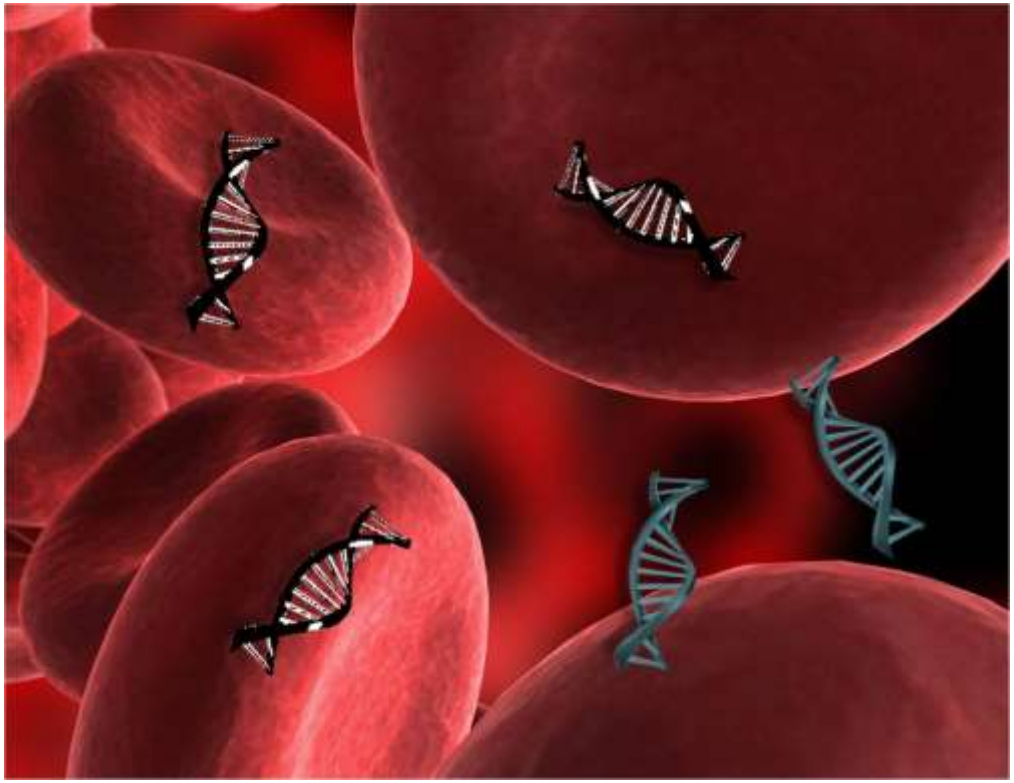
Sık görülen Anöploidilerin Noninvaziv Prenatal Taramasında cf-DNA Analizi



Prof. Dr. Seher Başaran
İstanbul Üniversitesi, İstanbul Tıp Fakültesi,
Tıbbi Genetik ABD

Prenatal Tanı?

Fetustaki, genetik veya nongenetik nedenlerin yol açtığı malformasyon ve hastalıkların gebeliğin mümkün olduğunca erken döneminde tanınmasıdır.

A. Malformasyonlar: Tanı ⟶ USG

B. Kromozom anomalileri:

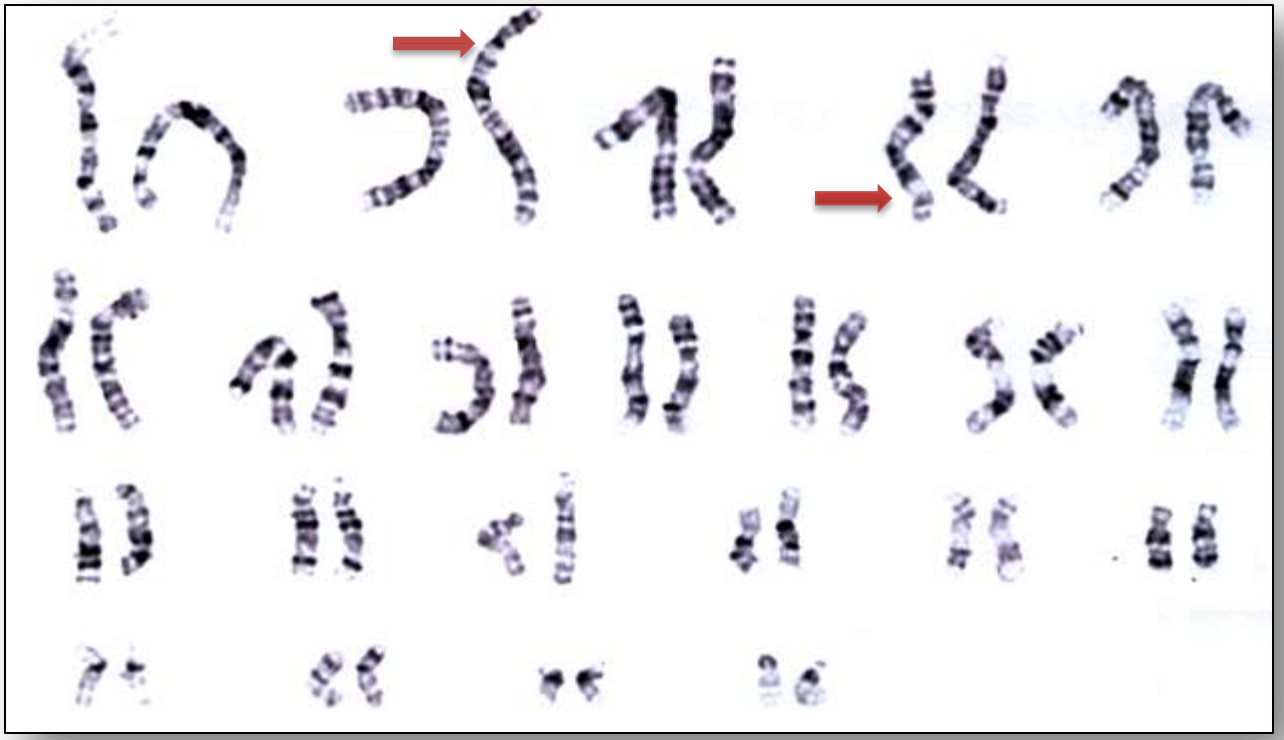
Tanı ⟶ Fetal kromozom analizleri/ aCGH/mikroarray

C. Tek gen hastalıkları:

Tanı ⟶ DNA/enzim analizleri

Prenatal tanı endikasyonu; İleri anne yaşı+2'li testte artmış risk+ICSI (donuk embriodan)

13.GH CVS Karyotip; **46,--,t(2;4)(p23;q31.1)dn**



a-CGH endikasyonu; Görünürde dengeli de novo resiprokal translokasyon

a-CGH sonucu normal

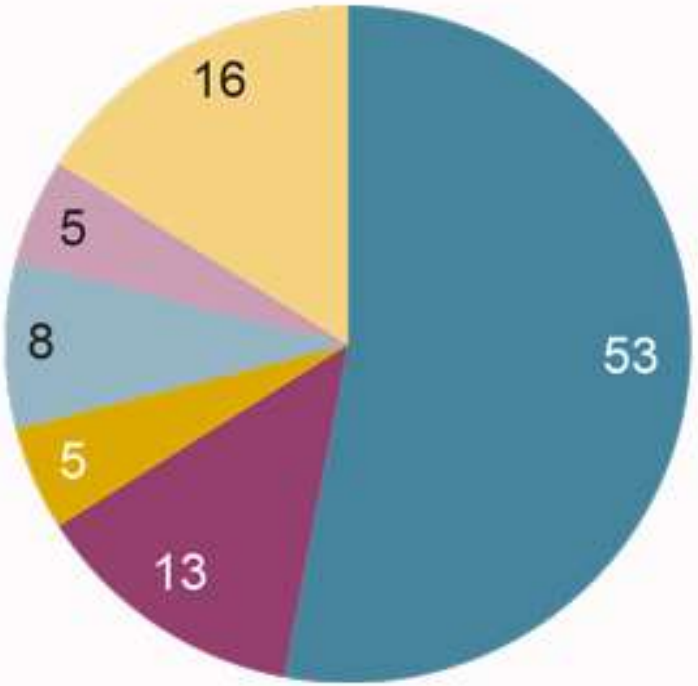
22. GH, 2. düzey USG; Ense plisi kalınlığında artış

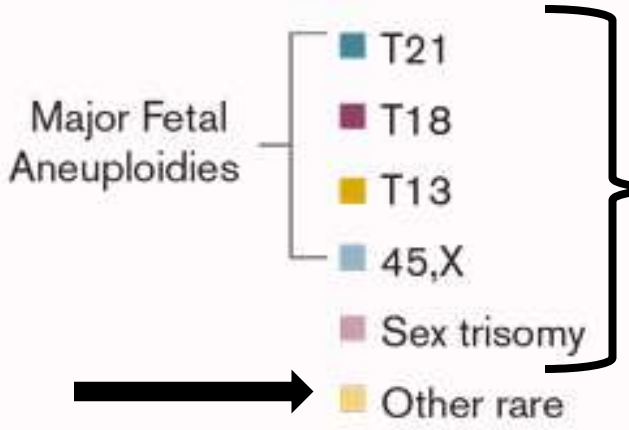
PTPN11 dizi analizi; **13. ekzonda heterozigot c.1529A>C de novo** (Leopard sendromu'nda tanımlanmış bir mutasyon)

Kromozom Anomalileri

- Yeni doğan sıklığı 1:156 (Karyotip analizi ile)
- Gebelik haftası ilerledikçe azalır
- Anne yaşı arttıkça artar
- Dengesiz kromozom anomalileri=fenotip etkilenir.
- Dengeli kromozom anomalileri=fenotip genellikle normal (*de novo*?)

Figure 1. Prenatal Prevalence of Chromosomal Abnormalities





Sık görülen Anöploidiler **%84**

%16

Data adapted from Wellesley, D, et al.[2], Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. *Eur J of Hum Gen*, 11 January 2012.

