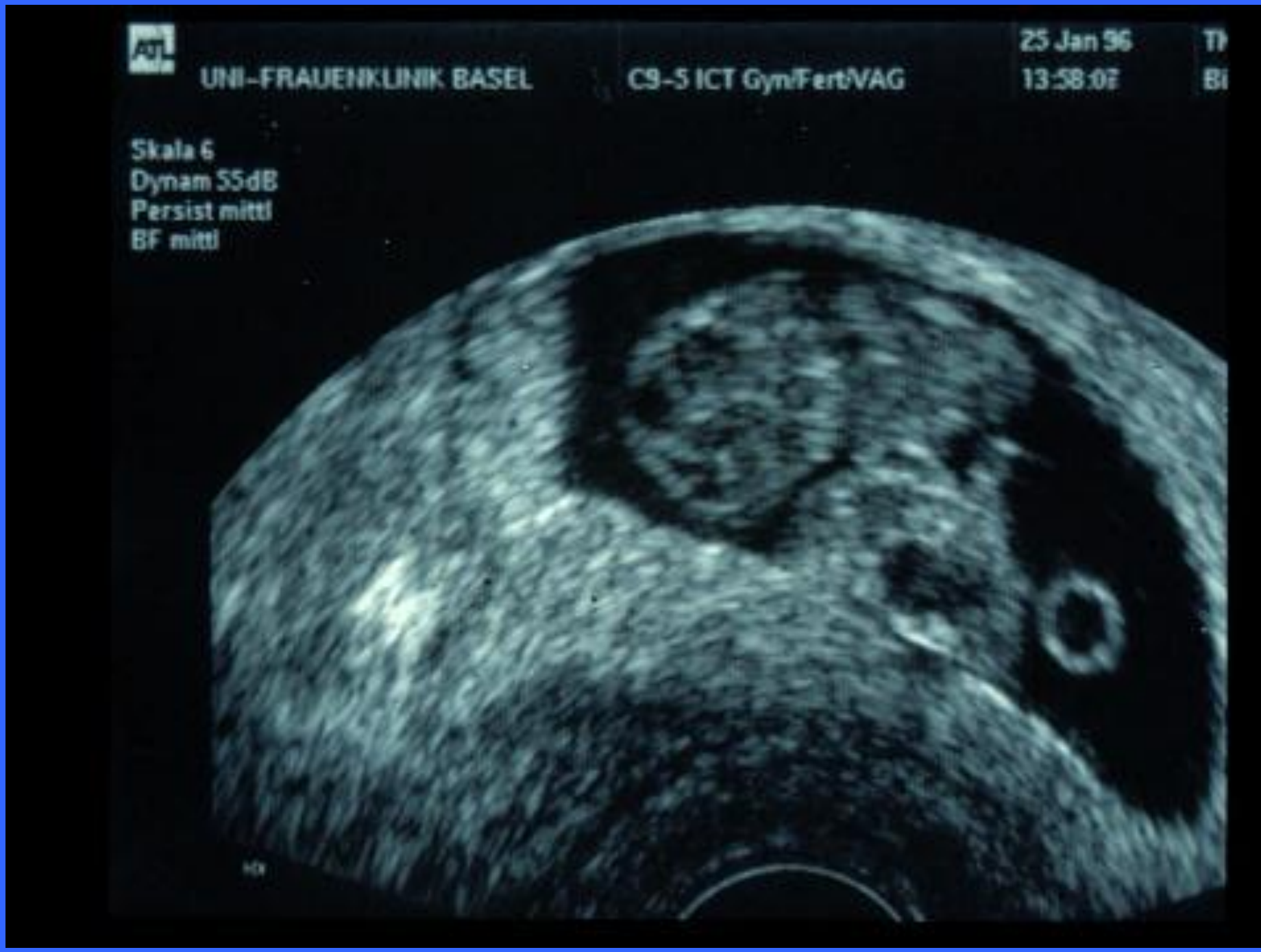


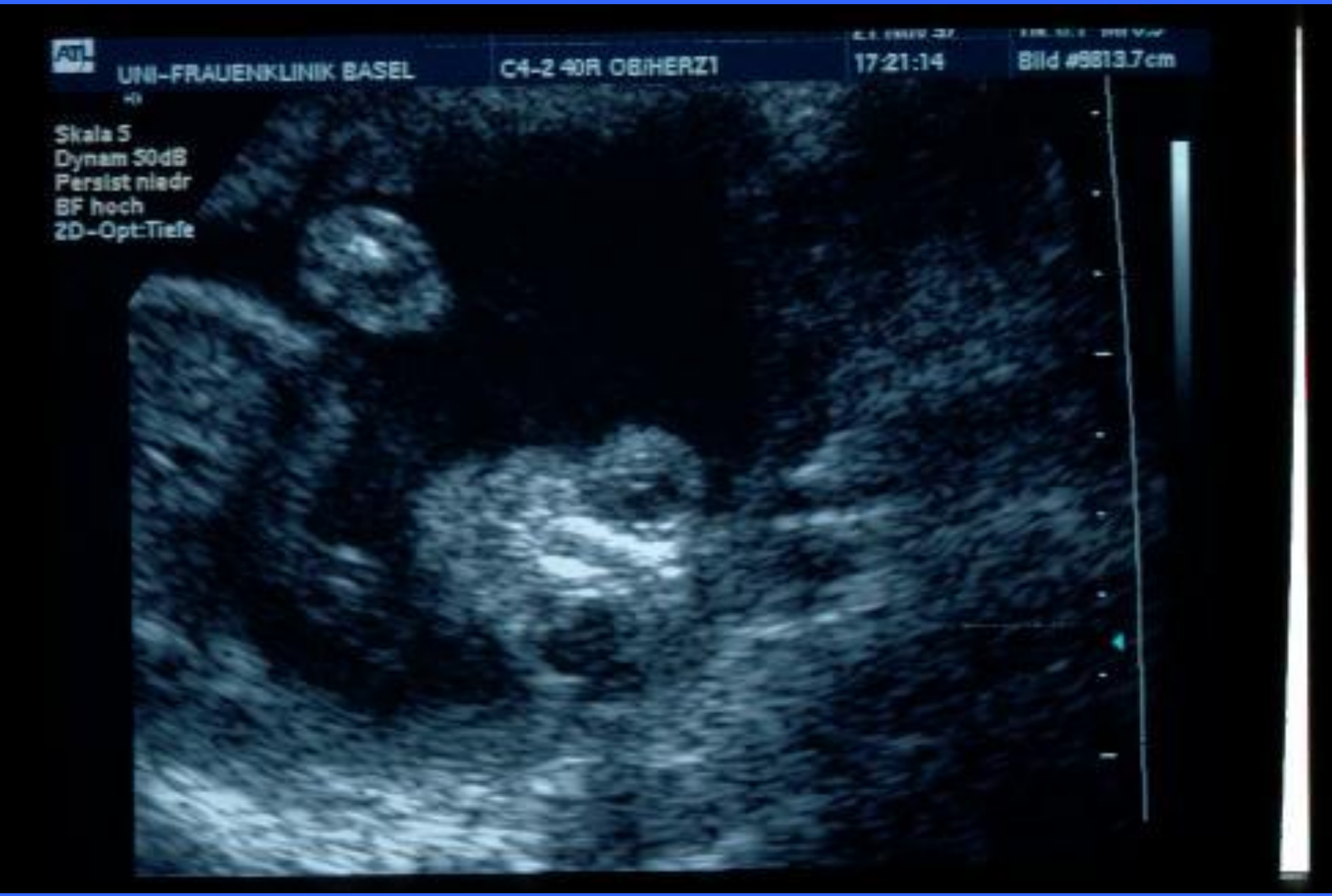
DiGeorge Critical Region 22q11.2



Oncor

DGCR & D22S39

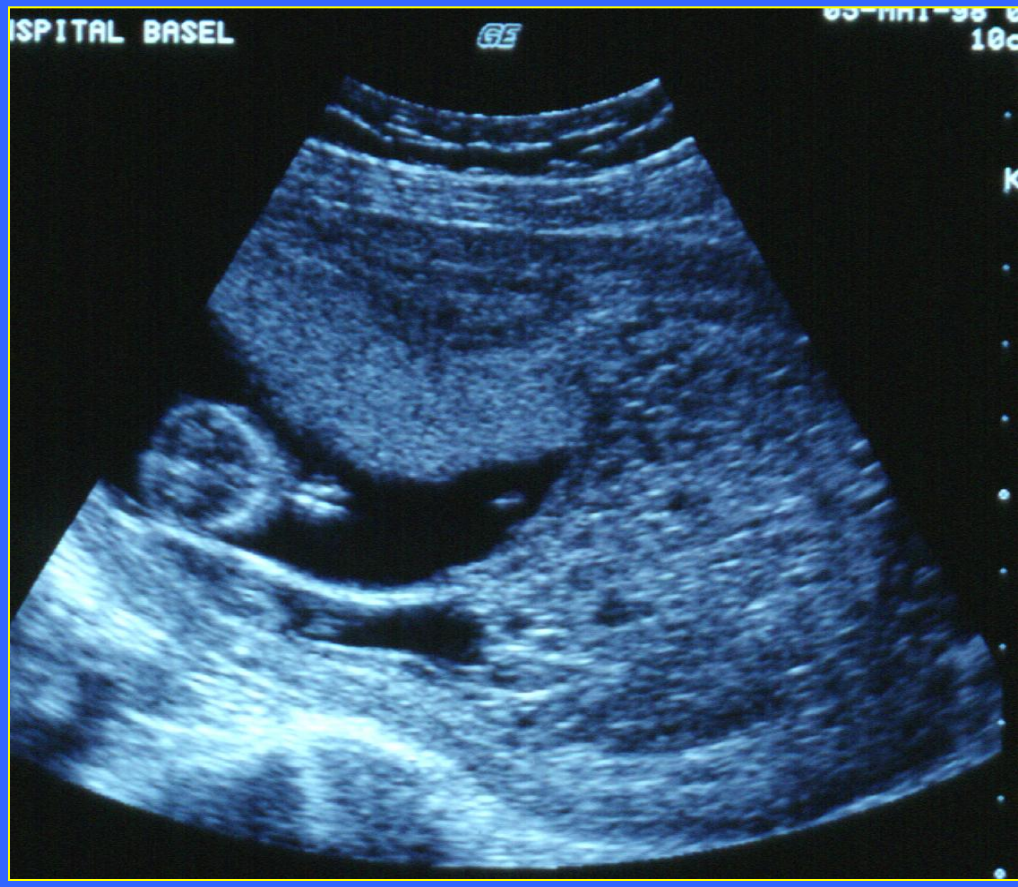

UNI-FRAUENKLINIK BASEL
C9-5 ICT Gyn/Fert/VAG
25 Jan 96
13:58:07
Skala 6
Dynam 55dB
Persist mittl
BF mittl


UNI-FRAUENKLINIK BASEL
C4-2 40R OB/HERZ1
17:21:14
Bild #8813.7cm
Skala 5
Dynam 50dB
Persist niedr
BF hoch
2D-Opt:Tiefe

Triploidy

Partial Mole (Pat. Imprimting):
Large placenta with
partial molar degeneration
Syndactyly
Ventriculomegaly

High AFP
High HCG



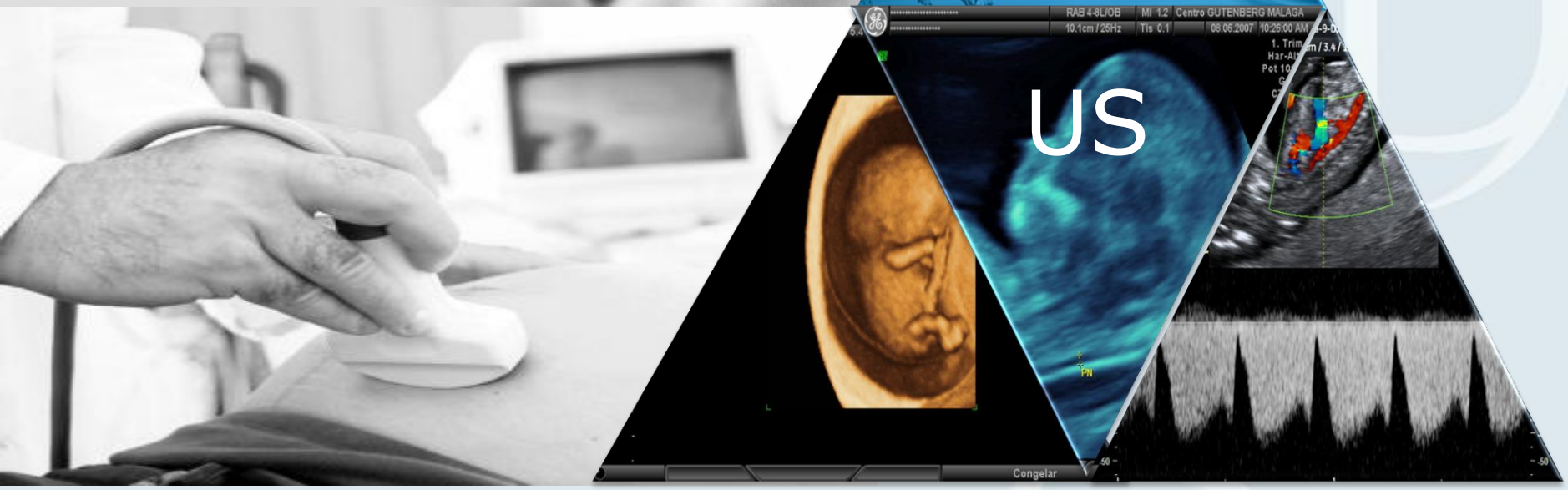
Non invasive prenatal testing for fetal aneuploidies

New pyramide of care?

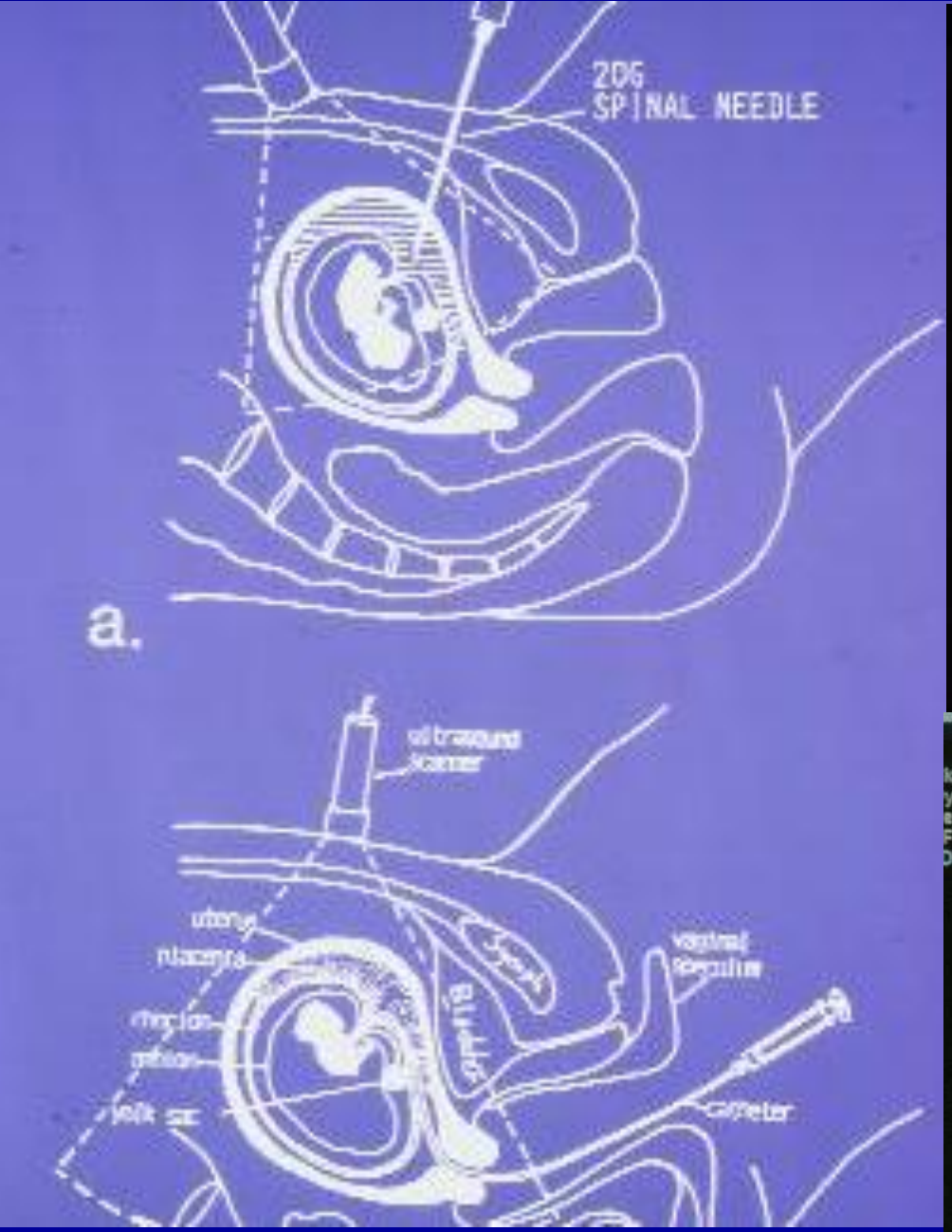
"The accuracy of NIPT obtained from the investigation of pregnancies at high risk for aneuploidies is applicable to the general population".