Kromozom anomalilerinin prenatal tanısı

TANI Testleri

İnvaziv Girişim (AS, CVS, KS) sonrası

- fetal kromozom analizi (Gold standart) (tüm genom)
- FISH/QF-PCR (sık görülen anomaliler ve hedefli mikrodelesyonlar)
- mikroarray/a-CGH (tüm genom submikroskobik anomaliler)

"İleri anne yaşı"

≥35 yaş tüm gebelerin %10'u

≥37 yaş tüm gebelerin %5'i risk grubunu oluşturacak

ve tümüne fetal karyotip yapılsa bile

"Tri 21 lerin %30'u saptanacak"

Çünkü, Tri 21 lerin %70 i <35 yaş annelerden doğar.

Amaç; daha az girişim ile daha geniş bir populasyona ulaşmak ve daha fazla anomali yakalamak.

"TARAMA TESTLERİ "

TARAMA Testleri

Tri 21 prevalansı 1:700 →100 Tri 21 doğumu için → 70 000 doğum

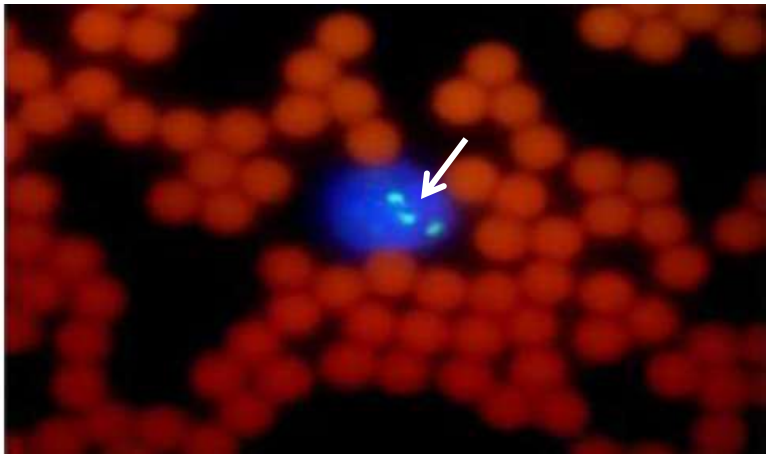
			Trizomi 21 "yakalanacak"	"doğacak"
Anne yaşı (>35)	% 10	7 000 AS	**30**	**70**
Üçlü test	% 5	3 500 AS	**60**	**40**
NT	% 5	3 500 AS	**75**	**25**
1. Trim. tarama	% 4,2	2 940 AS	**85**	**15**
Sequential	%4,2	2 940 AS	**90-95**	**5-10**

Hedef Trizomi 21 tanısı ise

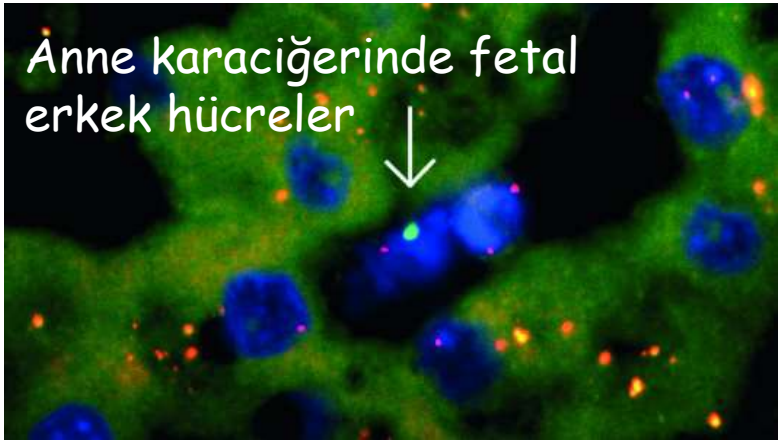
1. Trimester (CVS), 2. Trimester EP (AS) ve diğer USG bulgularının varlığında saptanan anomaliler ve oranları (2010-2014/8)

	T21	T18	T13	45,X	Poli X/Y	Diğer Otoz. Tri	Dengesiz Yapısal	Dengeli Yapısal	Toplam
CVS-NT	23%	4,6%	1,3%	2%	0,7%	0,7%	0	2,6%	34,9%
CVS-NT+ Diğer USG	20,7%	13,8%	4,8%	11%	0,7%	2,1%	3,5%	0	56,6%
AS-EP	1,33%	0	0	0	0	0	0	1,33%	2,7%
AS-EP+ Diğer USG	17,2%	0,9%	0	1,7%	0,9%	0,9%	1,7%	0	23,3%

80'ler ve 90'lar Maternal Kanda Fetal Kromozom Anomalilerinin Tanısı için fetal hücre avı ile geçti….



Anne kan dolaşımında fetal hücreler (T21)
1:1 000 000



PLAZMADA EKSTRASELLÜLER DNA

-1940 larda plazmada ekstraselluler DNA'nın varlığı bildirildi,
-1990 larda kanser hastalarının plazmalarından elde edilen serbest DNA da tümör kökenli genetik markerler saptandı,
-1997 de erkek çocuğa gebe olan kadınların plazmalarında fetusun Y kromozomuna ait markerler gösterildi (Lo et al., 1997).

Noninvasive testing for fetal aneuploidy using fetal nucleic acids

THE LANCET

Early report

Presence of fetal DNA in maternal plasma and serum

Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman, James S Wainscoat

1997

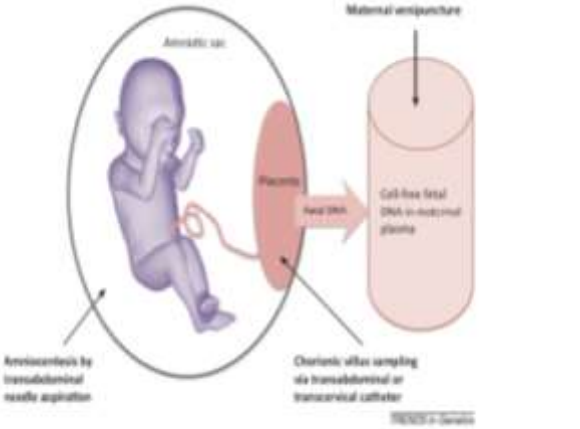
BMJ

RESEARCH

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

Rossa W K Chiu, professor,[1] Ranjit Akolekar, clinical research fellow,[1] 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,[3] Elizabeth T Lau, department manager and honorary assistant professor,[3] William W K To, consultant,[4] Wing C Leung, consultant,[5] Rebecca Y K Tang, consultant,[6] Sidney K C Au-Yeung, consultant,[6] Helena Lam, consultant,[6] Yu Y Kung, obstetrician,[6] Xiuqing Zhang, manager,[7,8] John M G van Vugt, professor,[9] Ryoko Minekawa, postdoctoral fellow,[1] Mary H Y Tang, consultant and honorary clinical associate professor,[1] Jun Wang, professor,[8] associate director,[7] Cees B M Oudejans, associate professor,[9] Tze K Lau, professor,[2] Kypros H Nicolaides, professor,[1] Y M Dennis Lo, professor[1,7]

2011



Hui & Bianchi 2012

Maternal Plazmada Dolaşan cell free DNA'nın Biyolojik Özellikleri

-Serbest dolaşımdaki cfDNA'nın % 80'i <200 bp ve plazmadaki tüm DNA'nın % 3-10 plasenta (sitotrofoblast) kaynaklıdır,

-Implantasyonu takip eden 18 inci günden itibaren plazmada saptanmaya başlanır ve gebelik ilerledikçe miktarı artar,

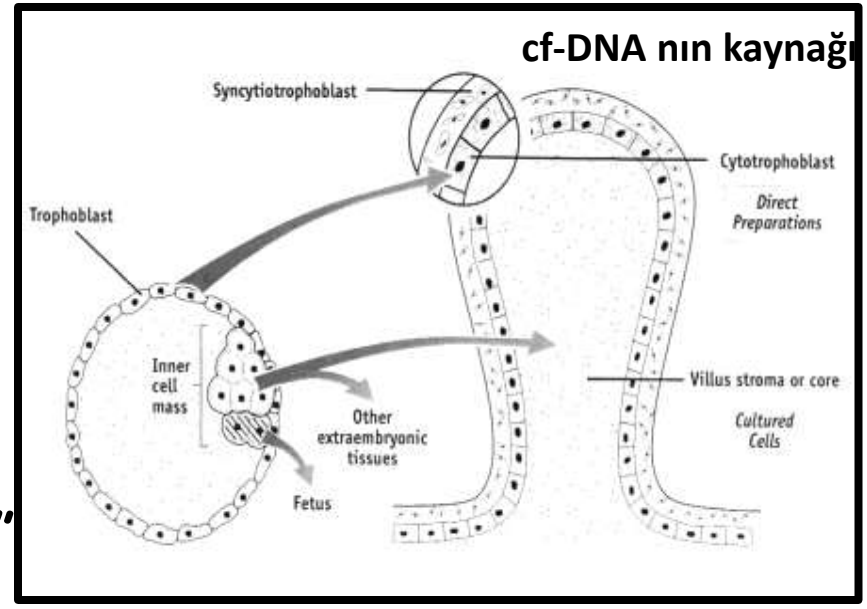
-Gebelik bittikten sonra, yarı ömrü 16 dakikadır,

-Genel görüş, cf-DNA nın kalıcı olmadığıdır; ancak bir yayın fetal DNA nın uzun süre (27 yıl) maternal kanda saptanabildiğini ve bu bazı fetal hücrelerin maternal kanda uzun yaşadığı ve plazma ayrıştırması esnasında tüm nükleuslu hücrelerin iyi uzaklaştırılamaması ile açıklanmaktadır.