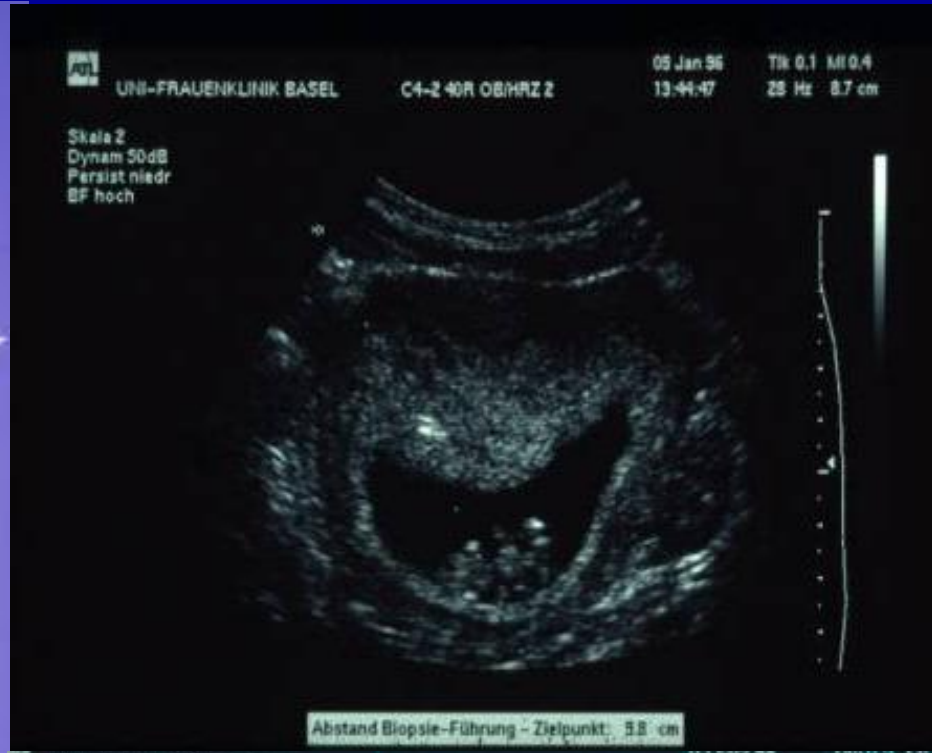
First experience in low-risk population

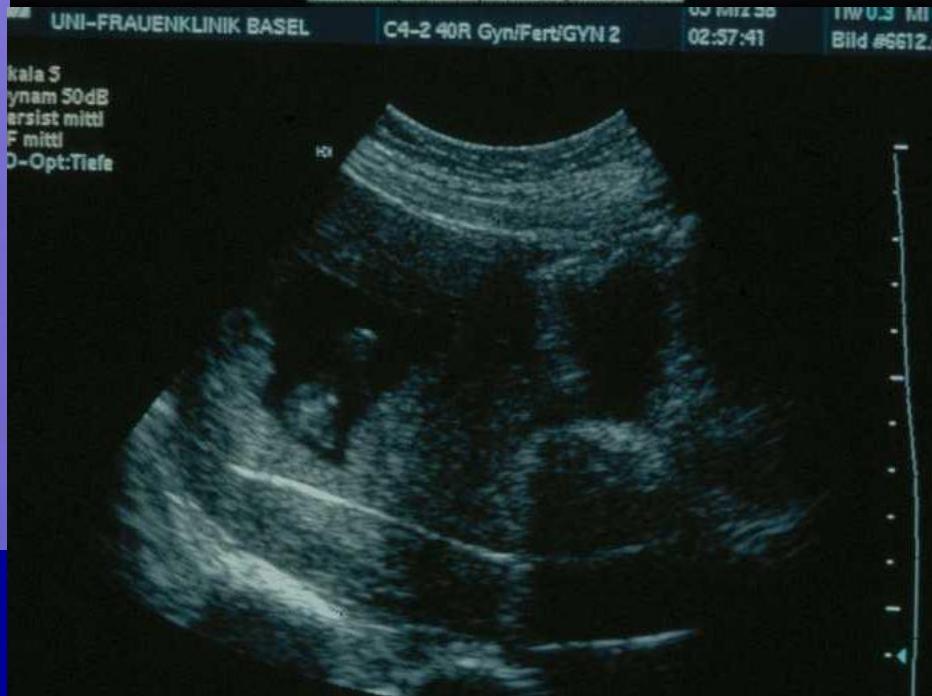
DR>99% FPR< 1%



Nicolaides KH, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1-6.







Procedure risk AC or CVS = 1%

CVS: Risks

Table 17 Total fetal loss rate in four randomized studies comparing first-trimester chorionic villus sampling with second-trimester amniocentesis

Study	*n*	*Chorionic villus sampling*	*Amniocentesis*
Canadian study[210]	2787	7.6%	7.1%
Danish study[211]	3079	6.3%	7.0%
Finnish study[212]	800	6.3%	6.4%
European study[213]	3248	14.0%*	9.0%

*$p < 0.01$

Procedure-Related Complications of Amniocentesis and Chorionic Villous Sampling

A Systematic Review

Faris Mujezinovic, MD, MSc, and Zarko Alfirevic, MD, FRCOG

OBJECTIVE: To compile a systematic review of complications related to genetic amniocentesis and chorionic villus sampling (CVS) to provide benchmark data for counseling and performance assessment of individual operators.

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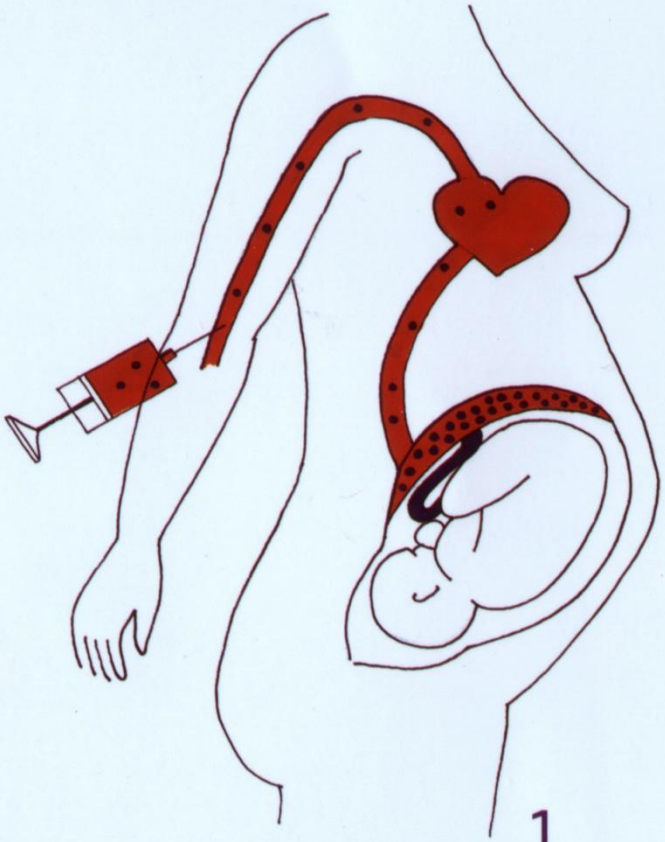
days was 0.6% (95% confidence interval [CI] 0.5–0.7), rising to 0.9% (95% CI 0.6–1.3) for pregnancy loss before 24 weeks and 1.9% (95% CI 1.4–2.5) for total pregnancy loss. Corresponding figures for CVS were before 28 weeks and total pregnancy loss were 1.46 (95% CI 0.86–2.49) and 1.25 (95% CI 1.02–1.53), respectively.

CONCLUSION: Although the risks of pregnancy loss are relatively low, lack of adequate controls tends to underestimate the true added risk of prenatal invasive procedures.

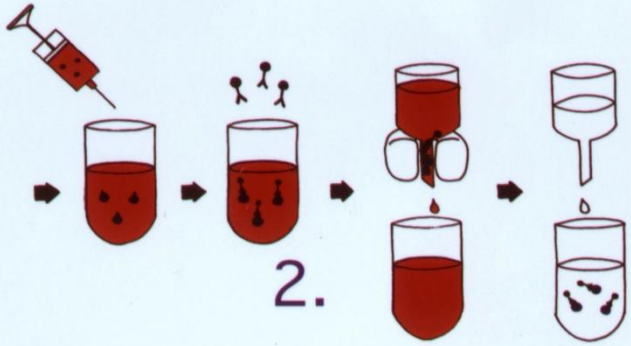
CONCLUSION: Although the risks of pregnancy loss are relatively low, lack of adequate controls tends to underestimate the true added risk of prenatal invasive procedures.

(Obstet Gynecol 2007;110:687–94)

ence of ultrasound markers of aneuploidy, or positive Down syndrome screening tests. On average, between 5% and 10% of pregnant women decide to



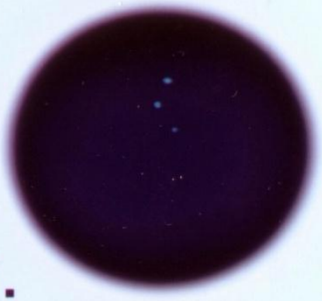
1.



2.

3.

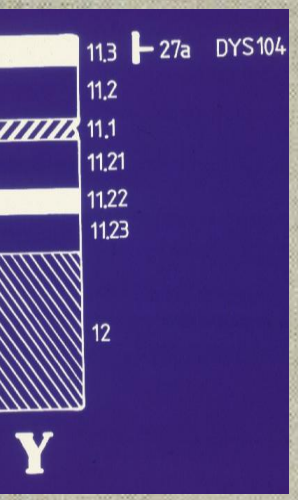
4.



5.

Result of quantifying studies: One of every 1 Mio. cells in maternal blood is fetal

Holzgreve et al., Lancet 335, 1220-1221, 1990



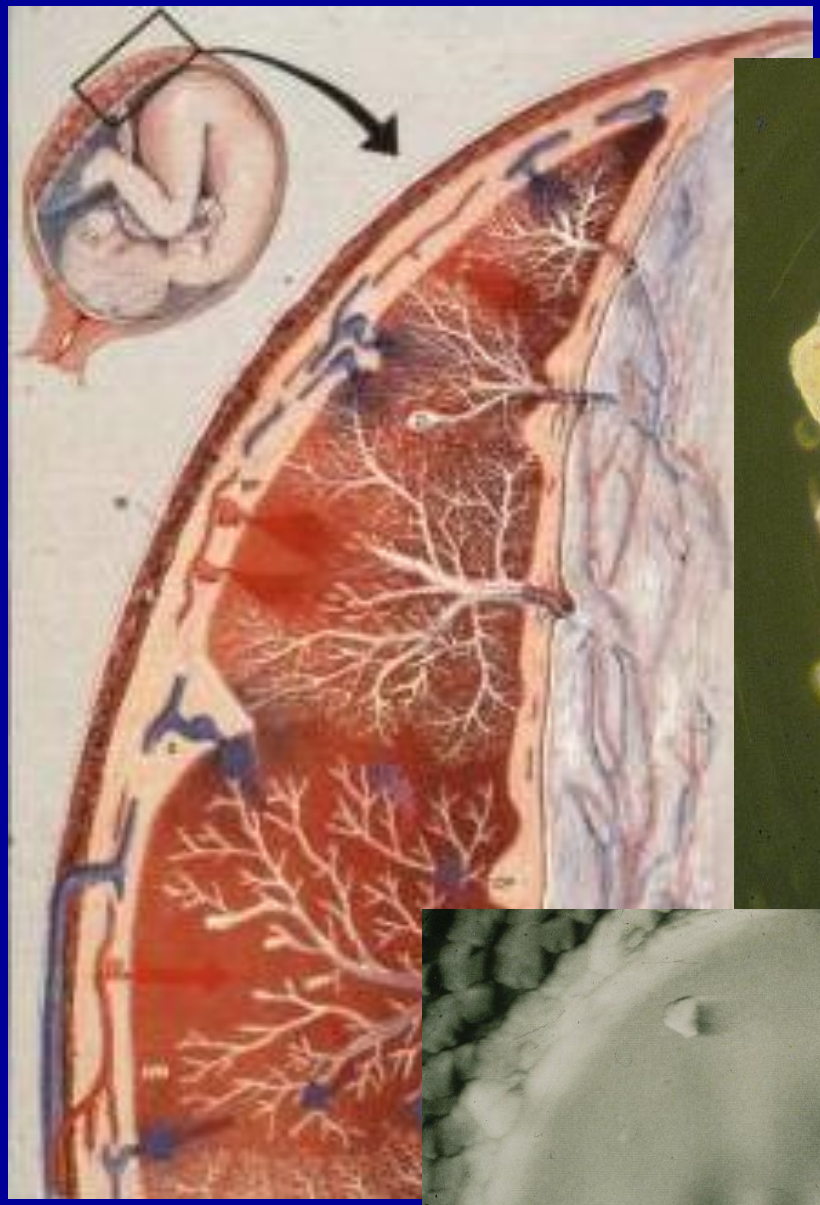
Autoradiogram of a Southern hybridisation with DNA probe 27A after amplification of 222 bp Y specific fragment.

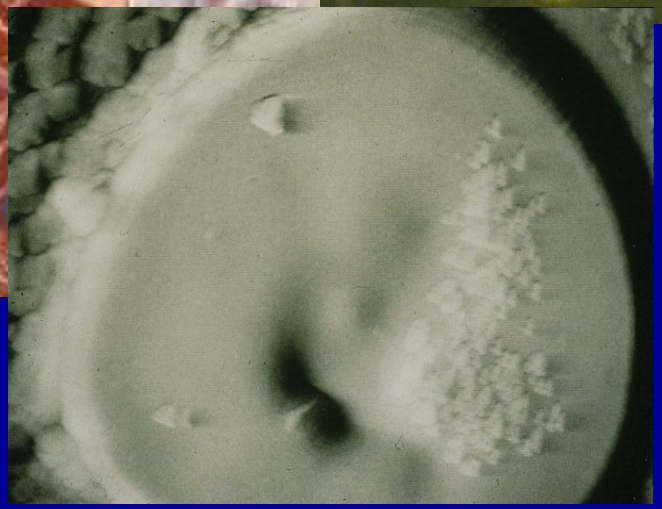
222 bp PCR product is indicated PCR products from no DNA; five controls (female DNA); 10^{-1} to 10^{-6} dilutions of 1 µg female DNA with corresponding amounts of admixed male DNA, and one male DNA.

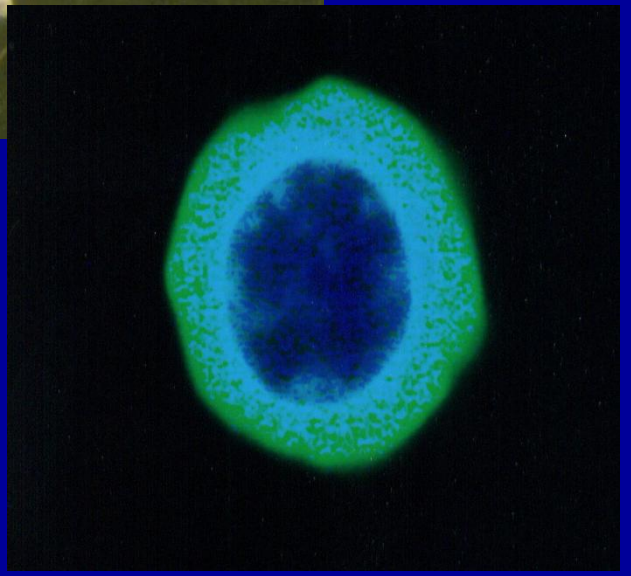
metimes it is worthwhile to stick to a topic in research formore than 20 years…

Birth of
Luise Joy
Brown
July 1978

Report of
R. Edwards
und P. Steptoe
in The Lancet







THE LANCET Vol 343 • April 23, 1994

Gänshirt D, Garritsen H, Miny P, Holzgreve W.

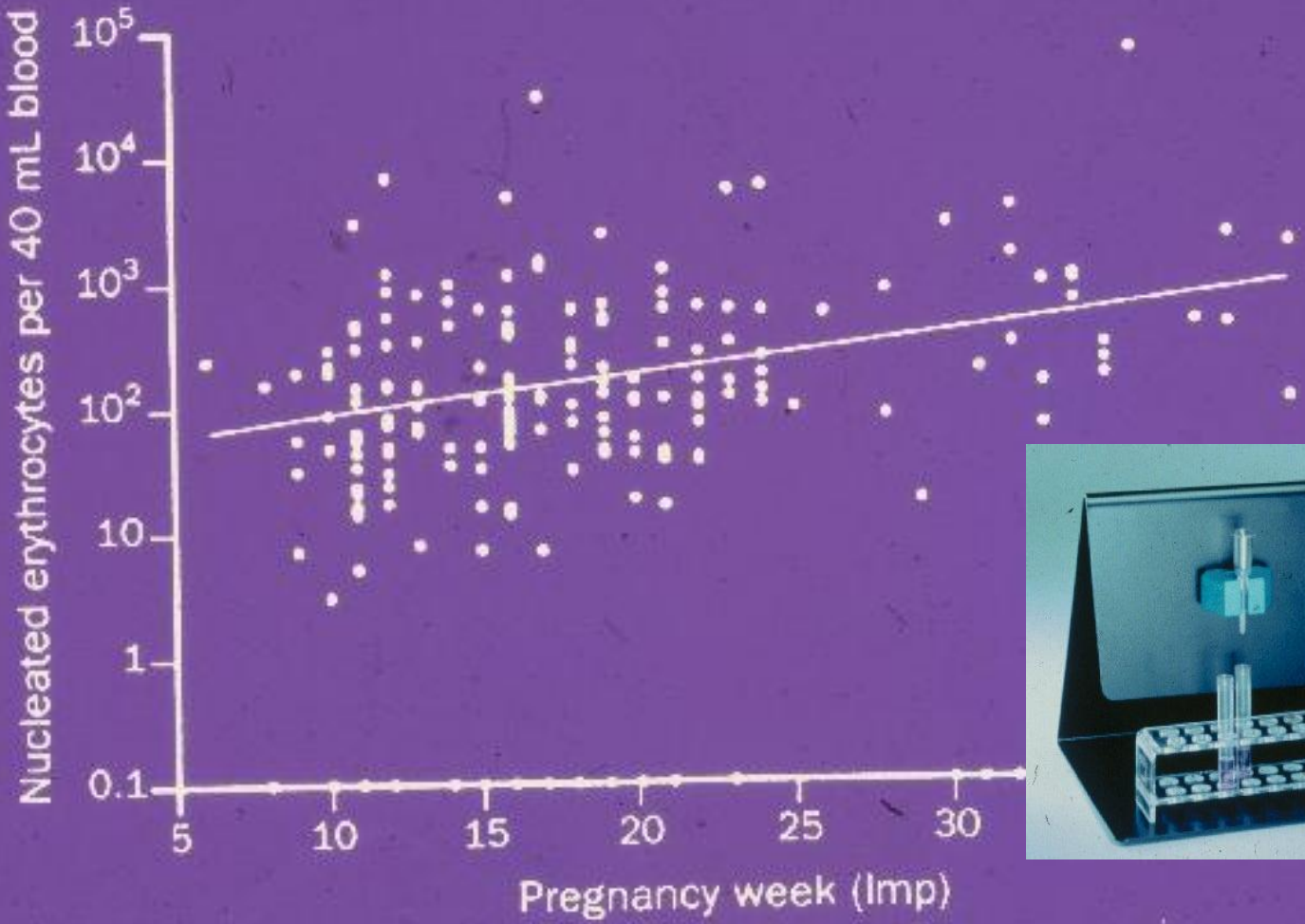


Figure: **Absolute enrichment of nucleated erythrocytes from venous maternal blood at different gestational ages (n = 219)**

Lmp = last menstrual period.

S. Hahn, R. Sant and W. Holzgreve, Molec.
Human Reproduction 4 (6), 515-521, 1998

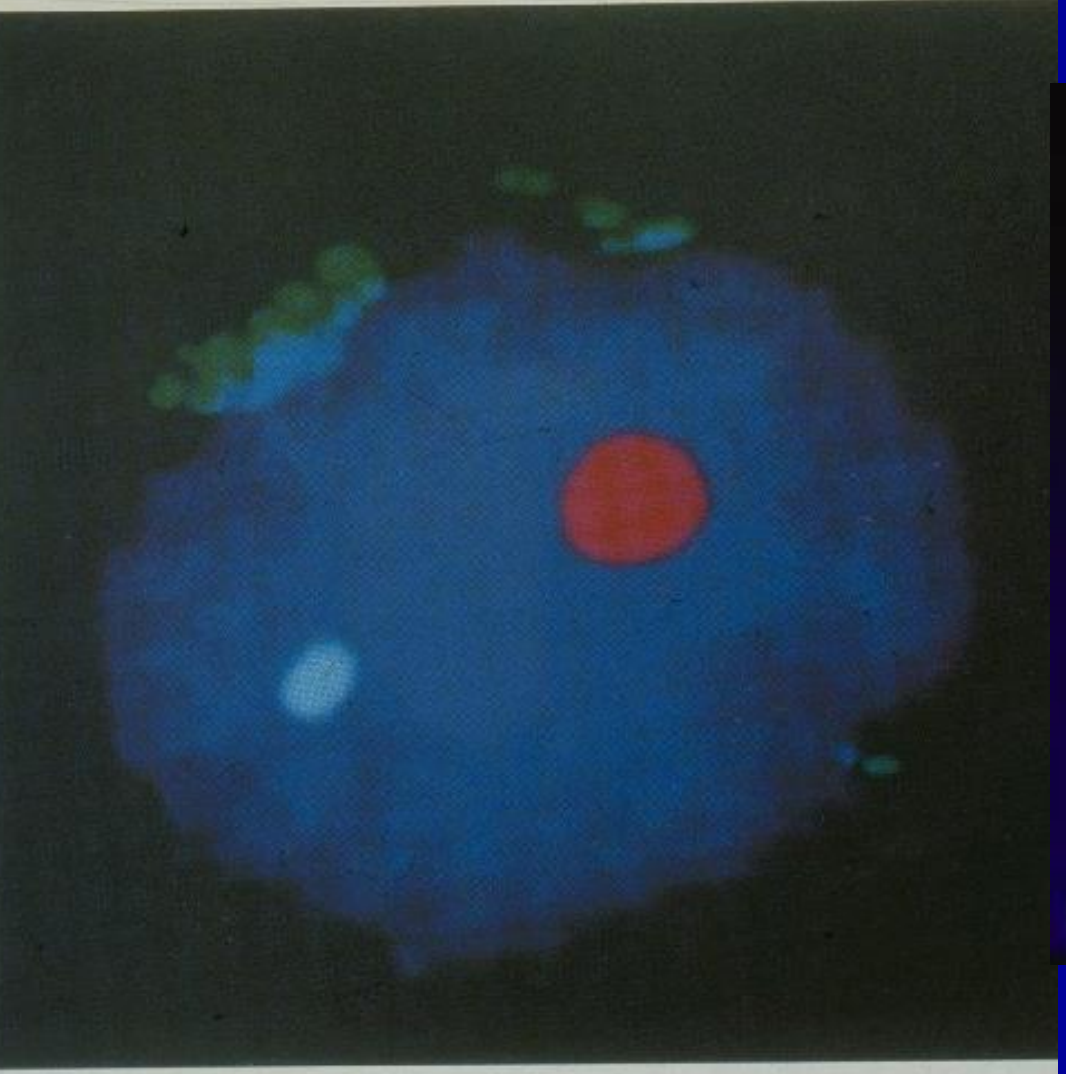
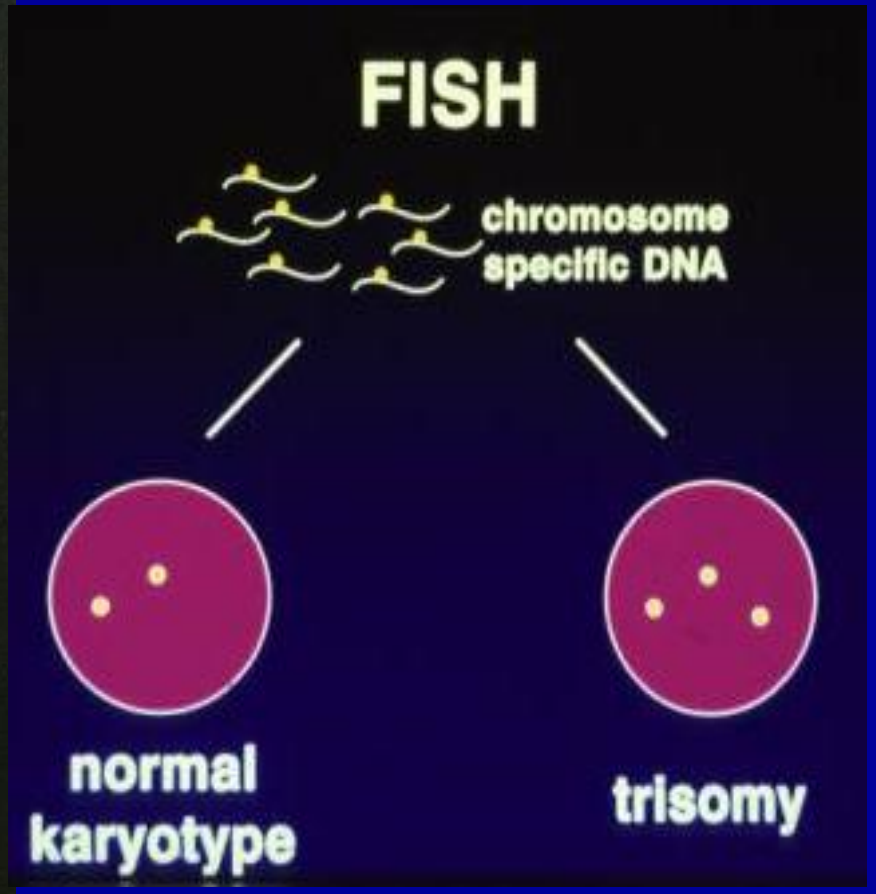


Figure 1. Male fetal cell identified by immunohistochemical staining for fluorescein-conjugated anti-fetal haemoglobin (Europa, Cambridge, UK) and simultaneous fluorescent in-situ hybridization for X (pale blue) and Y (pink) chromosomes (Genzyme Genetics, Framingham, MA, USA), following enrichment with anti-CD71 and



Nachweis von kindlichen Trisomien nicht-invasiv aus Blut der Schwangeren

NICHD NIFTY Study
1998 - 2003

- NIFTY - National Institute for Child Health and Development Fetal Cell Isolation Study.
- Large scale study (3000) cases to determine efficacy of fetal cells for detection of fetal aneuploidies.
- Comparison of FACS and MACS

- All lab results were compared to AC / CVS

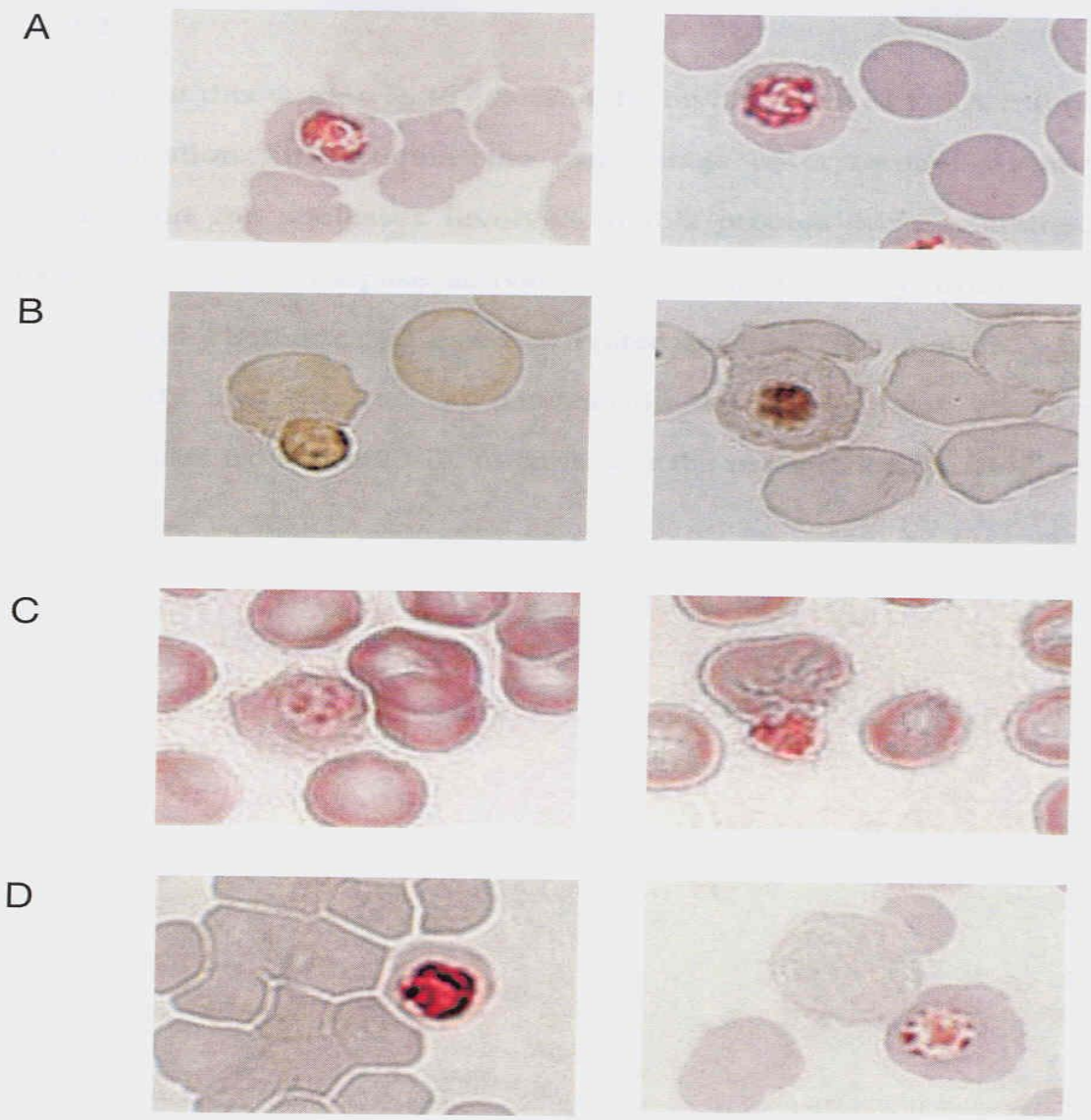
Intact fetal cells in maternal plasma: are they really there?

Farideh Z Bischoff, Sinuhe Hahn, Kirby L Johnson, Joe Leigh Simpson, Diana W Bianchi, Dorothy E Lewis, William D Weber, Katherine Klinger, Sherman Elias, Laird G Jackson, Mark I Evans, Wolfgang Holzgreve, Felix de la Cruz

Rare fetal cells can be recovered from maternal blood, which suggests that non-invasive prenatal diagnosis is possible. However, recovery and analysis of fetal cells from blood is complex, and sensitivity is low because of the rarity of these cells in the maternal circulation. An alternative strategy, which suggested that intact fetal cells can be found in maternal plasma by use of simple enrichment methods, has been reported. We aimed to replicate this technique. However, five independent laboratories were unable to identify any intact male cells from the plasma of 38 women known to be carrying male fetuses. Although apoptotic intact fetal cells could contribute to the detection of fetal DNA in maternal plasma, we believe that recovery of these cells is difficult and not clinically practical.

Bishoff, F.Z. et al., Lancet 361, 139 - 40, 2003


3



Babochkina T et al 2004.

Figure 5. **DNA fragmentation in late erythroblasts**

A - TUNEL-positive NRBCs in fetal blood; B – ISOL (In situ Oligo Ligation assay)–positive NRBCs in cord blood, note the ISOL-positive enucleating NRBC; C - TUNEL-positive NRBCs in bone marrow

D - TUNEL-positive/ Annexin V-negative NRBCs in maternal blood

Elevation in erythroblast count in maternal blood before the onset of preeclampsia

Wolfgang Holzgreve, MD,[a] Jin Chun Li,[a] Andrea Steinborn, PhD,[b] Thomas Külz, MD,[c] Christof Sohn, MD,[b] Markus Hodel, MD,[a] and Sinuhe Hahn, PhD[a]

Basel, Switzerland, and Rostock and Frankfurt, Germany

OBJECTIVE: We recently showed that both maternal and fetal erythroblast counts are elevated in the peripheral blood of pregnant women with preeclampsia. The purpose of this study was to examine whether this elevation actually occurs before the clinical onset of the disorder.

STUDY DESIGN: Erythroblasts were enriched and enumerated in 97 maternal blood samples obtained in the second trimester, and results were subsequently correlated with pregnancy outcomes.

RESULTS: Significantly higher quantities of erythroblasts (mean, 6041.7 vs 928.9; $P = .008$) were detected in blood samples obtained from women who later acquired preeclampsia (n = 15) than in blood samples from the control cohort (n = 72). Intrauterine growth restriction (n = 10) was not accompanied by a similar rise in erythroblast count.