"cf-DNA, ağırlıklı olarak plasenta kaynaklı olduğundan, plasentaya ait özellikleri (plasenta ile sınırlı mozaisizm, plasental epigenetik özellikler, vs) yansıtmaktadır ve bu nedenle

cfp-DNA ya da cf-DNA

olarak adlandırılması önerilmektedir."

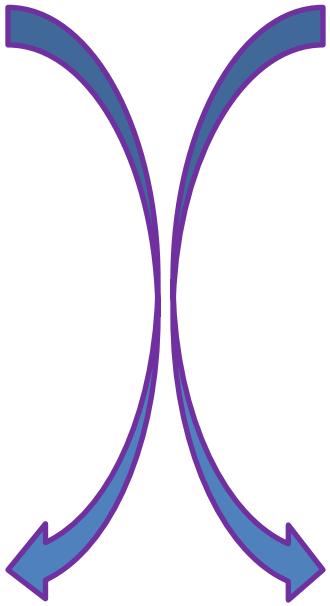


cf-DNA Testlerinin KLİNİK UYGULAMALARI

Maternal kandan alınan plazma örneğinde tüm hücre yapılarının uzaklaştırılması

↓

DNA nın ekstraksiyonu
(Minör oranda fetal DNA(%10) + Major oranda maternal DNA (%90))



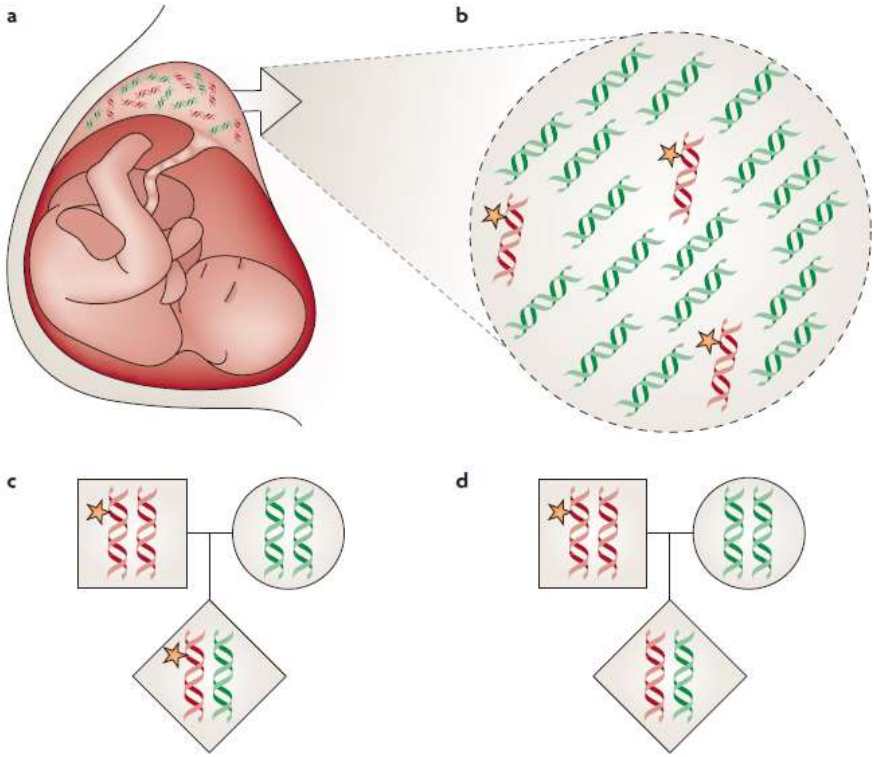
<u>Kalitatif Yöntemler;</u>
Maternal ve fetal DNA polimorfizm/değişimlerini tanır

TANI

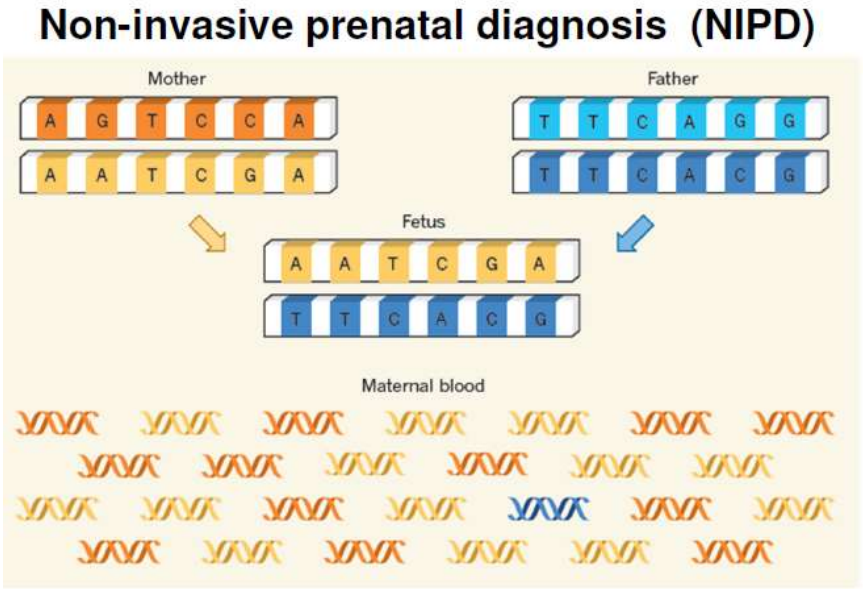
<u>Kantitatif Yöntemler;</u>
Normal ile karşılaştırma esasına göre tanır

TARAMA

Kalitatif yöntemle PATERNAL mutasyonun/özelliklerin fetusta TANISI



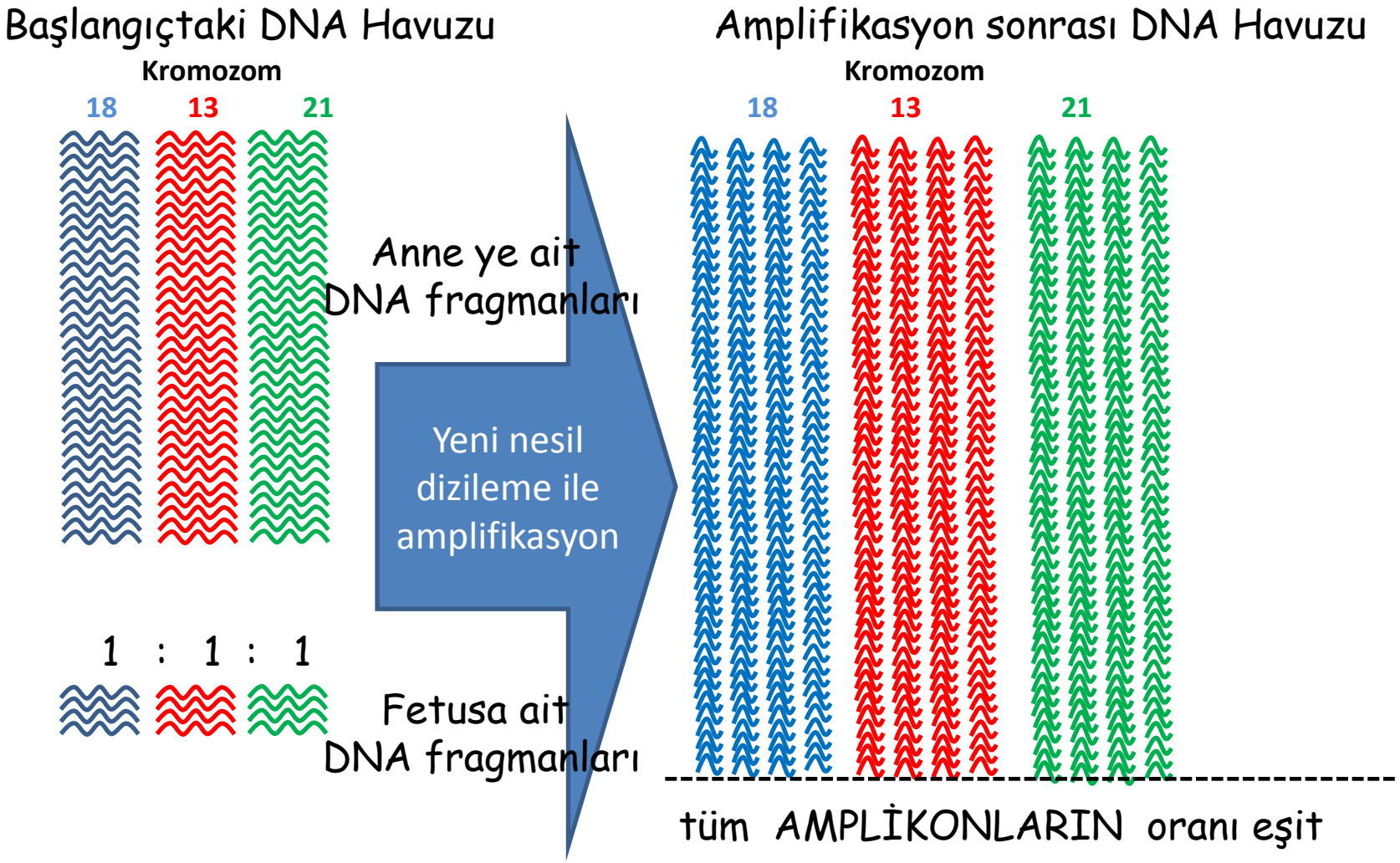
www.nature.com/reviews/genetics



Analysis by: Next Generation Sequencing (NGS)
Massive Parallel Shotgun Sequencing (MPSS)