CONCLUSION: Because a large proportion of the erythroblasts in maternal blood are fetal, our data suggest that fetal-maternal cell traffic is affected early in pregnancies that are later complicated by preeclampsia but not in those affected only by intrauterine growth restriction. (Am J Obstet Gynecol 2001;184:165-8.)

Key words: Erythroblasts, fetal cell traffic, preeclampsia,

Cells Exchanged During Pregnancy Live On

Microchimerism, viewed at first as an oddity, has been linked to autoimmune diseases and complications of pregnancy

A mother's love is enduring. But most mothers would be surprised to discover that there's a similarly enduring physical bond: Cells from a fetus can live on in the mother's body for decades after pregnancy, a situation called microchimerism. Likewise, a mother's cells can also survive for many years in her child.

When this phenomenon was first reported in the mid-1990s, scientists scoffed at the notion that these cells could persist for so long, tolerated by their host's immune system. "Everyone said it can't be true," says rheumatologist Michael Lockshin, director of the Barbara Volcker Center for Women and Rheumatic Disease at the Hospital for Special Surgery in New York City. "But now everyone who looks finds it."

In some cases, the cells might be benign guests: self-perpetuating lines of stem cells that can reproduce and even give rise to other types of cells, all without harming their host. But a growing body of research, still preliminary, suggests that the cells might also be at the root of some autoimmune diseases and other conditions.

Indeed, microchimerism might help explain one of the puzzles about autoimmune diseases: why many of them strike more women than men. No one knows how many women carry foreign cells around from past pregnancies, but several studies have shown that women with certain autoimmune diseases are more likely to harbor such cells than healthy women. "When you see that this is a real phenomenon, it gives you a different perspective," says pediatric hematologist William Reed of the Children's Hospital Research Institute in Oakland, California. "You begin to ask yourself whether a disease might have a pathogenesis that you've never considered before."

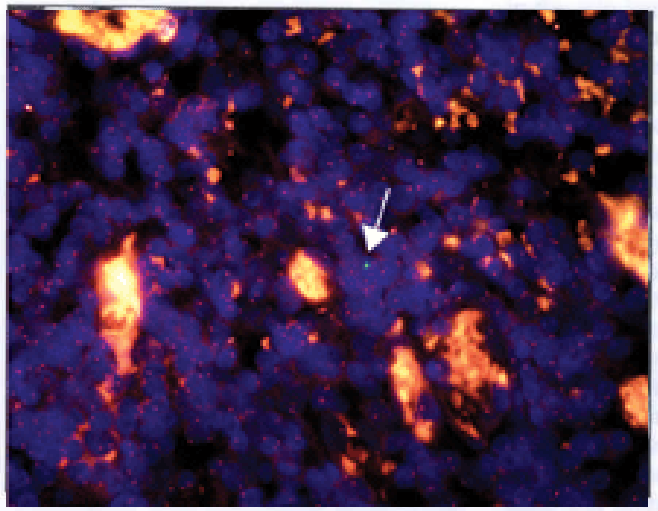
And it's not only the long-lived cells that might be making mischief. Reproductive biologists have known for some time that fetal cells course through the bloodstream of pregnant women, but in the past 4 years researchers have discovered that this temporary invasion might be implicated in two common complications of pregnancy.

Inner turmoil

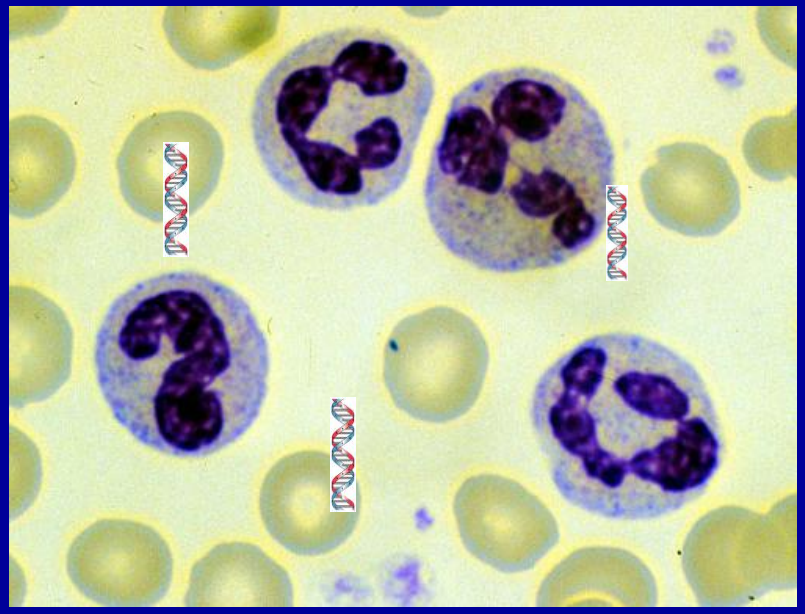
Fetal microchimerism was uncovered quite by chance. In 1992, medical geneticist Diana

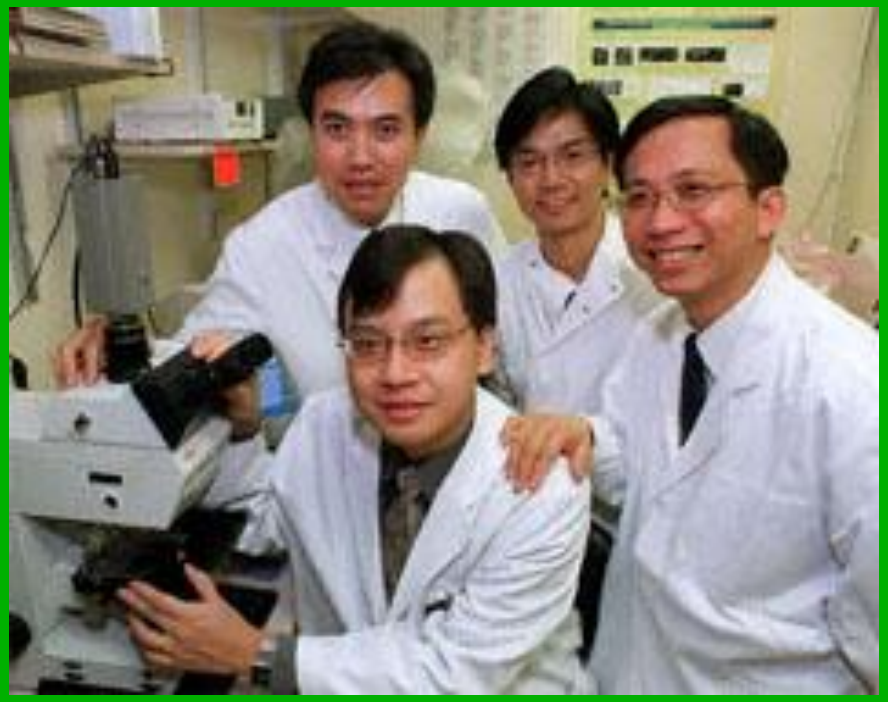
Boston, was tryi
prenatal diagnos
tal cells from the
Her team was s
ried a protein kn
so-called hemato
rise to cells of t
on a hunch tha
marker for fetal

Blood from
they studied con
with a Y chrom
fetuses from w
male. But amnio
nine of those w
fetuses. "We wer
who is now at Tu
Center in Boste
whether any of t
plained male ce
were male, and
two had previou
in which the s
known. "That is
to take shape," s



Under mom's skin. A cell with a green-stained Y chromosome, presumably from a son, was found in a skin biopsy from a woman with systematic sclerosis.

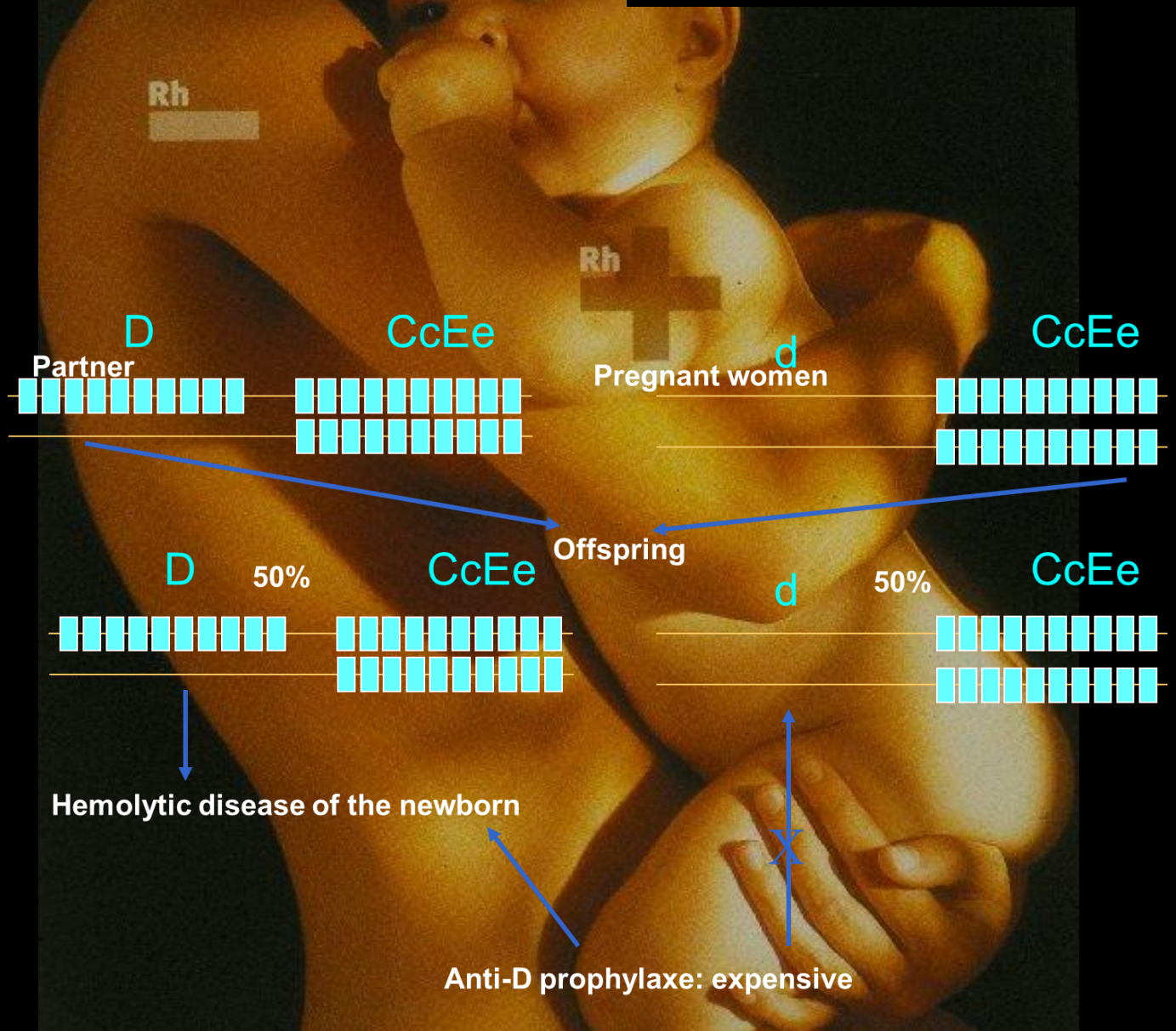




Lo YMD et al., Lancet 1997
Presence of fetal DNA in maternal plasma / serum

Example: 1. NIPD

Rhesus- Faktor



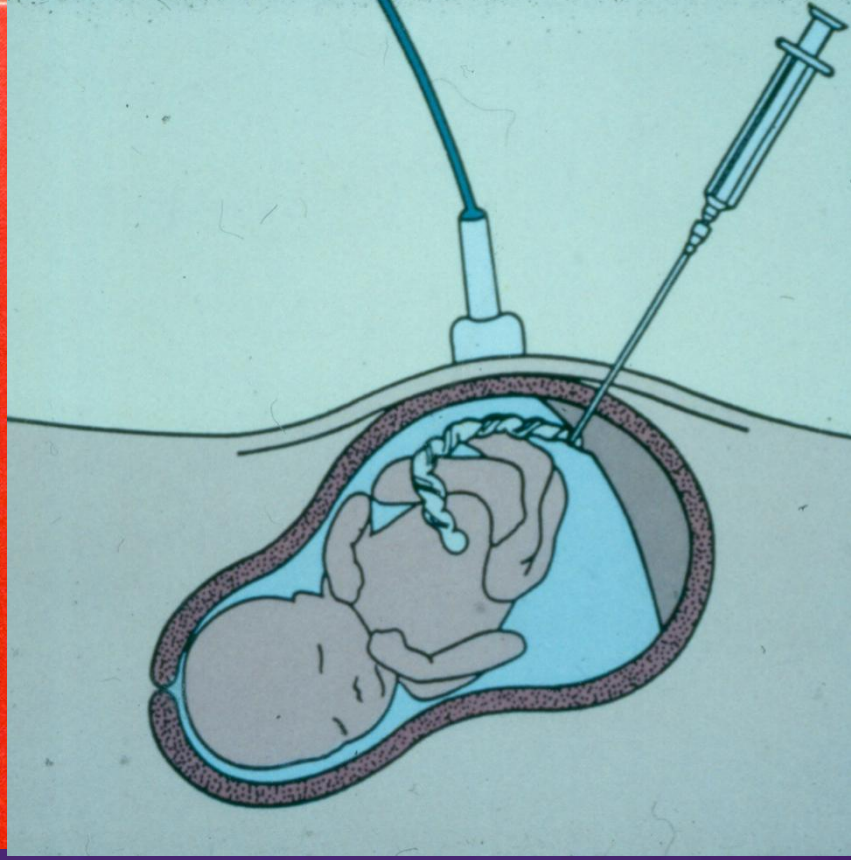
HARRISON

EVANS
ADZICK
HOLZGREVE

THE UNBORN PATIENT

The Art and Science of Fetal Therapy

THIRD EDITION



...antenatal diagnosis has aquired in the minds of some the sinister image of a "search and destroy" mission.

Sir William Liley, Auckland 1983

Vorwort zum Buch "The Unborn Patient"
Herausgegeben von M. Harrison, M. Golbus,
R. Filly, San Francisco.

RhD and Sex using fetal DNA

	Gestational Age	Sensitivity	Specificity
***PCR for SRY*:** (n=52)	11.5-34.6	94% (n=185)	100%
***PCR for RhD*:** (n=9)	13-17	96% (n=25)	100%
***SRY/RhD*:** (n=7)	13-17	92.6% (n=27)	100%

* Zhong XYZ,, Holzgreve W, Hahn S Lancet, 357, 310, 2001

E. Gautier, A. Benachi, Y. Giovangrandi, P. Ernault, M. Olivi, T. Gaillon, J-M. Costa:

Fetal RhD genotyping by maternal serum analysis: A two-year experience
AJOG 192, 666-9, 2005

285 pregnant women
283 with a complete gene deletion

0 false - positive
or
false - negative results

2013: > 25 000 Cases, Accuracy 99,9%

SensiGene® Fetal RHD Genotyping

universitäts klinikum bonn

A noninvasive prenatal blood test for the direct detection of fetal RHD genotype in RhD (-) mothers

About the Test

The SensiGene Fetal RHD Genotyping test, developed and validated by Sequenom CMM, is a laboratory-developed test (LDT) that analyzes circulating cell-free fetal DNA extracted from a maternal blood sample. The test detects fetal RHD status as early as the first trimester.

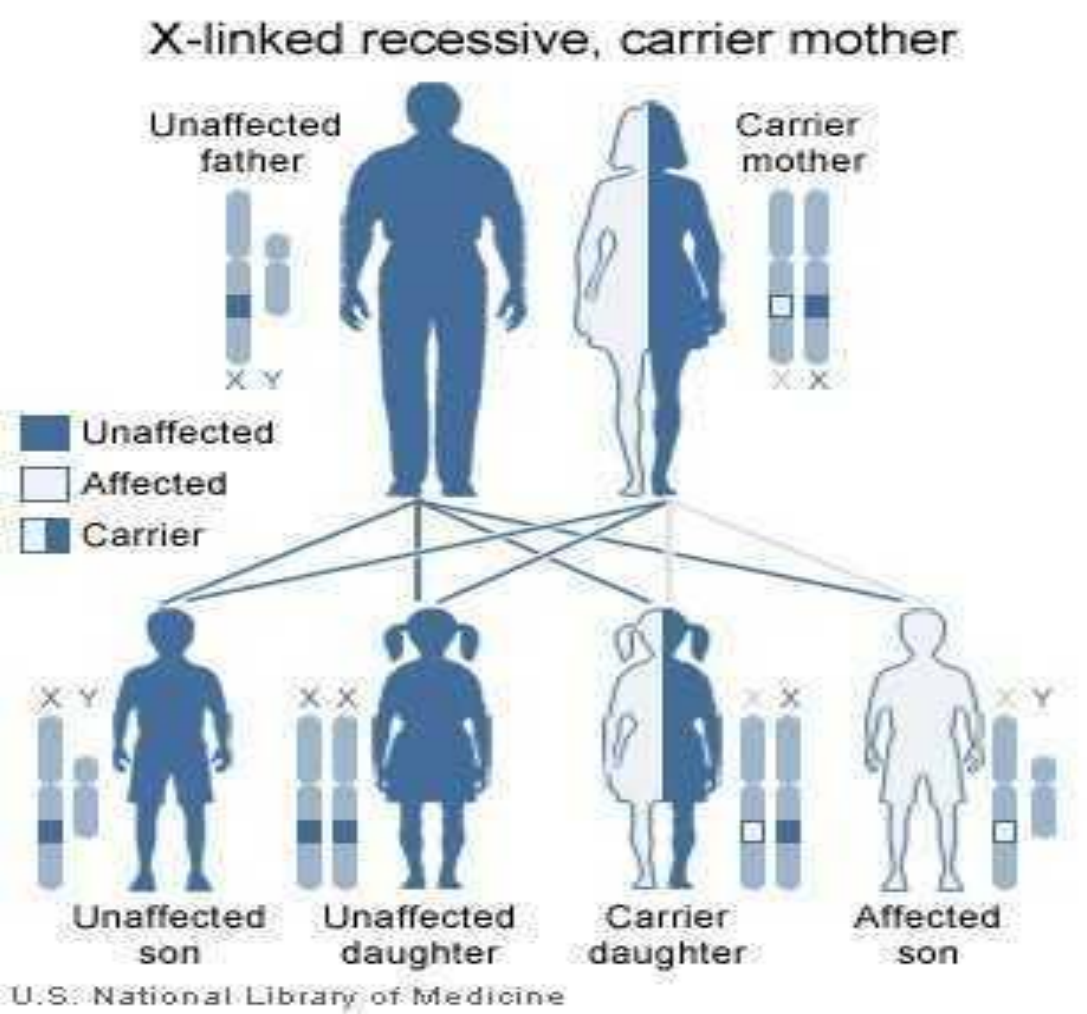
Why Use this Test?

- ✓ Direct determination of fetal RHD status without testing the father
- ✓ Fetal RHD status when maternal anti-D titers are unclear
- ✓ Identify RHD (+) fetus in RhD (-) mother
- ✓ RhD (-) sensitized patients

Unique, Proven Circulating Cell-Free Fetal (ccff) Nucleic Acid Technology

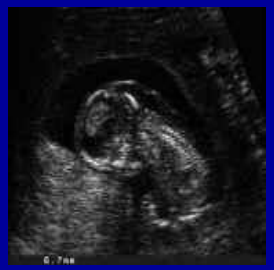
- Ccf-fetal DNA in maternal plasma is thought to derive from apoptosis of placental and fetal cells and possibly through the breakdown of fetal cells in circulation[1,3,4]
- Recent studies have shown >10% circulating fetal DNA concentration in maternal plasma[5]
- The ccff technology analyzes circulating cell-free fetal DNA from a maternal blood sample
- Sequenom holds an exclusive platform-independent license for fetal nucleic acid detection in serum and plasma, branded under the name SEQureDx® technology

Highly Sensitive. Noninvasive.

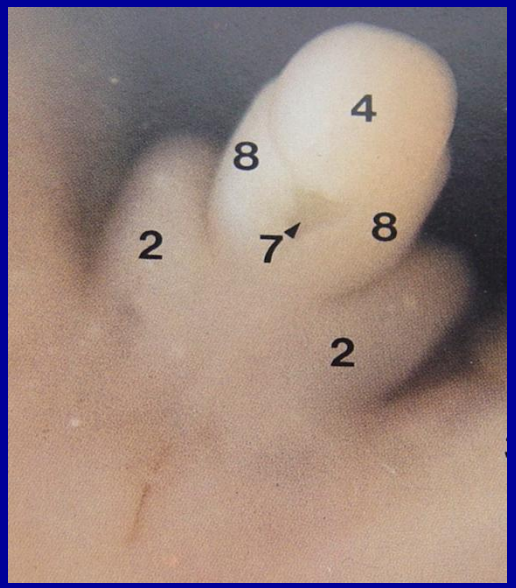


X-link disorders, e.g. Muskeldystrophy, Hemophilia A/B, Immunodeficiency, Ichthyosis etc

Non invasive sex assessment

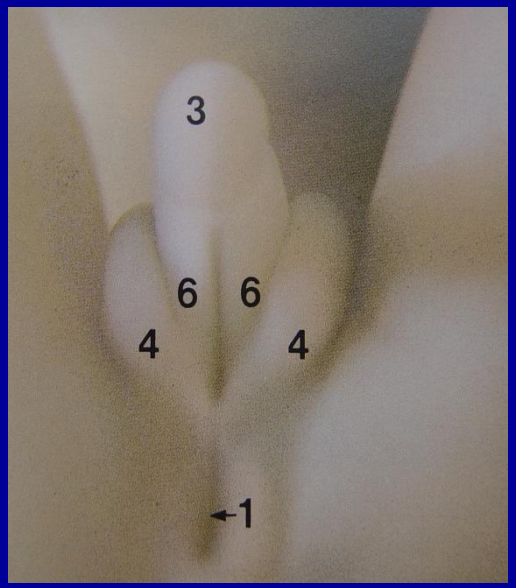


- **Ultrasound**



CRL =45mm

15 weeks



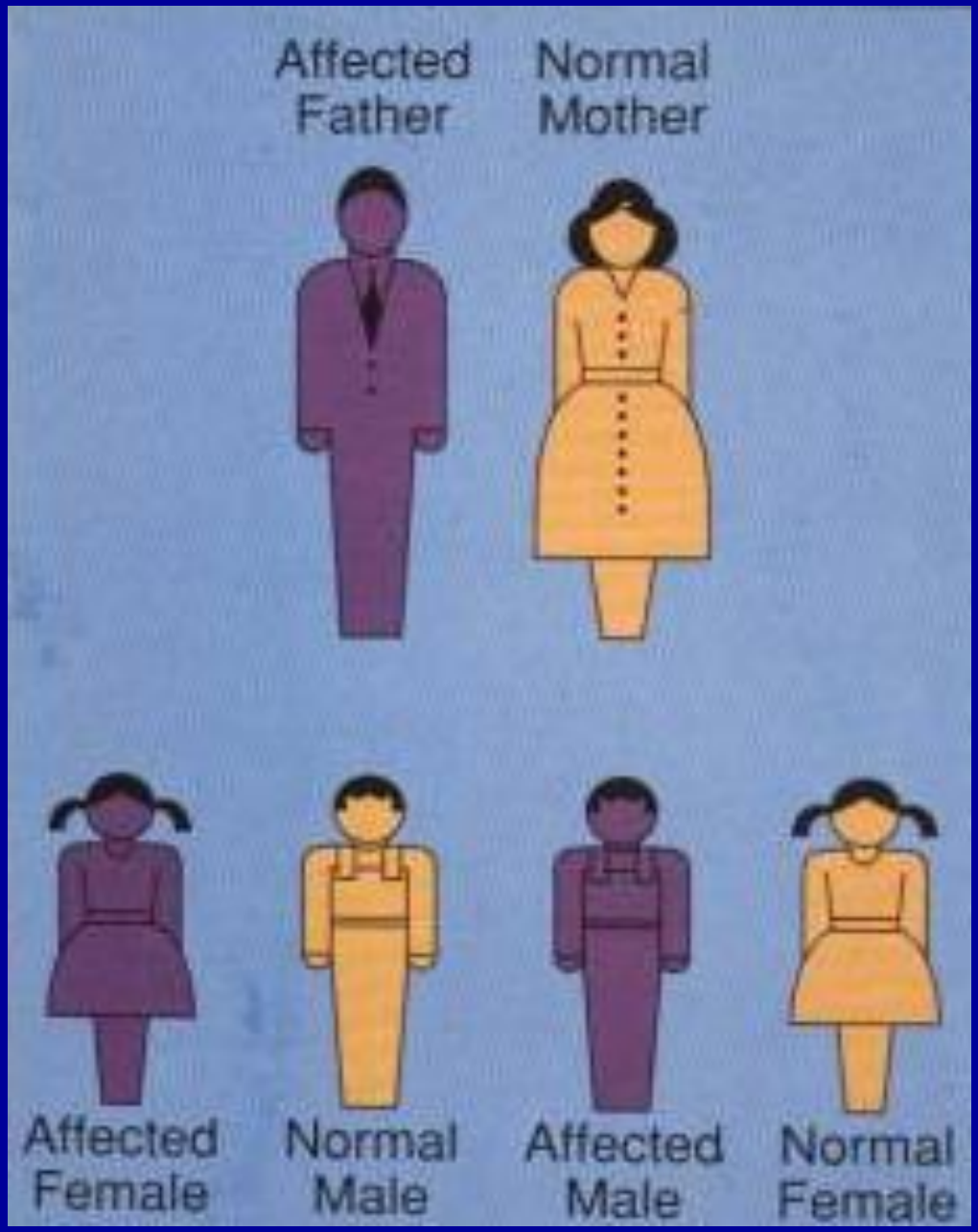
acc. To M.A. England

Fetal sex determination from maternal blood: Amer. Hosp. Paris (JM Costa et al.) 2002-2006

1851 determinations (1816 cases)

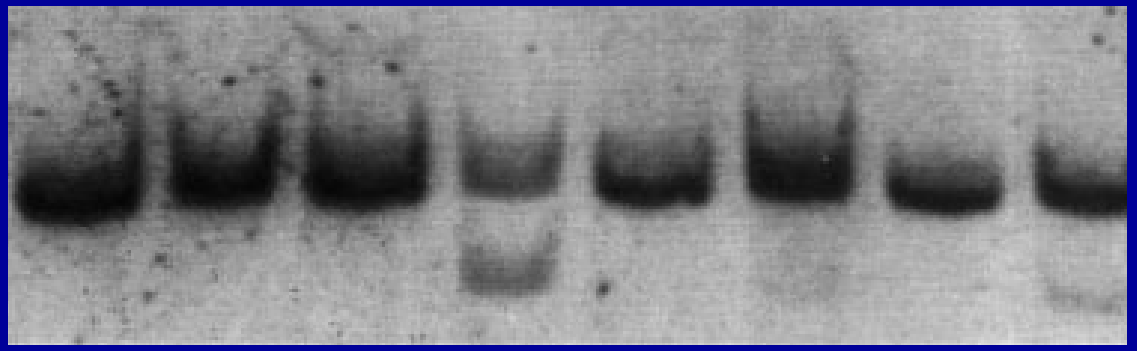
(gestational age 11.4SA, range: 5-40)

Syndromes	n
•Duchenne/Becker/ muscular dystrophy	549
•Hemophilia A and B	278
•X-linked mental retardation	89
•X-linked hydrocephalus	55
•Adrenoleucodystrophy	53
•Hunter	41
•Myotubular myopathy	38
•Menke's disease	28
•Granulomatous disease	23
•Retinitis pigmentosa	22
•Ectodermic anhydrotic dysplasia	19
•Lesch-Nyhan syndrome	17

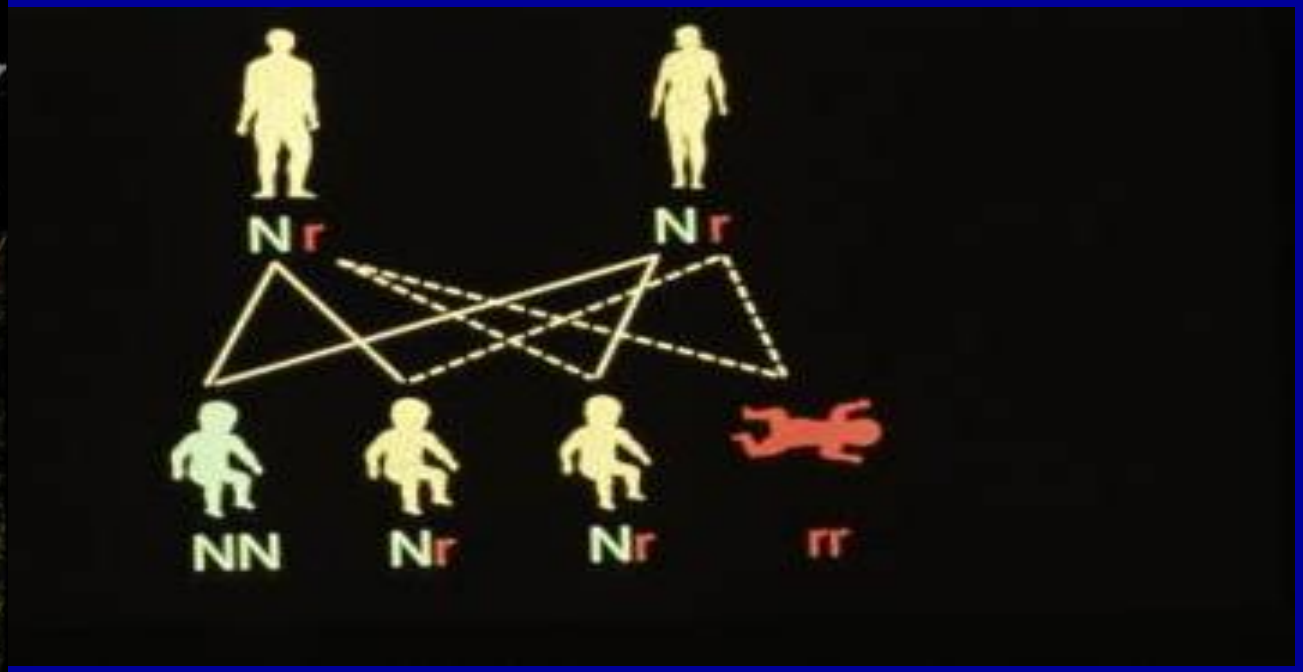

Affected Father
Normal Mother
Affected Female
Normal Male
Affected Male
Normal Female

Achondroplasia (ACH): G1138A mutation in FGFR3 gene

Li Y., Holzgreve W., Page-Christiaens G.C.M., et al., Prenat Diagn. 24, 896-8, 2004



Autosomal dominant disease: Myotonic dystrpphy, Marfan-syndrom, Polycystic kidney, Maligne Hyperthermie (M), Ehlers-Danlos-syndrom (B), M.....



Autosomal recessive mode of inheritance

Either both parents have the same mutation

or they have different mutations for the same disease =

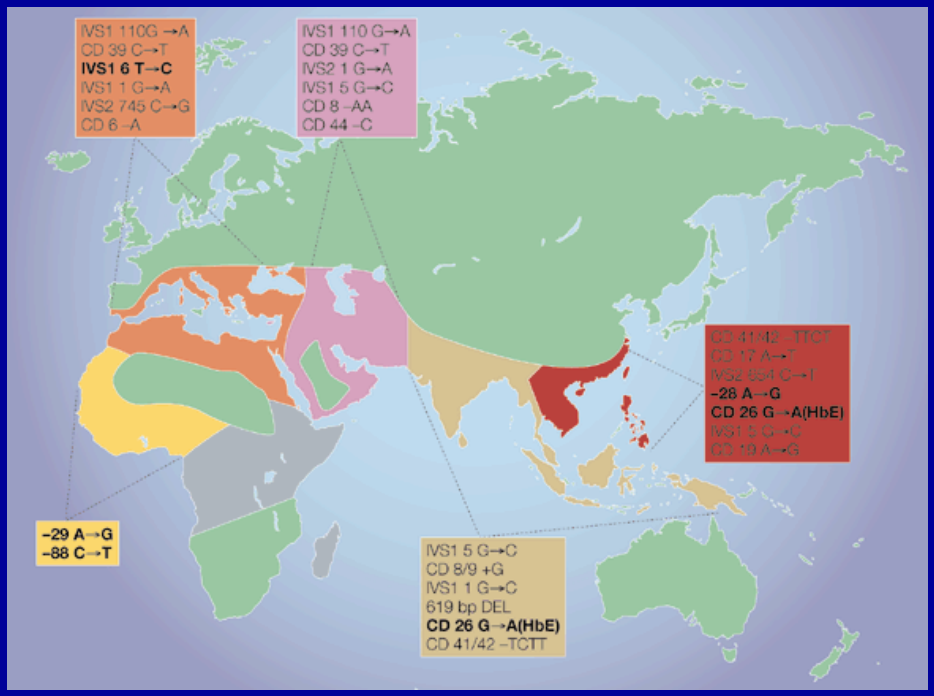
At risk to be „compound“ heterozygously affected

Hemoglobinopathies: b-Thalassemia

Caused by ß-globin gene mutations, > 500 mutations

At least 150,000 lethally affected homozygoutes of thalassemia are born annually

Weatherall DJ, Nature Reviews-Genetics 2002



Carrier couples frequently have different mutations

Therefore, detection of paternal mutation can aid in determining whether fetus will be affected or not,

“Compound heterozygously affected”

PRELIMINARY COMMUNICATION

Detection of Paternally Inherited Fetal Point Mutations for β-Thalassemia Using Size-Fractionated Cell-Free DNA in Maternal Plasma

Ying Li, PhD
Edoardo Di Naro, MD
Angeloantonio Vitucci, MD
Bernhard Zimmermann, PhD
Wolfgang Holzgreve, MD
Sinuhe Hahn, PhD

Context Currently, fetal point mutations cannot be reliably analyzed from circulatory fetal DNA in maternal plasma, due to the predominance of maternal DNA sequences. However, analysis of circulatory fetal DNA sequences in maternal plasma have been shown to selectively enrich for fetal DNA molecules on the basis of a smaller molecular size than maternal DNA.