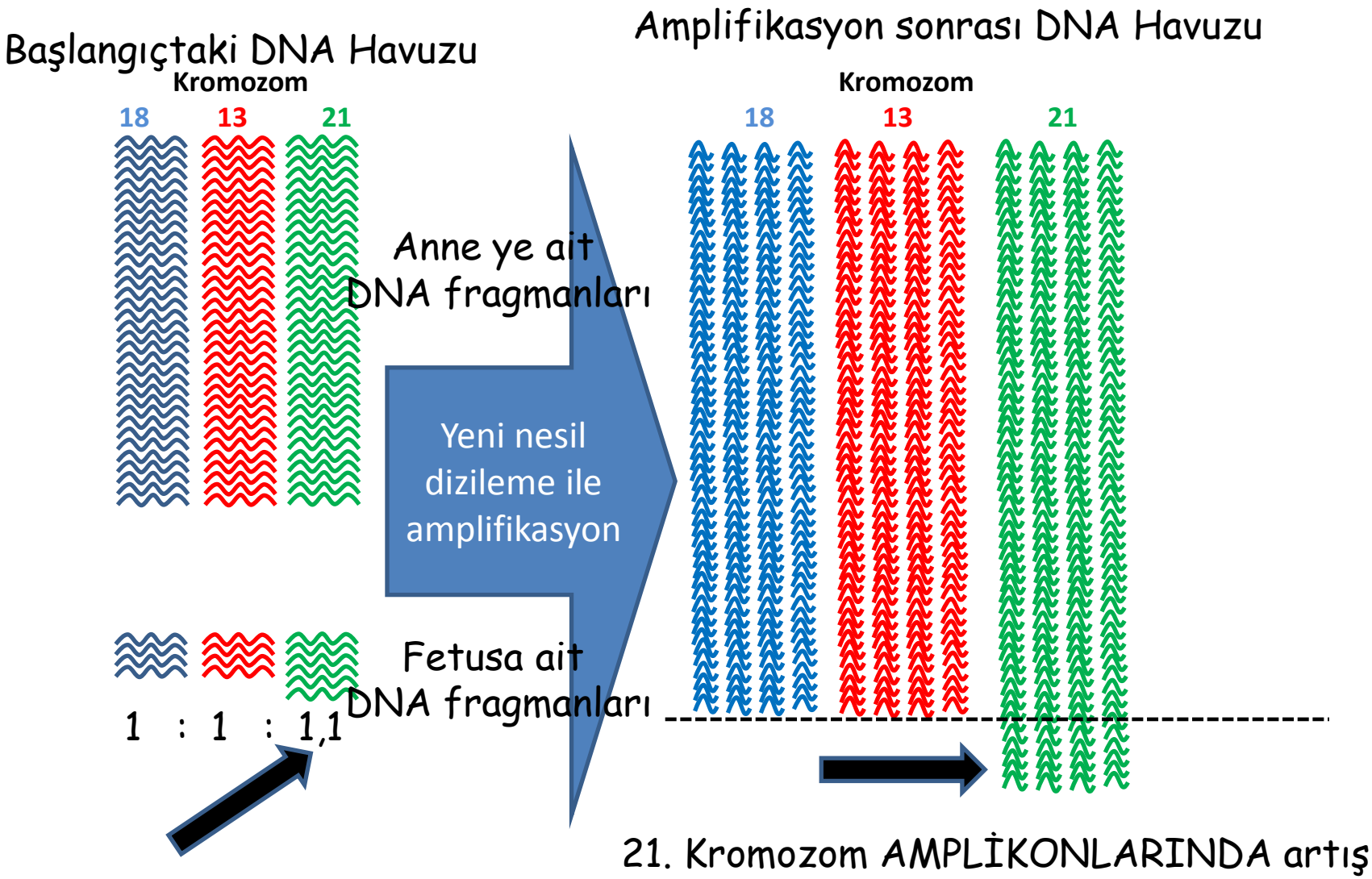
Kantitatif Yöntem; Fetus NORMAL

Anne 46 kromozom + fetus 46 kromozom



Kantitatif Yöntem; Fetus TRİZOMİK

Anne 46 kromozom + fetus 47 kromozom (47,+21)



454 | NATURE | VOL 486 | 28 JUNE 2012

GENETICS

Fetal tests spur legal battle

A newborn industry based on non-invasive genetic testing turns combative.

14 OCTOBER 2005 Sequenom licenses a patent for non-invasive prenatal diagnosis.

10 AUGUST 2010 Sequenom's lawyers send Verinata Health a letter warning that Verinata is developing tests that will infringe on Sequenom's patent and patent application.

30 AUGUST 2011 Stephen Quake — founder of Verinata — and Hei-Mun Fan, both at Stanford University in California, are issued a patent for 'Determination of fetal aneuploidies by massively parallel DNA sequencing'.

17 OCTOBER 2011 Sequenom launches the MaterniT21 test.

6 DECEMBER 2011 Sequenom sends Aria Diagnostics a letter warning about patent infringement.

19 DECEMBER 2011 Aria files a complaint against Sequenom.

6 JANUARY 2012 Natera files a complaint against Sequenom.

24 JANUARY 2012 Sequenom sues Aria.

22 FEBRUARY 2012 Verinata and Stanford University sue Sequenom.

1 MARCH 2012 Verinata launches the Verifi prenatal test.

7 MAY 2012 Ariosa (formerly Aria) launches the Harmony prenatal test.

Commercial landscape of noninvasive prenatal testing in the United States

Ashwin Agarwal, Lauren C. Sayres, Mildred K. Cho, Robert Cook-Deegan, and Subhashini Chandrasekharan

Prenatal Diagnosis 2013, 33, 521–531

Table 1 **Commercial landscape of noninvasive prenatal testing for fetal aneuploidy in the United States**

	Sequenom	Verinata Health	Ariosa Diagnostics	Natera
Test name	MaterniT21 Plus[13]	Verifi[15]	Harmony Prenatal Test[18]	Panorama Prenatal Test[24]
Platform	SEQureDx technology incorporating massively parallel shotgun sequencing[13]	Massively parallel sequencing using SAFeR algorithm[15]	DANSR technology incorporating targeted sequencing and FORTE algorithm[79,80]	Next-generation SNP-based Targeted Aneuploidy Testing[24]
Conditions	Trisomy 13, trisomy 18, trisomy 21, and sex chromosome aneuploidies[13]	Trisomy 13, trisomy 18, trisomy 21, sex chromosome aneuploidies, and fetal sex[15]	Trisomy 13, trisomy 18, and trisomy 21[18]	Trisomy 13, trisomy 18, trisomy 21, and sex chromosome aneuploidies[24,81,82]
Cost	$1700 out-of-pocket, $235 co-pay with insurance coverage, and $2900 directly billed to insurers[83,84]	$295 out-of-pocket, $200 co-pay with insurance coverage, and $1200 directly billed to insurers[16,97]	$795 out-of-pocket and $95 co-pay with insurance coverage[18–20,84]	Unknown out-of-pocket and $1495 directly billed to insurers[24]
Turnaround	8 to 10 days[10]	8 to 10 days[15]	8 to 10 days[18]	15 days[24]
Market entry	October 2011[83]	March 2012[15]	May 2012[6]	December 2012[24]
Marketing	Through physicians[13]	Through physicians[15]	Through physicians[18]	Through physicians[24]
Regulatory status	CAP accredited, CLIA certified, plans to submit an IVD PMA application[13,85,86]	CAP accredited, CLIA certified, plans to submit an IVD PMA application[15]	CAP accredited, CLIA certified[18]	CAP accredited, CLIA certified[82]
Primary publications	Ehrich *et al.*[87], Bombard *et al.*[88], Palomaki *et al.*[89], Palomaki *et al.*[90]	Sehnert *et al.*[91], Bianchi *et al.*[98]	Sparks *et al.*[79], Sparks *et al.*[80], Ashoor *et al.*[92], Norton *et al.*[93], Ashoor *et al.*[94], Nicolaides *et al.*[95]	Zimmermann *et al.*[81]

CLIA, Clinical Laboratory Improvement Amendments; CAP, College of American Pathologists; DANSR, Digital Analysis of Selected Regions; IVD, *In vitro* diagnostic product; PMA, Pre-market Approval; SNP, Single nucleotide polymorphism.

Noninvasive prenatal testing for aneuploidy–ready for prime time?

Lyn S. Chitty, Melissa Hill, Helen White, David Wright, Stephen Morris,

American Journal of Obstetrics & Gynecology, APRIL 2012

Study	Method	Total no. samples tested	No. normal samples tested (true negatives)	No. aneuploid samples tested (true positive)	Sensitivity, % (95% CI)[a]	Specificity, % (95% CI)[a]
Lo et al[16]	RNA allelic ratio	67	57 (55)	10 (9)	90 (60.6–99.5)	96.5 (89.4–99.4)
Tsui et al[17]	RNA allelic ratio	62	58 (51)	4 (4)	100 (47.3–100)	89.7 (80.6–95.4)
Fan et al[18,19]	MPS	18	T21 cohort: 9 (9) T18 cohort: 16 (16) T13 cohort: 17 (17)	T21: 9 (9) T18: 2 (2) T13: 1 (1)	T21: 100 (71.8–100) T18: 100 (22.4–100) T13: 100 (5–100)	T21: 100 (71.8–100) T18: 100 (82.9–100) T13: 100 (83.8–100)
Chiu et al[20]	MPS	28	14 (14)	14 (14)	100 (80.7–100)	100 (80.7–100)
Ghanta et al[21]	Tandem SNP	27	20 (20)	7 (7)	100 (65.2–100)	100 (86.1–100)
Tong et al[22]	Differential methylation	29	24 (23)	5 (5)	100 (55–100)	95.8 (81.7–99.8)
Papageorgiou et al[23]	Differential methylation	40	26 (26)	14 (14)	100 (80.7–100)	100 (89.2–100)
Deng et al[24]	RT-MLPA	113	87 (87)	25 (23)	92 (77–98.6)	100 (96.6–100)
Chiu et al[25]	MPS	15	10 (10)	5 (5)	100 (54.9–100)	100 (74.2–100)
Sehnert et al[26]	MPS	47	T21 cohort: 34 (34) T18 cohort: 39 (39) T13 cohort: 46 (46)	T21: 13 (13) T18: 8 (8) T13: 1 (no call)	T21: 100 (79.5–100) T18: 100 (68.8–100) T13: –	T21: 100 (91.6–100) T18: 100 (92.6–100) T13: 100 (93.7–100)
Chen et al[27]	MPS (2 plex)	289	T13 cohort: 264 (261) T18 cohort: 252 (248)	T18: 37 (34) T13: 25 (25)	T18: 91.9 (80.4–97.8) T13: 100 (88.8–100)	T18: 98.9 (97.1–99.7) T13: 98.4 (96.4–99.4)
Ehrich et al[28]	MPS (4 plex)	449	410 (409)	39 (39)	100 (92–100)	99.7 (98.8–99.9)
Lau et al[29]	MPS (12 plex)	108	T21 cohort: 97 (97) T18 cohort: 98 (98) T13 cohort: 106 (106)	T21: 11 (11) T18: 10 (10) T13: 2 (2)	T21: 100 (76.2–100) T18: 100 (74.5–100) T13: 100 (22.4–100)	T21: 100 (97–100) T18: 100 (97–100) T13: 100 (97.2–100)
Chiu et al[8]	MPS (8 plex)	657	571 (6)	86 (68)	79.1 (70.6–86)	98.9 (97.9–99.5)
Chiu et al[8]	MPS (2 plex)	232	146 (3)	86 (86)	100 (96.6–100)	97.9 (94.8–99.8)
Palomaki et al[9]	MPS	1683	1471 (1468)	212 (209)	98.6 (96.4–99.6)	99.8 (99.5–99.9)
Sparks et al[14]	Targeted MPS	167	123 (123)	T21: 36 (36) T18: 8 (8)	T21: 100 (92.1–100) T18: 100 (68.8–100)	T21: 100 (97.6–100) T18: 100 (97.6–100)
Ashoor et al[15]	Targeted MPS	397	297 (297)	T21: 50 (50) T18: 50 (49)	T21: 100 (94.2–100) T18: 98 (90.1–99.9)	T21: 100 (99–100) T18: 100 (99–100)

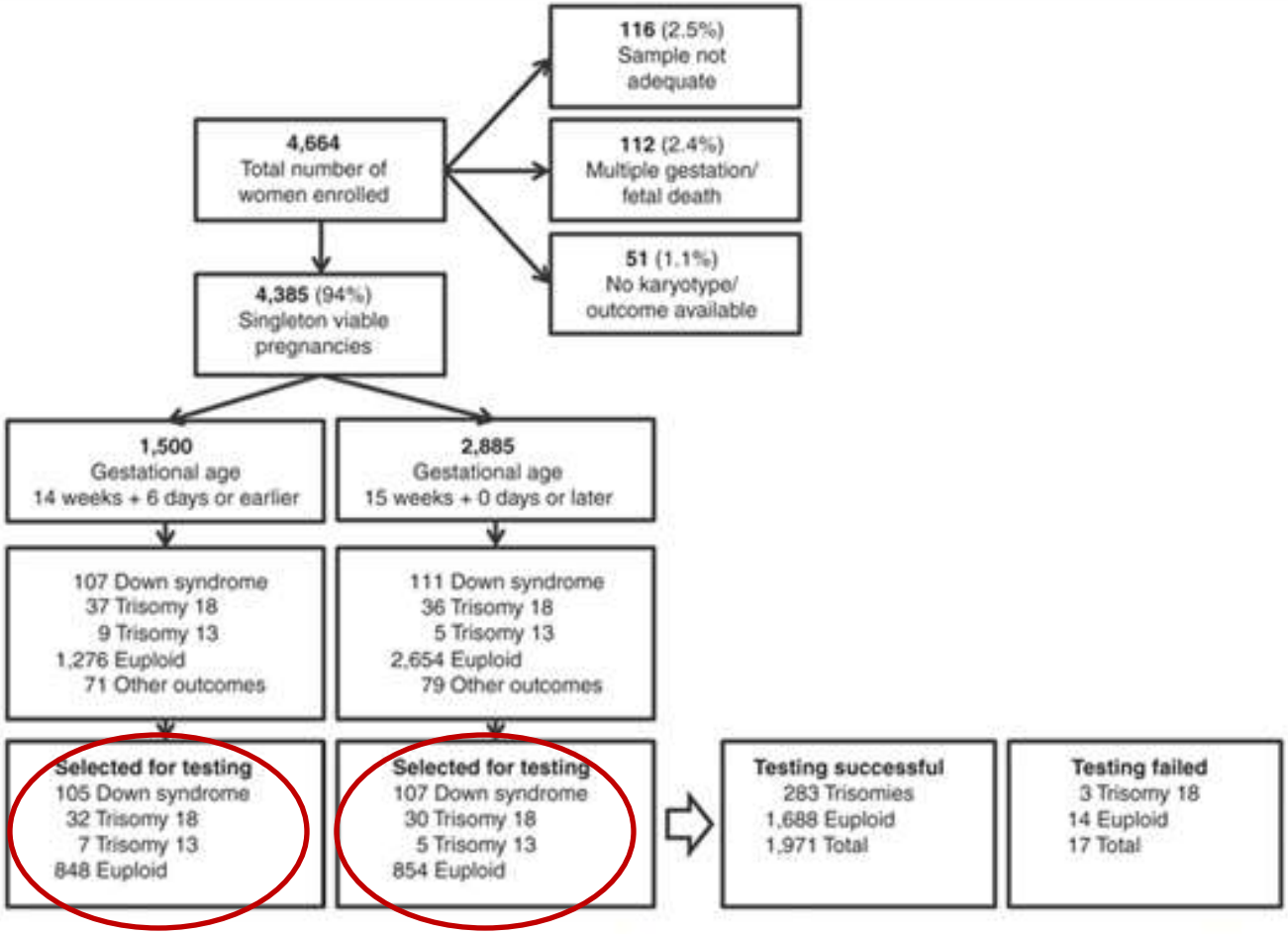
CI, confidence interval; *MLPA*, multiplex ligation-dependent probe amplification; *MPS*, massively parallel sequencing; *RT*, reverse transcriptase; *SNP*, single nucleotide polymorphisms; *T*, trisomy.
[a]All CIs have been calculated by one of the authors (H.W.) using data published in the articles quoted.

Chitty. Noninvasive prenatal testing for aneuploidy. Am J Obstet Gynecol 2012.

DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study

Glenn E. Palomaki PhD, Cosmin Deciu MS, Edward M. Kloza MS, Geralyn M. Lambert-Messerlian PhD, James E. Haddow MD, Louis M. Neveux BA, Mathias Ehrich MD, Dirk van den Boom PhD, Allan T. Bombard MD, MBA, Wayne W. Grody MD, PhD, Stanley F. Nelson MD & Jacob A. Canick PhD

Genetics in Medicine (2012) 14, 296–305 | doi:10.1038/gim.2011.73



Flow chart showing the cohort of samples collected, those selected for testing, and the numbers for which testing was successful. This report focuses on 1,988 pregnancies subject to testing. Of these, 286 were common trisomies (Down syndrome, trisomy 18, and trisomy 13), along with 1,702 matched euploid controls.

Firma Son yayın	Sequenom Palomaki, 2011 ve 2012	Verinata Bianchi, 2012, 2014	Ariosa Norton, 2012 Nicolaides, 2012	Natera Zimmermann, 2012 Nicolaides, 2013	BGI Jiang, 2012
Test adı	Materni21/plus	Verifi	Harmony	Panorama	NIFTY
Teknik	MP shotgun S	MPSS	DANSR- targeted	NGS –SNP targeted	MPSS
Hedef Anomaliler	21, 18, 13, X, Y Plus +DGS,5p-, 1p36 -, PW/AS	21, 18, 13, X, Y	21, 18, 13 X,Y	21, 18, 13, X, Y, triploidi	21, 18, 13, X, Y
Olgu sayısı	**1971**	**532/1914**	**3080**	**185**	**903**
Duyarlılığı	T21 %99,1 T18 >%99,9 T13 %91,7 X/Y %96,2	T21 %100 T18 %97,2 T13 %78,6 X, Y %93,8-100	T21 %100 T18 %97,4 T13 %80	T21 >%99 T18 >%99 T13 >%99 45,X %91,7	T21 %100 T18 %100 T13 %100 45,X %75
Özgüllüğü	T21 %99,9 T18 >%99,6 T13 %99,7 X/Y %99,7	T21 %100 T18 %100 T13 %100 X ,Y %99,6-100	T21 %99,97 T18 %99,93 T13 >%99	Tümü %100	T21 %100 T18 %99,7 T13 %100
Yanlış negatif	**T21 3/212 T13 1/12 X/Y 1/26**	**T18 1/36 T13 3/14 XX 1/233**	**T21 0/81 T18 1/38 T13 2/10**	**45,X 1/12**	**45,X 1/4**
Yanlış pozitif	**T21, T18, T13 24/1688 (%1,4)**	**T21 6/1909 T18 3/1905**	**T21 1/2888 T18 2/2888 T13 1/1939**	**0**	**T18 1/891**

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

cf-DNA Endikasyonları;

- İleri anne yaşı (≥35)
- Fetal USG de anöploidi riskini arttıran bulgu varsa!!
- Trizomili gebelik öyküsü
- MS- tarama testlerinde trizomi için artmış risk
- 21 ve 13. kromozomlarının katıldığı Robertson tipi translokasyon için taşıyıcı ebeveynlerin gebelikleri

ACMG POLICY STATEMENT | Genetics inMedicine

2013

ACMG statement on noninvasive prenatal screening for fetal aneuploidy

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WHAT ARE THE CURRENT LIMITATIONS OF NIPS?