Objective To examine the prenatal analysis of 4 common β-thalassemia point mutations: *IVSI*-1, *IVSI*-6, *IVSI*-110, and codon 39.

Design, Setting, and Patients A total of 32 maternal blood samples were collected at 10 to 12 weeks of gestation (mean, 10.7 weeks) between February 15, 2003, and February 25, 2004, in Bari, Italy, from women with risk for β-thalassemia in their newborns immediately prior to chorionic villous sampling. Samples in which the father and mother did not carry the same mutation were examined. Circulatory DNA was size-fractionated by gel electrophoresis and polymerase chain reaction (PCR) amplified with a peptide-nucleic-acid clamp, which suppresses amplification of the normal maternal allele. Presence of the paternal mutant allele was detected by allele-specific real-time PCR.

Main Outcome Measure Detection of paternally inherited β-globin gene point mutations.

Results Presence or absence of the paternal mutant allele was correctly determined in 6 (86%) of 7 cases with the *IVSI*-1 mutation, 4 (100%) of 4 with the *IVSI*-6 mutation, 5 (100%) of 5 with the *IVSI*-110 mutation, and 13 (81%) of 16 with the codon 39 mutation. One false-positive test result was scored for the *IVSI*-1 mutation. Two cases with the codon 39 mutation were classified as uncertain and 1 case was excluded due to lack of a diagnostic test result at the time of analysis. These results yielded an overall sensitivity of 100% and specificity of 93.8%, with classified cases removed.

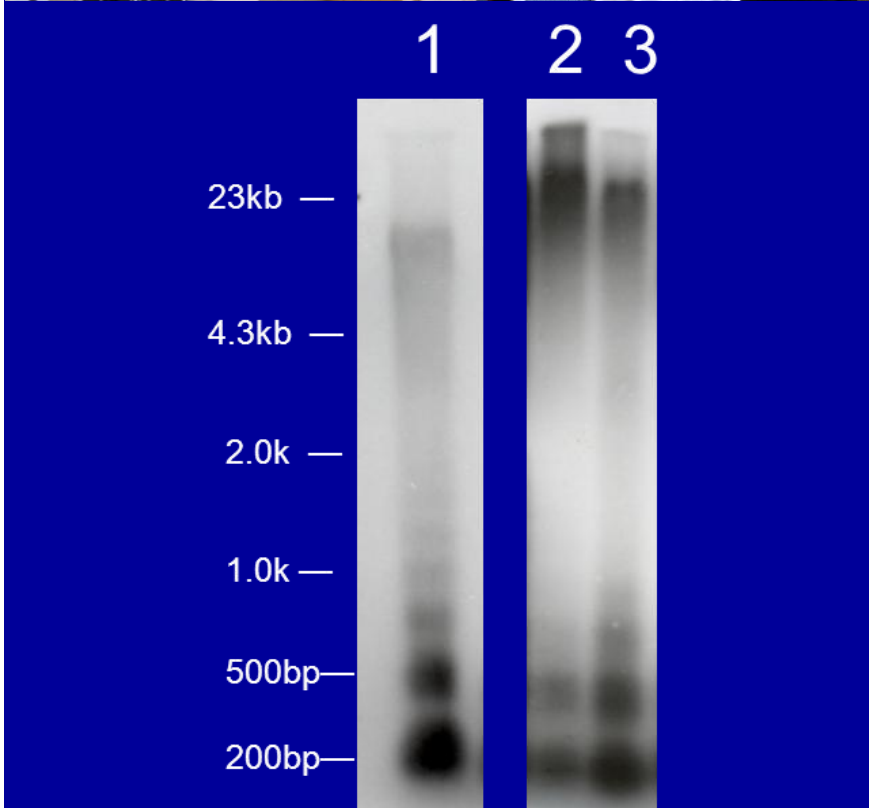
Conclusion Our recently described technique of the size-fractionation of circulatory DNA in maternal plasma may be potentially useful for the noninvasive prenatal determination of fetal point mutations.

JAMA. 2005;293:843-849 www.jama.com

MONOGENIC DISORDERS frequently involve point mutations. This single nucleotide exchange makes the analysis of point mutations more complex as stringent assays need to be established that permit a clear distinction between normal and mutant alleles. The prenatal diagnosis of this multitude of hereditary genetic disorders currently relies on invasive procedures,[1] such as amniocentesis or chorionic villous sampling, which are associated with a small but significant risk of fetal loss.[2,3] To avoid this procedure-related risk, several strategies have been considered for noninvasive assessment of fetal genetic traits, including the isolation of rare fetal cells from the maternal circulation and the analysis of circulatory fetal DNA in maternal plasma.[1,4-6]

Although proof-of-principle studies have indicated that the analysis of isolated fetal cells by single-cell polymerase chain reaction (PCR) can be used for the noninvasive prenatal diagnosis of hemoglobinopathies,[7,8] this strategy is too complex, labor intensive, and not sufficiently efficient for routine clinical settings. The analysis of fetal genetic traits by the analysis of cell-free fetal DNA in maternal plasma has proven to be remarkably reliable for the assessment of fetal loci absent from the maternal genome, such as Y-chromosome–specific sequences or the RhD gene in pregnant women who are Rh-negative, especially in European medical centers.[1,4]

Author Affiliations: Laboratory for Prenatal Medicine, University Women's Hospital/Department of Research, University Hospital, Basel, Switzerland (Drs Li, Zimmermann, Holzgreve, and Hahn); and Department of Obstetrics and Gynecology (Dr Di Naro) and Division of Hematology II (Dr Vitucci), University of Bari, Bari, Italy.
Corresponding Author: Sinuhe Hahn, PhD, Laboratory for Prenatal Medicine, University Women's Hospital/Department of Research, Spitalstrasse 21, CH 4031 Basel, Switzerland (shahn@uhbs.ch).



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Arnold W. Cohen, M.D.

Arnold W. Cohen, M.D. trained in Ob/Gyn and MFM at the Hospital of the University of Pennsylvania. He has been Chairman, Division of Maternal-Fetal Medicine at Albert Einstein Medical Center, Director of Obstetrics and Maternal- Fetal Medicine at the Hospital of the University of Pennsylvania and now Chairman of Ob/Gyn at Albert Einstein Medical Center.
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Wolfgang Holzgreve, M.D.

Dr. Holzgreve graduated from the School of Medicine, the Westfälische Wilhelm University, Münster, Germany in 1975. In 1976, he earned the degree of Master of Science in Health and Medical Sciences at the University of California, Berkeley.
Read more...

Dennis Lo, DM DPhil

Dennis Lo, DM DPhil is the Li Ka Shing Professor of Medicine and Professor of Chemical Pathology of the Chinese University of Hong Kong. He received his BA from the University of Cambridge and his DM and DPhil degrees from the University of Oxford. He discovered the presence of cell-free fetal DNA in maternal plasma in 1997.
Read more...

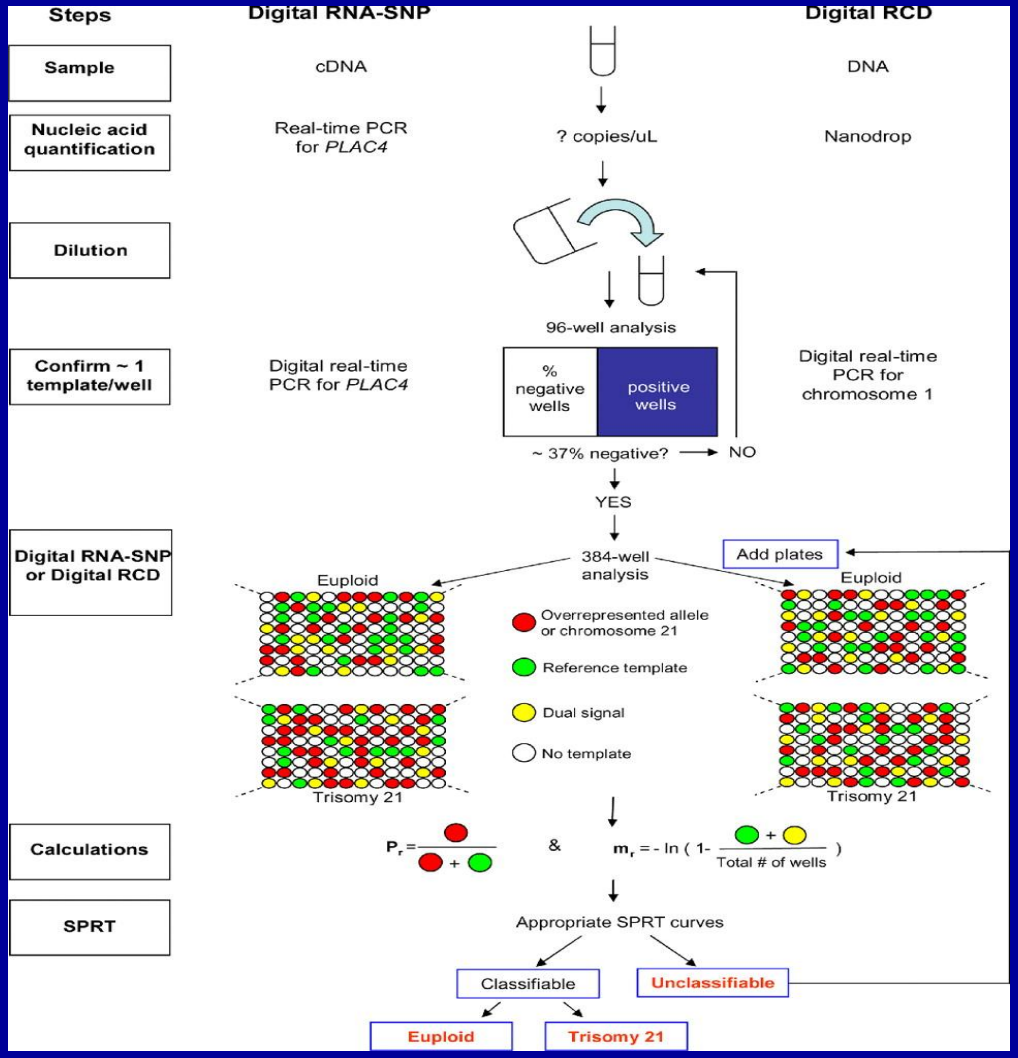
Lee P. Shulman, M.D. FACMG FACOG

Lee P. Shulman M.D. is the Anna Ross Lapham Professor in Obstetrics and Gynecology and Chief of the Division of Reproductive at the Feinberg School of Medicine at Northwestern University in Chicago, Illinois.
Read more...

Yves Ville, M.D.

Yves Ville, M.D. currently serves as Professor and Chairman of the Department of Obstetrics and Fetal Medicine at Necker-Enfants-Malades Hospital, Paris Descartes University.
Read more...

NIPD durch Digital PCR



Lun et al. PNAS 08

Nicht-invasiver Nachweis fetaler Chromosomenstörungen

Next Generation Sequencing (NGS) = massively parallel (shotgun) sequencing

1. Blutabnahme

2. Plasmagewinnung

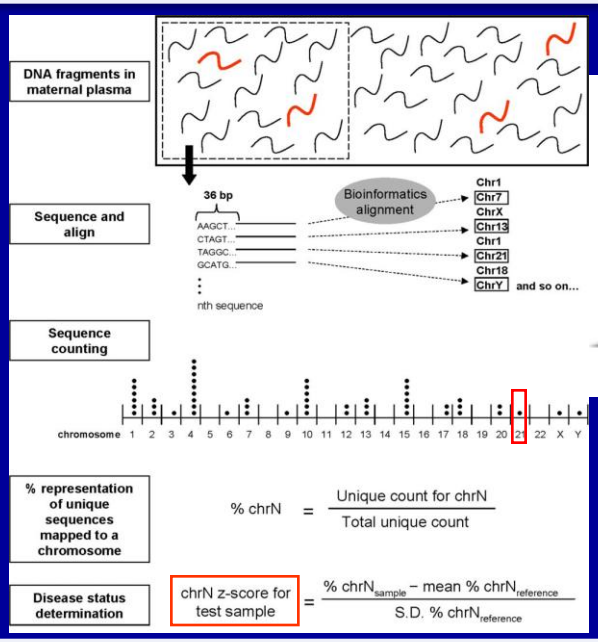
3. Extraktion zellfreier DNA

4. Erstellung genomischer Bibliothek

5. Amplifizierung/Quantifizierung Bibliothek

6. Next Generation Sequencing

7. Bio-IT Datenanalyse



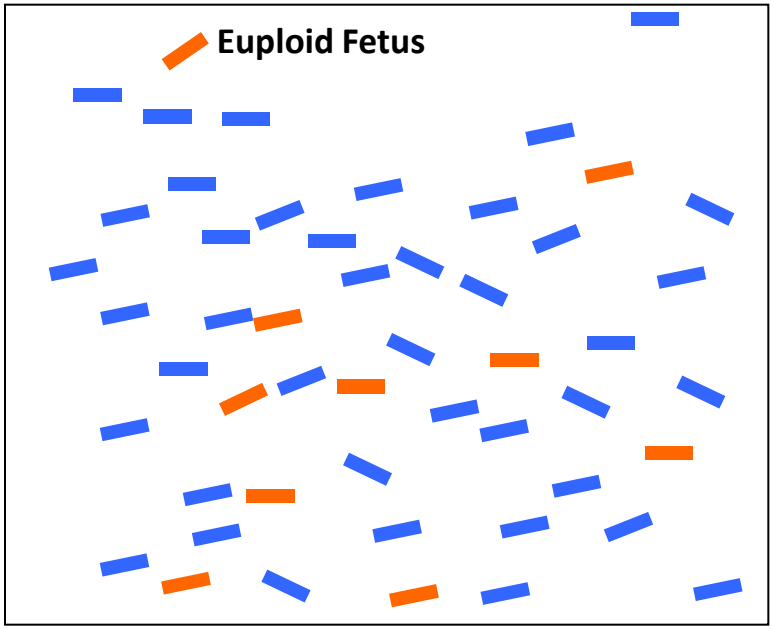
Chiu et al. 2008

8. z-score Berechnung

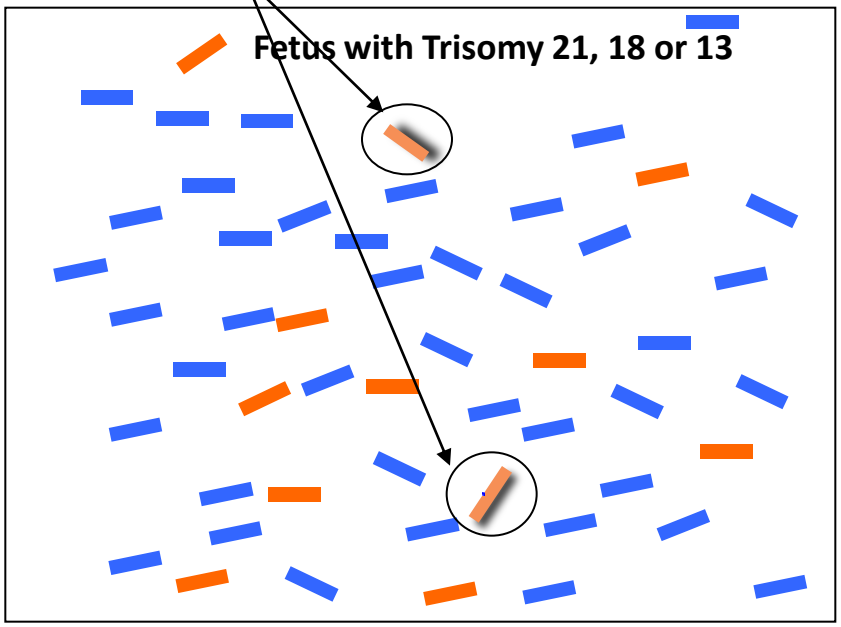
Principles of Fetal Trisomy Testing From a Maternal Blood Sample Using DNA Sequencing

- ~10% of the DNA fragments in a pregnant woman's blood are from the fetus (—)
- ~90% are from the mother (—)

Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA and Euploid Fetal DNA



Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA, Fetal DNA and Extra Fragments of Chromosome 21, 18 or 13 Contributed by a Fetal Trisomy 21, 18 or 13



Principles of Fetal Trisomy 21 Testing From a Maternal Blood Sample Using DNA Sequencing

The total number of ccf-fetal fragments vs. ccf-maternal fragments of any one chromosome is proportional to the size of the chromosome, and is consistent from sample to sample, and patient to patient.