1. Risk assessment is limited to specific fetal aneuploidies (trisomy 13, 18, and 21) at this time. Some platforms also screen for sex chromosome abnormalities. Approximately 50% of cytogenetic abnormalities routinely identified by amniocentesis will not be detected when trisomy 21, 18, and 13 are the only aneuploidies being screened. When patients <35 years or >35 years are considered separately, 75 and 43% of cytogenetic abnormalities will be missed, respectively.[11,12]
2. Chromosomal abnormalities such as unbalanced translocations, deletions, and duplications will not be detected by NIPS. Therefore, when fetal anomalies are detected, invasive diagnostic testing and cytogenomic microarray analysis are more likely to detect chromosomal imbalances than NIPS and may be a better testing option.[13]
3. NIPS is not able to distinguish specific forms of aneuploidy. For example, NIPS cannot determine if Down syndrome is due to the presence of an extra chromosome (trisomy 21), a Robertsonian translocation involving chromosome 21, or high-level mosaicism. Identification of the mechanism of aneuploidy is important for recurrence risk counseling and emphasizes the importance of diagnostic testing following NIPS.
4. NIPS does not screen for single-gene mutations.
5. Uninformative test results due to insufficient isolation of cell-free fetal DNA could lead to a delay in diagnosis or eliminate the availability of information for risk assessment. Biologic factors associated with reduced available cell-free fetal DNA include a high body mass index and early gestational age (<10 weeks gestation).[14,15]
6. Currently, it takes longer for NIPS test results to be returned than for test results on maternal serum analytes. Providers should keep this in mind when offering patients NIPS if timing is important for reproductive decision making. In

1-2. Risk değerlendirmesi T21, T18 ve T13 ile sınırlı (bazıları X ve Y için de tarama yapıyor). Eğer tarama bu üç trizomi için yapılırsa AS ile saptanabilecek anomalilerin ~%50 si yakalanamayacaktır (diğer trizomiler, yapısal anomaliler, mozaikler ve aCGH ile tanınabilecek anomaliler). <35 yaş gebelerde bu oran %75 ve >35 yaş gebelerde %43 olacaktır.

3. Trizomilerin translokasyon tipi olup olmadığı genetik danışma için önemlidir bu yüzden NIPS de trizomi saptanırsa kromozom analizi önemlidir.
4. NIPS tek gen mutasyonlarını taramaz.

5-6. NIPS başarısız olabilir, bu da tanının gecikmesine yol açar.

7. NTD-AFP
8. NIPS, 1. trimester USG nin kazançlarını karşılamaz.

SHOULD PRETEST OR POSTTEST GENETIC COUNSELING ABOUT ANEUPLOIDY SCREENING BE PERFORMED?

Pretest information should be provided by a prenatal care provider, a trained designee, or a genetic counselor to ensure patients make informed decisions. Aneuploidy screening is not a routine prenatal test; it is acceptable for patients to decline screening.

Pretest information should include:

1. A brief explanation of the purpose of NIPS.
2. Advantages of NIPS as compared with maternal serum analyte screening.
   - On the basis of available data, detection rates appear to be higher.
   - There is a high negative predictive value for Down syndrome. This may be important for patients seeking to avoid the risks (e.g., fetal loss) inherent with invasive testing.
   - NIPS has a lower false-positive rate, meaning fewer women will receive a "positive" screen, necessitating fewer invasive procedures.
   - Risk assessment is less dependent on gestational age.
3. Considerations for follow-up invasive testing if NIPS indicates an increased risk for aneuploidy.
4. Limitations of NIPS.

Posttest counseling is recommended when NIPS indicates that a patient is at high risk or has a "screen-positive" result. When a "screen-negative" result is encountered, residual

Test öncesi danışma şunları içermeli;

1. NIPS in amacı kısaca açıklanmalı,
2. Diğer anne kanı tarama testlerine karşı avantajları
   1. Eldeki bilgi birikimi ile yakalama oranı daha yüksek
   2. Negativ prediktif değer yüksek
   3. Yanlış pozitiflik oranı daha az
   4. Risk hesaplaması gebelik haftasından bağımsız
3. Eğer NIPS pozitif ise invaziv girişim,
4. NIPS 'in limitleri (Yanlış negatifler, CPM, diğer kromozom anomalileri, vd)

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Opinion

Non-invasive prenatal diagnosis for Down syndrome: the paradigm will shift, but slowly

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1. Down sendromu için "tanı" mı yoksa tarama mı?
   NIPT çok yüksek duyarlığı olan TARAMA testidir,
2. Tarama testi + ise, NIPT invaziv prenatal tanının yerini alabilir mi?
   Diğer tarama testi + ve NIPT+ ise Down sendromu riski 290 kez artmıştır,
   NIPT – ise risk 110 kez azalmıştır ancak "rezidual risk" önemli ve ciddidir,
3. Fetal kromozom analizinde (CVS ve AS) saptanan diğer anöploidileri saptayabilir mi?
   T21 anomalilerin yarısını oluşturur, tarama testleri ile diğer anöploidi ve triploidi için risk hesaplanabilir, ancak NIPT çalışmalarında klinik süreç tamamlanmadı,
4. FISH veya mikroarray teknikleri ile saptanan mikrodelesyon/duplikasyon sendromları?
   NIPT ile mikrodelesyon/duplikasyon tanısının yakın gelecekte gerçekleşmesi bekleniyor,
5. Klasik tarama testlerinin yerini alır mı? Evet ise biokimyasal ve USG nin konjenital malformasyonların erken gebeliklerdeki potansiyel faydalarını kaybeder miyiz?
   USG'nin yerini alamaz.

cf-DNA özellikle non-viable trofoblast kökenli olduğundan "CPM" riskini taşır.
NIPT testleri yüksek riskli gruplarda yapılmış ve yapılmaktadır.
NIPT'nin düşük ve orta risk grubunda performansı daha düşüktür,
buna karşın maliyeti artıracaktır.

EDITORIALS www.AJOG.org

Noninvasive prenatal testing for aneuploidy–ready for prime time?

Lyn S. Chitty, MRCOG; Melissa Hill, PhD; Helen White, PhD; David Wright, PhD; Stephen Morris, PhD

Tarama testi > 1:150 ise
->NIPT + ise ->CVS

1:380 Down s. Prevalansı
500 000 gebelikte 1 389 Down s.

Taramada >1:150 ise

↓

13 646 NIPT %99 DR ve %1 FPR

↓

1 305 CVS

↓

13 düşük ve 1 169 Down s. ⟶ NIPT 200 £ ise ⟶ tarama+tanı 29,7 milyon £

TABLE 2
Projected numbers undergoing noninvasive prenatal diagnosis

Screening			NIPD						CVS	
Cut-off (1 in)	DR_S	FPR_S	NIPD	DR_N	FPR_N	TP_N	FP_N	FN_{S+N}	CVS	Miscarriages
150	85.0%	2.5%	13,646	99.0%	1.0%	1169	136	220	1305	13
500	94.0%	7%	37,704	99.0%	1.0%	1293	377	96	1670	17
1000	96.0%	12%	60,668	99.0%	1.0%	1320	607	69	1927	19
2000	98.0%	19%	94,103	99.0%	1.0%	1348	941	41	2289	23
5000	99.0%	31%	155,944	99.0%	1.0%	1361	1559	28	2921	29
10,000	99.5%	43%	215,785	99.0%	1.0%	1368	2158	21	3526	35

Figures calculated for hypothetical population of 500,000 women. With Down syndrome prevalence at screening of 1 in 360 number of affected fetuses is 1389. Using invasive prenatal diagnosis rather than NIPD with screening cut-off of 1 in 150, 13,646 women would undergo CVS, detecting 1181 Down syndrome prevalence cases with estimated 136 miscarriages.
CVS, chorionic villus sampling; DR_N, detection rate with NIPD; DR_S, detection rate with screening; FN_{S+N}, combined false-negative results with screening and NIPD = number of Down syndrome prevalence cases – TP_N; FP_N, false-positive results with NIPD; FPR_N, false-positive rate with NIPD; FPR_S, false-positive rate with screening; *NIPD*, noninvasive prenatal diagnosis; TP_N, true positives with NIPD.

Chitty. Noninvasive prenatal testing for aneuploidy. Am J Obstet Gynecol 2012.

REVIEW | Genetics inMedicine

Noninvasive prenatal testing: limitations and unanswered questions

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Prior screening algorithms for aneuploidy involve ultrasound and/or serum screening in the first and/or second trimesters, with trisomy 21 detection rates of 81–96% with false-positive rates set at 5%.[7] Initial reports of NIPT describe detection rates of 98–99% or higher for trisomy 21, 44–91% for trisomy 13, and 83–95% for trisomy 18, with false-positive rates of 1–2%.[8–11] Sensitivity and specificity are high; however, the positive predictive value (reliability of a positive test) and the negative predictive value (reliability of a negative test) are affected by the prevalence of the disease in the population tested. The lower the disease prevalence, the higher the negative predictive value (true negatives) and the lower the positive predictive value (true positives). For example, with sensitivity of 100% and specificity of 99% (1 false-positive in 100), if the disease prevalence is high (risk of Down syndrome of 10 per 100, 10%), screening 1,000 patients would result in a positive predictive value of 91%. With a lower disease prevalence (risk of Down syndrome of 1 per 1,000), screening 1,000 patients would result in a positive predictive value of 9%. Therefore, the prevalence of the condition being screened for must be taken into account when interpreting test results, particularly the positive predictive value, as with a lower disease prevalence, a positive result is less reliable (more likely to be a false-positive result).

Duyarlılık %100
Özgüllük %99 (100' de 1 yanlış pozitif)

Prevelans Down sendromu %10 ise 1000 olguda;
Pozitif prediktif değer %91

Prevelans Down sendromu %0,1 ise 1000 olguda;
Pozitif prediktif değer %9

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DNA Sequencing versus Standard Prenatal Aneuploidy Screening

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"The study's endpoint was a comparison of false positive rates for trisomies 21 and 18 between the two methods. The false positive rate for combined trisomies 18 and 21 among those undergoing DNA testing was 0.45 percent while the rate for standard testing was 4.2 percent, a statistically significant difference.

Another comparison was made for positive predictive value of test results: DNA results for ***trisomy 21 had a predictive value of 45.5 percent*** *compared to 4.2 percent in standard testing; DNA results for* ***trisomy 18 had a predictive value of 40.8 percent*** *compared to 8 percent for standard testing, a significant improvement."*

TARAMA Testleri

Tri 21 prevalansı 1:700 →100 Tri 21 doğumu için → 70 000 gebelik

			Trizomi 21 "yakalanacak"	"doğacak"	"fetal kayıp" 0,5-1%
Anne yaşı (>35)	% 10	7 000 AS	**30**	**70**	**35-70**
Üçlü test	% 5	3 500 AS	**60**	**40**	**17-35**
NT	% 5	3 500 AS	**75**	**25**	**17-35**
1. Trim. Tarama	% 4,2	2 940 AS	**85**	**15**	**15-30**
Sequential	%4,2	2 940 AS	**90-95**	**5-10**	**15-30**
cf-DNA	%0,45	315 AS	**98**	**2**	**2-3**

Hedef Trizomi 21 tanısı ise

cf-DNA konfirmasyon sonuçları-1

Trizomi 21		Fetal doku	Gebelik akıbeti	Yorum
1. A1/14	AY+cf-T21	47,+21	tahliye	
2. A86/14	PatUSG+1.TST+AY+cf-T21	47,+21	tahliye	
3. A210/13	PatUSG+1.TST+AY+cf-T21	47,+21	tahliye	
4. A238/13	cf-T21	47 +21	tahliye	
5. A479/13	1.TST+AY+cf-T21	47 +21	tahliye	
6. T56/13	NT+cfT21	47,+21	tahliye	
7. A325/14	PatUSG+AY+4'lü+cf-T21	47,+21	tahliye	
8. A193/14	cf- T21	46/47,+21(35/20)	tahliye	
9. T7/14	NT+ cf-T21	46,der(21q;21q),+21	tahliye	
10.A218/14	PatUSG+1.TST+AY+cf-T21	FISH Tri21	tahliye	
11.191/14	PatUSG+AY+cf **normal**	AS 46/ 47,+21 (2/32) I-FISH AS 10/65 KS 24/46	tahliye Plasenta I-FISH 1. Bölge 50/50 2. Bölge 147/3 3. Bölge 104/9 4. Bölge 130/20	**YANLIŞ NEGATİF** **CPM**

Trizomi 18				
1. A218/13	PatUSG+AY+cfT18	47,+18	tahliye	
Trizomi 13				
1. A92/14	1.TST+AY+**cfT13**	AS N female	doğdu N Plasenta N Karyotip+FISH (145)	**YANLIŞ POZİTİF**

cf-DNA konfirmasyon sonuçları-2

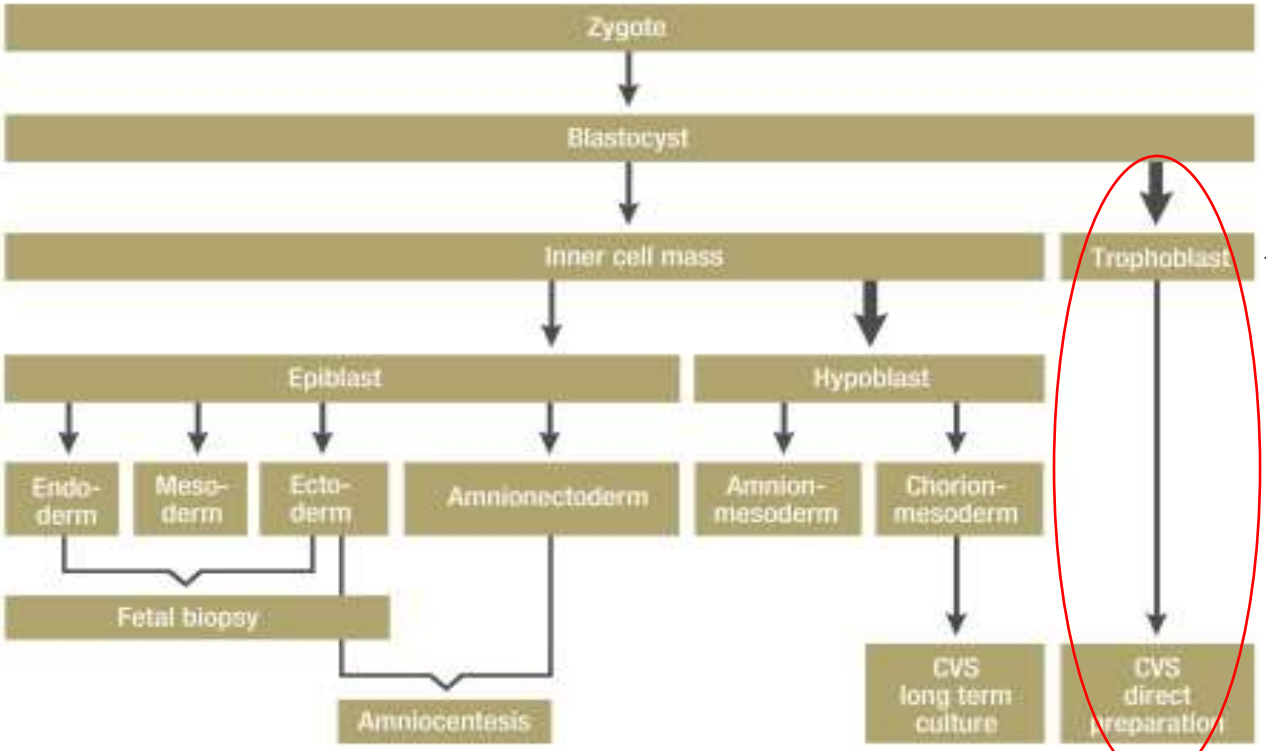
Monozomi X	Fetal doku	Gebelik akıbeti	Yorum
1. A30/14 cf monozomi X	45,X/46,X,i(Xq) (TS)	tahliye	
2. A2/14 AY +cf monozomi X	N female	doğdu	**YANLIŞ POZİTİF**
3. A104/144'lü+ cf monozomi X	N female	devam	**YANLIŞ POZİTİF**
4. A137/14AY+2'li+ cf monozomi X	N female	doğdu	**YANLIŞ POZİTİF**
	Plasenta I-FISH 1-4/100 (4 bölge) ve Kordon fibro 2/98		
5. A114/14 AY+4'lü +cf monozomi X	45,X/46,XX (9/50) I-FISH 2/88	doğdu N	
	Plasenta FISH	Karyotip	
	1. Bölge X/XX/XXX 91/22/1	0/9/5	
	2. Bölge 97/17/0	0/0/18	
	Kordon kanı 2/111/4	8/25/0	
47,XXY			
1.A189/14 AY+cf XXY	47,XXY	devam	
2. A98/14 AY+cf XXY	N male	devam	**YANLIŞ POZİTİF**
3. A282/13 cf XXY	N male	doğdu	**YANLIŞ POZİTİF**

TOPLAM 21 olgudan;
6 sı "Yanlış pozitif"
1 i "Yanlış negatif"
14 ü "doğru"

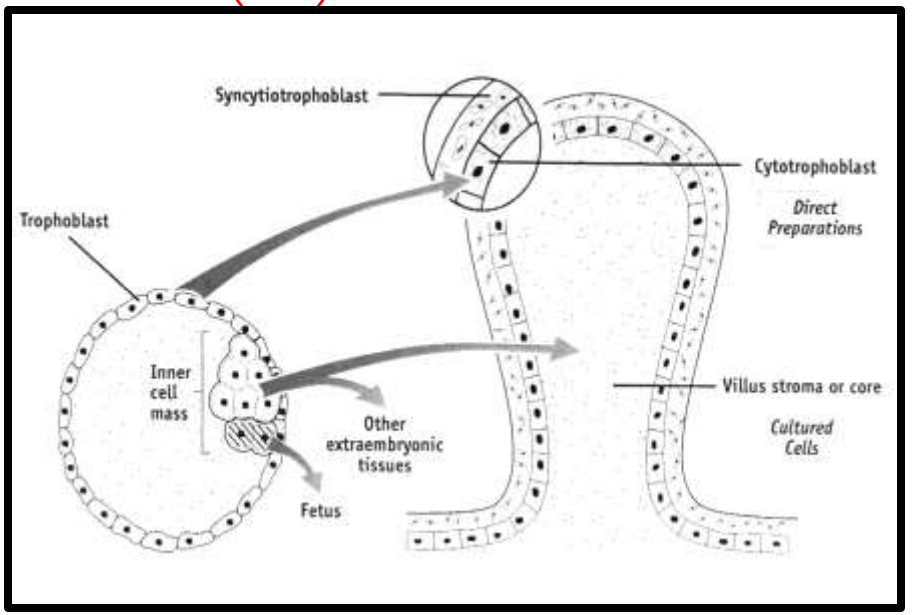
Yapısal Kromozom Anomalisi		
1.A290/13 AY+1.TST+cf DNA normal	45,XY,t(13q;14q)	doğdu

cf-DNA da yanlış pozitif veya yanlış negatif sonuçlanmasının nedenleri;