Sequencing tells you <u>which</u> chromosome the combined maternal and fetal fragments come from.

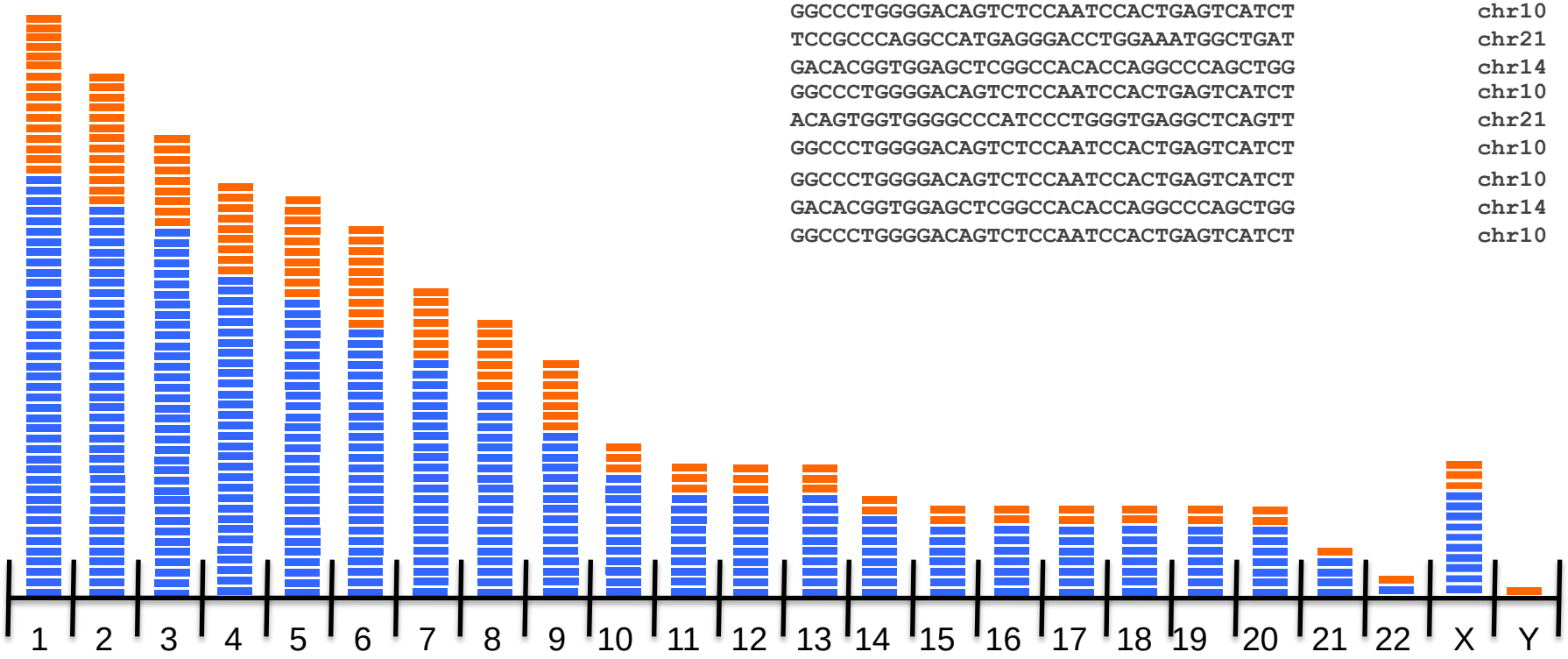
Chromosome 1

Chromosome 21

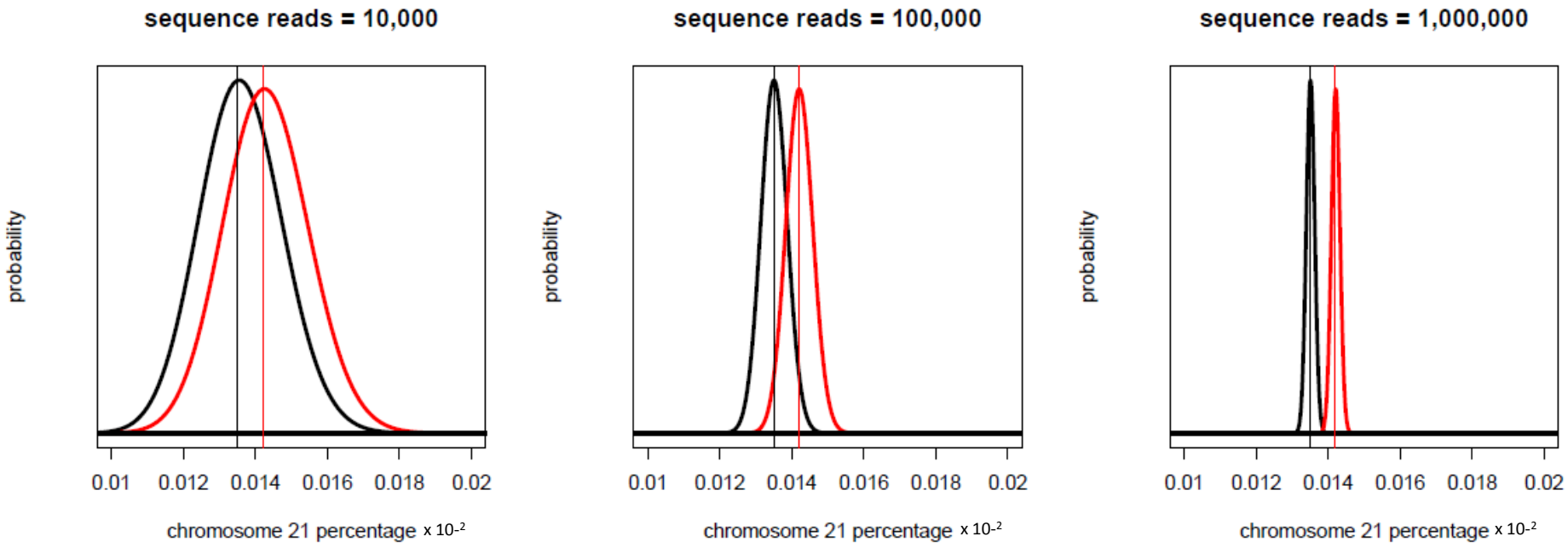
Principles of Fetal Trisomy Testing From a Maternal Blood Sample Using DNA Sequencing

Sequencing tells you which chromosome the ccf fragment comes from.

TCCGCCCAGGCCATGAGGGACCTGGAAATGGCTGAT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
ACAGTGGTGGGGCCCATCCCTGGGTGAGGCTCAGTT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
TCCGCCCAGGCCATGAGGGACCTGGAAATGGCTGAT	chr21
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
ACAGTGGTGGGGCCCATCCCTGGGTGAGGCTCAGTT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10



Using ccf DNA as the Analyte: The Power of Sequencing



Graphic key: Expected chromosome 21 material; **Excess chromosome 21 material**

Terabyte of data: a minimum of 9 million unique reads per sample

REPORTS OF MAJOR IMPACT www.AJOG.org

Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting

Mathias Ehrich, MD; Cosmin Deciu, MSc; Tricia Zwiefelhofer; John A. Tynan, DPhil; Lesley Cagasan, MSc; Roger Tim, DPhil; Vivian Lu; Ron McCullough, DPhil; Erin McCarthy; Anders O. H. Nygren, DPhil; Jarrod Dean; Lin Tang, DPhil; Don Hutchison, MSc; Tim Lu, DPhil; Huiquan Wang, DPhil; Vach Angkachatchai, DPhil; Paul Oeth, MSc; Charles R. Cantor, DPhil; Allan Bombard, MD; Dirk van den Boom, DPhil

Cite this article as: Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 2011;204:205.e1-11.

Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study

BMJ 2011;342:C7401

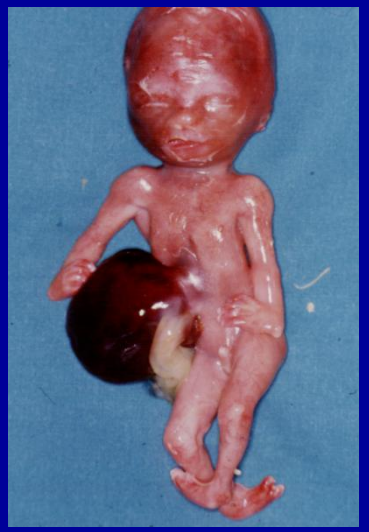
Rossa W K Chiu, professor,[1] Ranjit Akolekar, clinical research fellow,[3] Yama W L Zheng, student ,[1] Tak Y Leung, professor,[2] Hao Sun, assistant professor,[1] K C Allen Chan, associate professor,[1] Fiona M F Lun, postdoctoral fellow,[1] Attie T J I Go, professor,[4] Elizabeth T Lau, department manager and honorary assistant professor,[5] William W K To, consultant,[6] Wing C Leung, consultant,[7] Rebecca Y K Tang, consultant,[8] Sidney K C Au-Yeung, consultant,[9] Helena Lam, consultant,[10] Yu Y Kung, obstetrician,[11] Xiuqing Zhang, manager,[12,13] John M G van Vugt, professor,[4] Ryoko Minekawa, postdoctoral fellow,[3] Mary H Y Tang, consultant and honorary clinical associate professor,[5] Jun Wang, professor,[12] associate director,[13] Cees B M Oudejans, associate professor,[4] Tze K Lau, professor,[2] Kypros H Nicolaides, professor,[3] Y M Dennis Lo, professor[1,12]

Next Generation Sequencing (NGS) für nicht-invasiven Nachweis fetaler Trisomie 21

Studie	Fallzahl	Fetale T21	Sensitivität (Falsch neg.)	Spezifität (Falsch pos.)
proof-of-concept				
Fan et al. 2008	18	9	100%	100%
Chiu et al. 2008	28	14	100%	100%
Chiu et al. 2010	15	5	100%	100%
Sehnert et al. 2011	47	13	100%	100%
Sparks et al. 2012	298	89	100%	100%
Stumm et al. 2012	43	8	100%	100%
clinical setting				
Chiu et al. *BMJ* 2011	232	86	100%	97,9% (3)
Ehrich et al. *AJOG* 2011	449	39	100%	99,7% (1)
Palomaki et al. 2011	1696	212	99,1% (2)	99,9% (1)
Bianchi et al. 2012	493	39	100%	100%

Trisomie 18

Studie	Fallzahl	Fetale T18	Sensitivität (Falsch neg.)	Spezifität (Falsch pos.)
Fan et al. *PNAS* 2008	18	2	100%	100%
Sehnert et al. 2011	47	8	100%	100%
Chen et al. 2011	392	37	91,9%% (3)	98,0% (5)
Sparks et al. 2012	298	7	100%	100%
Palomaki et al. 2012	1971	59	100%	99,7% (5)
Bianchi et al. 2012	499	36	97,2% (1)	100%

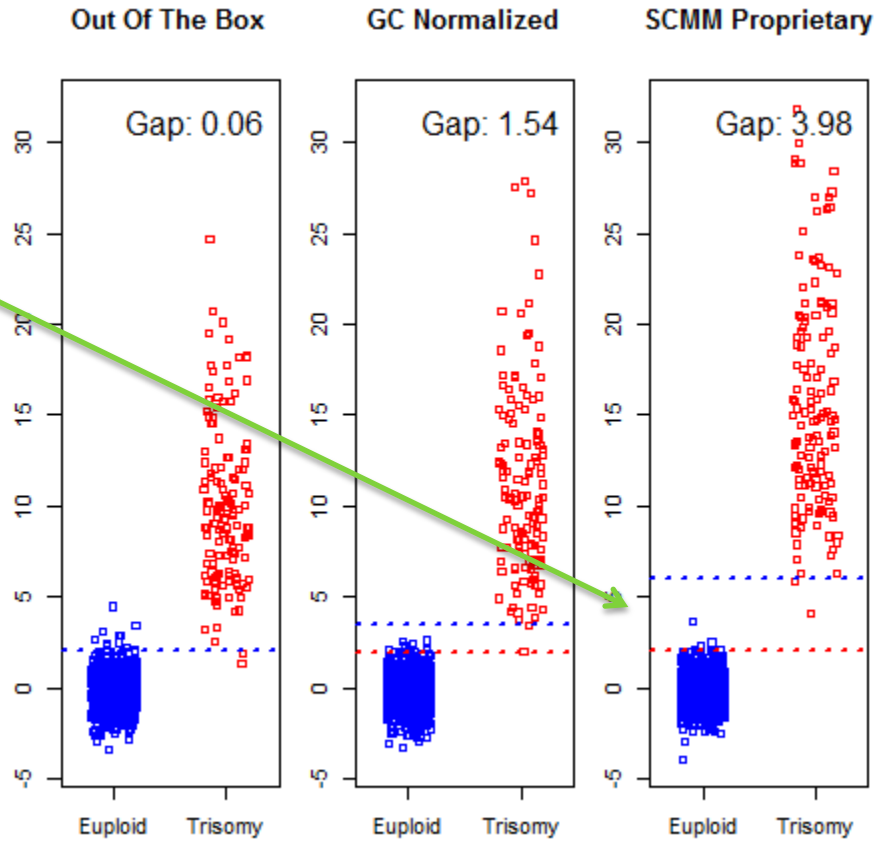


Trisomie 13

Studie	Fallzahl	Fetale T13	Sensitivität (Falsch neg.)	Spezifität (Falsch pos.)
Fan et al. *PNAS* 2008	18	1	100%	100%
Sehnert et al. 2011	47	1	0% (1)	100%
Chen et al. 2010	392	25	100%	98,9% (3)
Palomaki et al. 2012	1971	12	91,7% (1)	99,1% (16)
Bianchi et al. 2012	499	14	78,6% (3)	100%

Sequencing & Quality – v2 Biochemistry

- **Chromosome 21**:
  - Sensitivity: 99.6 %
  - Specificity: 99.9 %
- **Chromosome 18**:
  - Sensitivity: 99.0 %
  - Specificity: 99.9 %
- **Chromosome 13**:
  - Sensitivity: 98.9 %
  - Specificity: 99.9 %



MaterniT21 PLUS

Non invasive prenatal testing for fetal aneuploidies

NIPT methodologies

Counting method			Non-counting method
MPSS		Targeted sequencing	Targeted Sequencing
Sequenom MaterniT21Plus™	Verinata Verifi™	BGI	Ariosa Test Harmony™	Natera (NATUS) Test Panorama™

data are not yet available comparing the performance and cost of these tests directly to each other

NATUS: Next-Generation Aneuploidy Testing Using SNPs

THE SEQUENCE FACTORY

The bold ambitions of one institute could make China the world leader in genome sequencing. **David Cyranoski** asks if its science will survive the industrial ramp-up.

In 2006, Li Yingrui left Peking University for the BGI, China's premier genome-sequencing institute. Now, freckled and fresh-faced at 23 years old, he baulks at the way a senior BGI colleague characterized his college career — saying Li was wasting time playing video games and sleeping during class. "I didn't sleep in lectures," Li says. "I just didn't go."

He runs a team of 130 bioinformaticians, most no older than himself. His love of games has served him well when deciphering the flood of data spilling out of the BGI's sequencers every day. But "science is more satisfying" than video games, he says. "There's more passion."

The people at the BGI — which stopped officially using the name Beijing Genomics Institute in 2007 after moving its headquarters to Shenzhen — brim with passion, and an ambition so naked that it unsettles some. In the past few years the institute has leapt to the forefront of genome sequencing with a bevy of papers in top-tier journals. Some recent achievements include the genomes of the cucumber[1], the giant panda[2], the first complete sequence of an ancient human[3] and, in this issue of *Nature*[4], the genomes of more than 1,000 species of gut bacteria, compiled from 577 billion base pairs of sequence data.

The mission, BGI staff say with an almost rehearsed uniformity, is to prove that genomics matters to ordinary people. "The whole institute feels this huge responsibility," says Wang Jun, executive director of the BGI and a professor at the University of Copenhagen. The strategy is to sequence — well, pretty much anything that the BGI or its expanding list of collaborators wants to sequence. It has launched projects to tackle 10,000 microbial genomes and those of 1,000 plants and animals as part of an effort to create a genomic tree of life cov-

D. CYRANOSKI

The BGI's sequencing room, where thousands of projects will contribute to building a genomic tree of life.

> "In Shenzhen, the mountains are high and the emperor is far away."

of expensive equipment. In January, the BGI announced the purchase of 128 of the world's newest, fastest sequencers, the HiSeq 2000 from Illumina, each of which can produce 25 billion base pairs of sequence in a day. When all are running at full tilt, the BGI could theoretically sequence more than 10,000 human genomes in a year. This puts it on track to surpass the entire sequencing output of the United States, says David Wheeler, director of the Molecular Biology Computational Resource at Baylor College of Medicine in Houston, Texas. "It is clear there is a new map of the genomics world," he says.

The charge that the BGI has reduced science to brute mechanization does little to ruffle feathers in Shenzhen. Wang himself quips that the BGI brings little intellectual capital into projects: "We are the muscle, we

decide whether the BGI is a business or a non-profit research institute. Genome scientists around the world are watching to see how it will strike a balance. Edison Liu, director of the Genome Institute of Singapore and head of the Human Genome Organization warns: "If they are just a sequence-for-money operation, they will not be remembered."

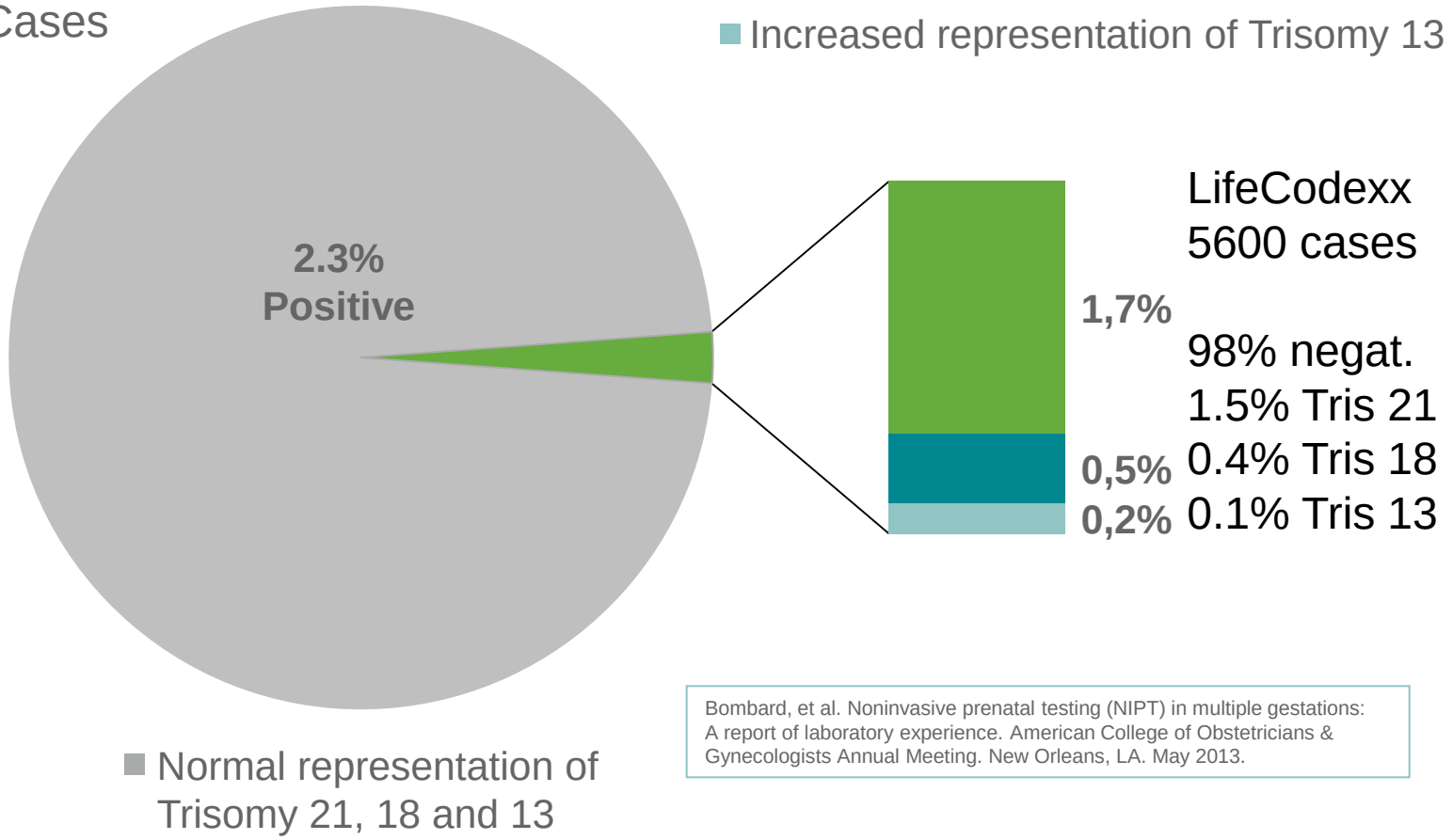
Getting far from the emperor

China was late to the genomics frenzy of the 1990s that led to the sequencing of the human genome. The fact that the country didn't miss out altogether is thanks largely to the BGI's determined, charismatic and sometimes abrasive leader Yang Huanming ('Henry'). As the human genome project was nearing completion, Yang and a small group of sequencing advocates tried to get China involved. They found support from the Chinese Academy of Sciences (CAS), which secured a building

MaterniT21™ PLUS LDT
Laboratory Experience: > 100,000 Patients

Results of Testing

Updated March 31, 2013
N = 96,681 Cases



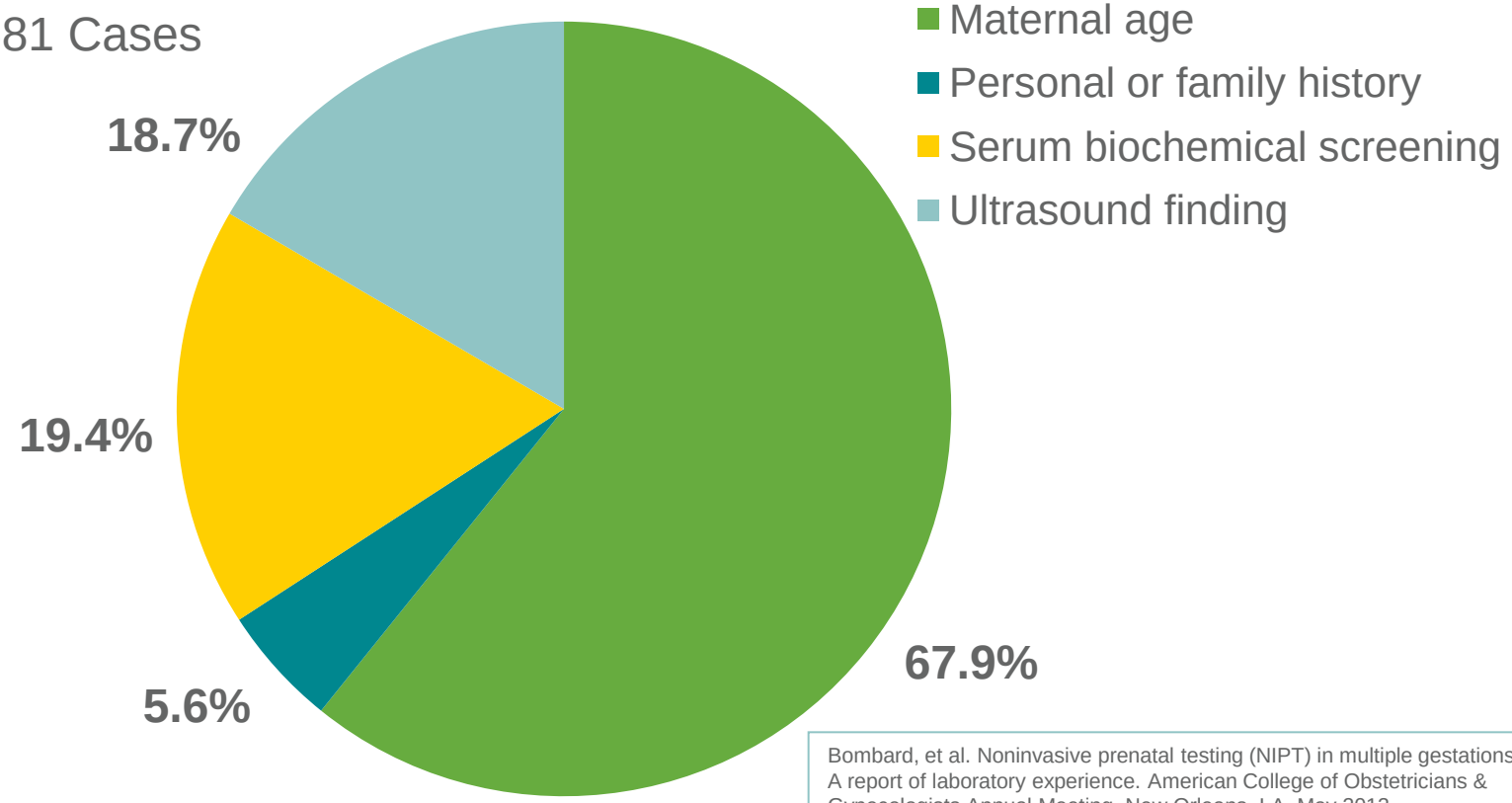
Bombard, et al. Noninvasive prenatal testing (NIPT) in multiple gestations: A report of laboratory experience. American College of Obstetricians & Gynecologists Annual Meeting. New Orleans, LA. May 2013.

MaterniT21 PLUS

MaterniT21™ PLUS LDT
Laboratory Experience: > 100,000 Patients

Indications for Use

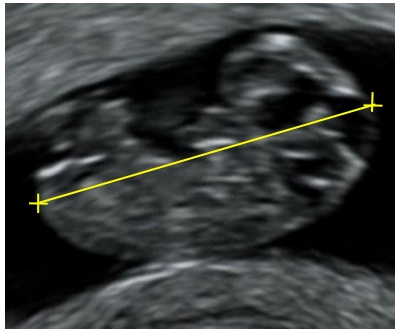
Updated March 31, 2013
N = 96,681 Cases

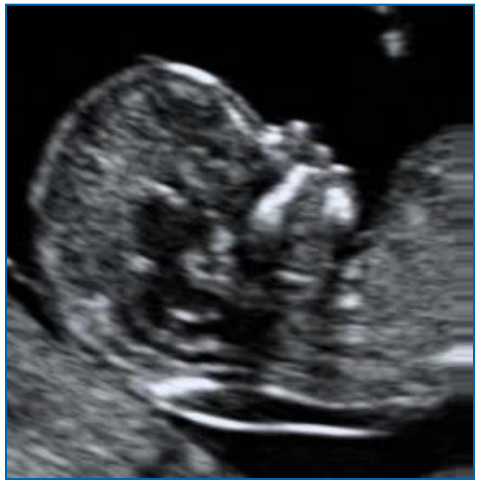


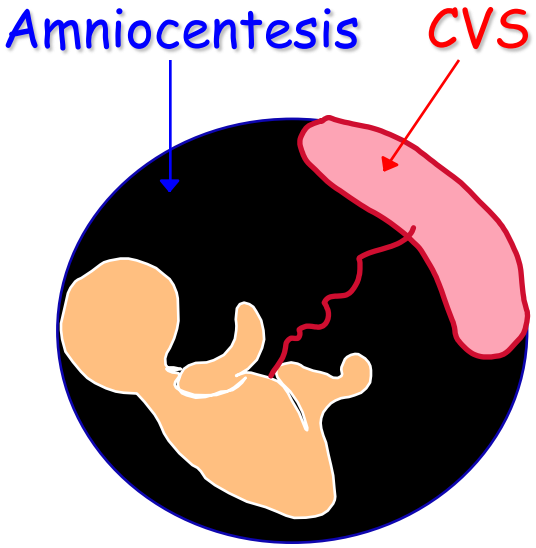
Bombard, et al. Noninvasive prenatal testing (NIPT) in multiple gestations: A report of laboratory experience. American College of Obstetricians & Gynecologists Annual Meeting. New Orleans, LA. May 2013.

**Total equals greater than 100% as certain patients have multiple indications for testing*

Future prenatal strategy







10 weeks:
- Scan to measure the fetus
- Blood for cfDNA test

12 weeks:
- Detailed ultrasound scan
- Discuss results
- Decide if CVS is necessary

- +ve NIPT test
- Fetal defects
- NT ≥3.5 mm

K Nicolaides

Professional recommendations

- Adequate counseling
- Singleton
- High-risk population

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

The Society for Maternal-Fetal Medicine

COMMITTEE OPINION

Number 545 • December 2012

The American College of Obstetricians and Gynecologists Committee on Genetics
The Society for Maternal-Fetal Medicine Publications Committee

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.

Noninvasive Prenatal Testing for Fetal Aneuploidy

ispd

Position Statement from the Aneuploidy Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis, April 2013

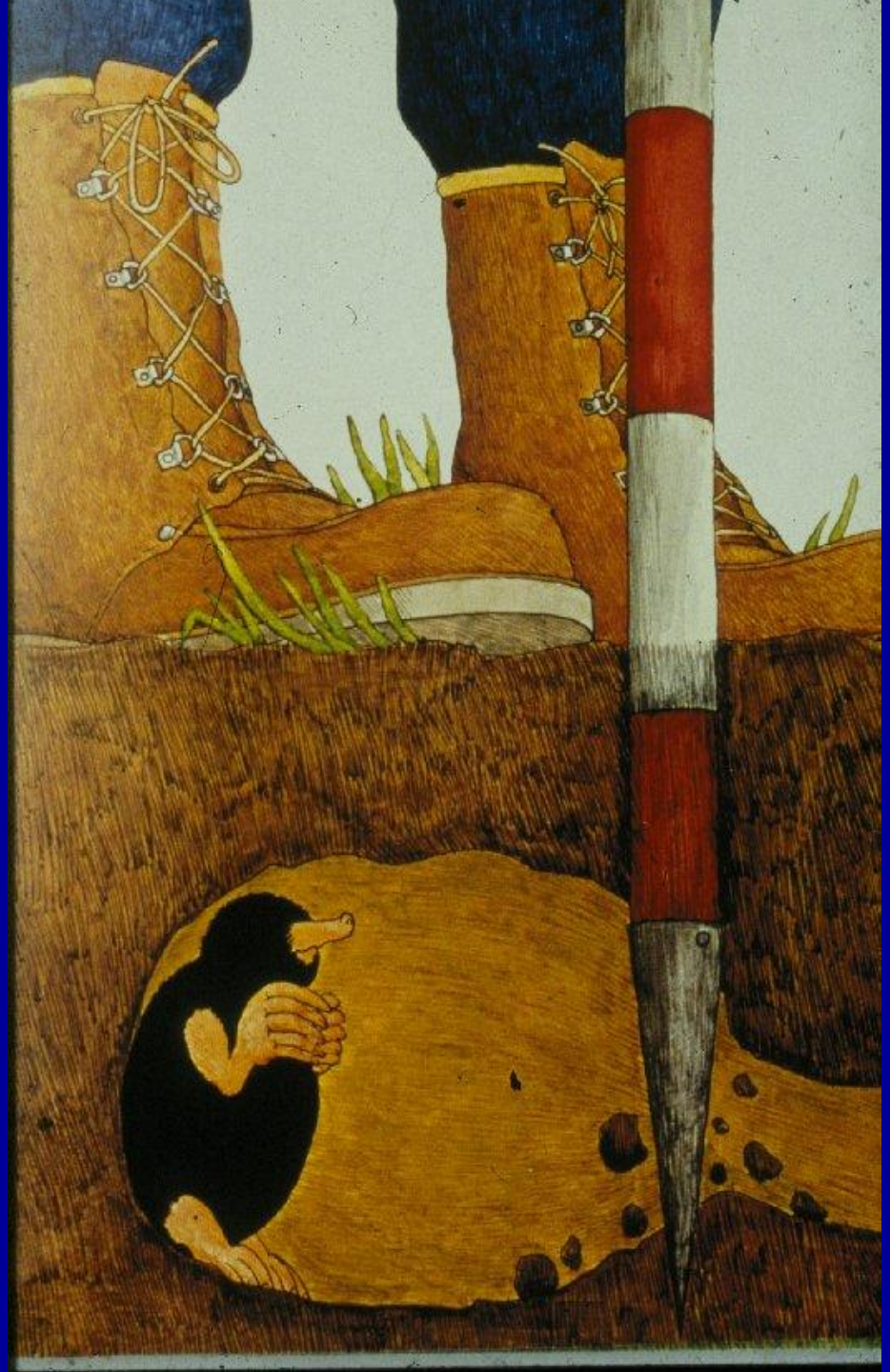
Peter Benn (Chair), Antoni Borell, Rossa Chiu, Howard Cuckle, Lorraine Dugoff, Brigitte Faas, Susan Gross, Joann Johnson, Ron Maymon, Mary Norton, Anthony Odibo, Peter Schielen, Kevin Spencer, Tianhua Huang, Dave Wright, Yuval Yaron.

ACMG

ACMG POLICY STATEMENT | Genetics inMedicine

ACMG statement on noninvasive prenatal screening for fetal aneuploidy

Non-invasive prneatal diagnosis (NIPD) from blood of pregnant women finally available



RHESUS-FAcTOR/
KELL/
X-linked diseases

AUTOSOMAL DOMINANT diseases (PATERNAL MUTATIOS)

COMPOUND HETEROZYG. AUTOSOM. RECESSIVE DISEASES

FINALLY ALSO AVAILABLE FOR CHROMOSOMAL ANOMALIES