1. Plasenta ile sınırlı mozaisizm
2. Gerçek düşük oranlı mozaisizm
3. UPD
4. Vanishing twin
5. Maternal mozaisizm
6. Maternal sorunlar (kanser, obesite, vs)
7. Teknik sorunlar



cf-DNA nın kaynağı



CPM tiplerinin sıklıkları
EUCROMIC 1986-1992

48 laboratuvar n:62 865 CVS

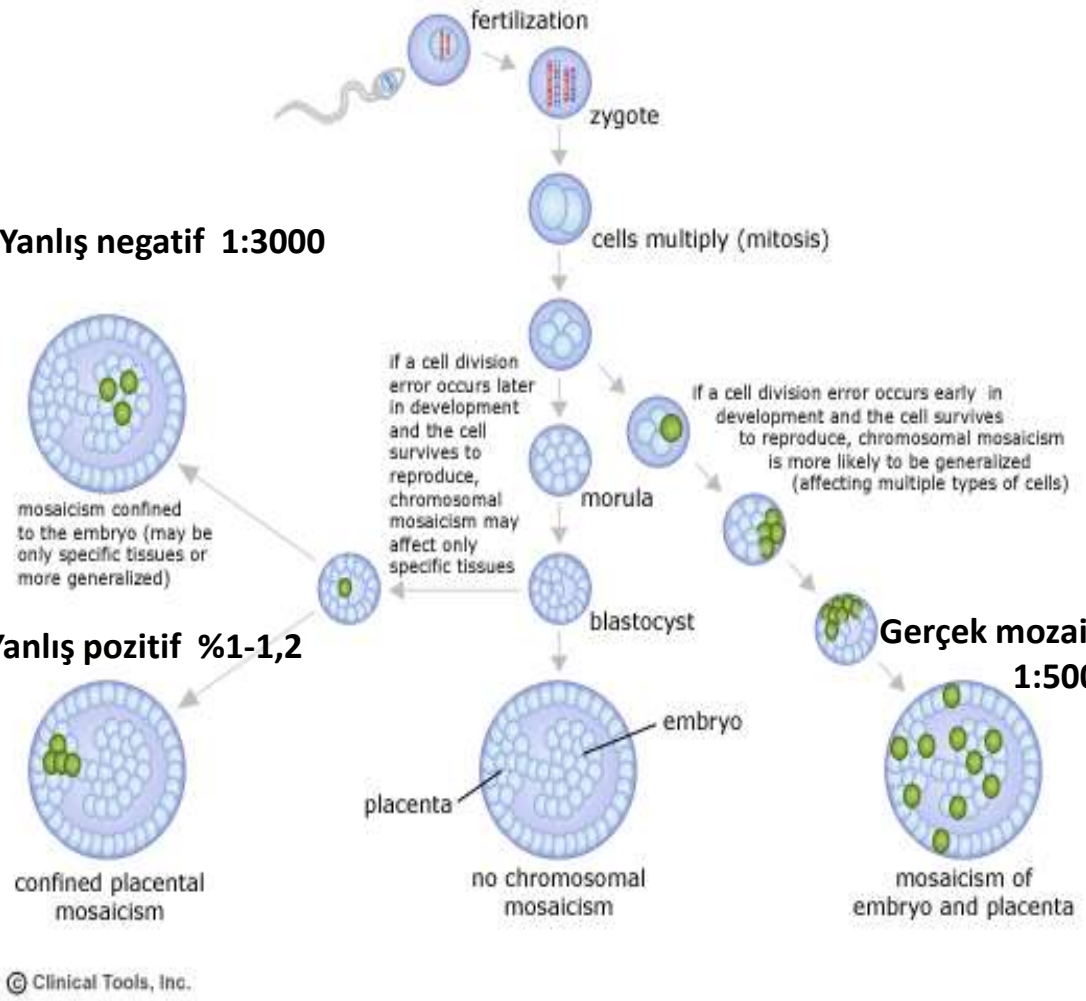
Hahnemann JM&Vejerslev LO, 1997, Prenat Diagn:17;801

normal	59 592	% 94.8	
nonmozaik anormal	2 321	% 3.7	
şüpheli sonuç	***952***	***% 1.51***	
gerçek mozaik	77	% 0.12	cf ??
nonmoz. CV/moz. Fetus	12	% 0.02	
yanlış pozitif CPM	656	% 1.04	cf ->CVS/AS
nonmozaik CPM	96	% 0.15 !!	
yanlış negatif CPM	19	% 0.03!!	cf Normal
sınıflandırılamayan	92	% 0.15	

PLASENTA İLE SINIRLI MOZAİSİZM

Fetus - mozaik Plasenta ve CVS - normal

Yanlış negatif 1:3000



Fetus - normal Plasenta ve CVS - mozaik

Yanlış pozitif %1-1,2

Gerçek mozaik 1:500

Fetus – mozaik Plasenta ve CVS - mozaik

1989-2014/8 ay İTF-TGABD+PREMED Mozaik CVS (n:25, %0,8)

	Endikasyon	CVS-DP	CVS-HK	Fetal doku	Sonuç	Gebelik akıbeti
1	PatUSG	46	47,+21	yok	DP YN	IUMF
2	PatUSG	46	47,+21	AS IFISH T21	DP YN	tahliye
3	PatUSG+1.TRİ	46	47,+18	yok	DP YN	tahliye
4	1.TRİ	46 IFISH N	47,+18	AS IFISH T18	DP YN	tahliye
5	PatUSG	92	47,+13/92	AS 47,+13	DP YN	tahliye
6	PatUSG	46	47,+7	yok	DP YN	tahliye
7	PatUSG	46	46/47,+8	yok	DP YN	tahliye
8	İAY+KAÇ	46	46/47,+9	yok	DP YN	tahliye
9	PatUSG	46	46/47,+9	AS 46	DP YN	tahliye
10	KAE+PatUSG	46,t(11q;22q) IFISH mos	46,t(11q;22q)/47,t(11q;22q)+9	yok	DP YN	tahliye
11	PatUSG	46	46/47,+10	yok	DP YN	tahliye
12	ÜT	46	46/47,+15	AS 46	DP YN	tahliye
13	1.TRİ	46 IFISH mos	47,+16	AS IFISH mos	DP YN	tahliye
14	ÜT+İAY	46	46/47,+16	yok	DP YN	IUMF
15	PatUSG	46	69,XXX	yok	DP YN	tahliye
16	PatUSG	46	69,XXX	yok	DP YN	tahliye
17	PatUSG	46	46/46,dup(11)(p11.2p15.5)dn	46,dup(11)(p11.2p15.5)	DP YN	tahliye
18	PatUSG+1.TRİ	46	45,der(15;18)(p10;p10)dn	45,der(15;18)(p10;p10)	DP YN	tahliye
19	PatUSG+İAY	46,5p+	46,del(5)(p13.3->pter)dn	46,del(5)(p13.3->pter)	DP YN	tahliye
20	1.TRİ	47,+16	üreme yok	AS 46/47+16	GM	tahliye
21	PatUSG+İAY	46/47,+18	47,+18	yok	GM	tahliye
22	PatUSG+1.TRİ	4n	2n/4n	yok	GM	tahliye
23	PatUSG	2n/4n	2n/4n	yok	GM	tahliye
24	PatUSG+1.TRİ	46/47,+idic(14/22)	46/47,+idic(14/22)	yok	GM	doğdu
25	PatUSG+İAY	47,+22	46	yok	HK YN?	tahliye

CVS "DP-Yanlış Negatif" yani cfDNA "yanlış negatif" olacak

19/3090 %0,6 yani ~1:167

1989-2014/8 ay İTF-TGABD+PREMED Mozaik CVS (n:25, %0,8)

	Endikasyon	CVS-DP	CVS-HK	Fetal doku	Sonuç	Gebelik akıbeti
1	PatUSG	**46**	**47,+21**	**yok**	**DP YN**	IUMF
2	PatUSG	**46**	**47,+21**	**AS IFISH T21**	**DP YN**	tahliye
3	PatUSG+1.TRİ	**46**	**47,+18**	**yok**	**DP YN**	tahliye
4	1.TRİ	**46 IFISH N**	**47,+18**	**AS IFISH T18**	**DP YN**	tahliye
5	PatUSG	**92**	**47,+13/92**	**AS 47,+13**	**DP YN**	tahliye
6	PatUSG	46	47,+7	yok	DP YN	tahliye
7	PatUSG	46	46/47,+8	yok	DP YN	tahliye
8	İAY+KAÇ	46	46/47,+9	yok	DP YN	tahliye
9	PatUSG	46	46/47,+9	AS 46	DP YN	tahliye
10	KAE+PatUSG	46,t(11q;22q) IFISH mos	46,t(11q;22q)/47,t(11q;22q)+9	yok	DP YN	tahliye
11	PatUSG	46	46/47,+10	yok	DP YN	tahliye
12	ÜT	46	46/47,+15	AS 46	DP YN	tahliye
13	1.TRİ	46 IFISH mos	47,+16	AS IFISH mos	DP YN	tahliye
14	ÜT+İAY	46	46/47,+16	yok	DP YN	IUMF
15	PatUSG	46	69,XXX	yok	DP YN	tahliye
16	PatUSG	46	69,XXX	yok	DP YN	tahliye
17	PatUSG	46	46/46,dup(11)(p11.2p15.5)dn	46,dup(11)(p11.2p15.5)	DP YN	tahliye
18	PatUSG+1.TRİ	46	45,der(15;18)(p10;p10)dn	45,der(15;18)(p10;p10)	DP YN	tahliye
19	PatUSG+İAY	46,5p+	46,del(5)(p13.3->pter)dn	46,del(5)(p13.3->pter)	DP YN	tahliye
20	1.TRİ	47,+16	üreme yok	AS 46/47+16	GM	tahliye
21	PatUSG+İAY	46/47,+18	47,+18	yok	GM	tahliye
22	PatUSG+1.TRİ	4n	2n/4n	yok	GM	tahliye
23	PatUSG	2n/4n	2n/4n	yok	GM	tahliye
24	PatUSG+1.TRİ	46/47,+idic(14/22)	46/47,+idic(14/22)	yok	GM	doğdu
25	PatUSG+İAY	47,+22	46	yok	HK YN?	tahliye

1989-2014/8 ay İTF-TGABD+PREMED
Fetal dokuda konfirme edilemeyen "Yanlış Pozitif" CVS olguları;

	Endikasyon	CVS-DP	CVS-HK	Fetal doku	"	Gebelik akıbeti
1	1.TRİ+İAY	48,+7,+2/47,+2	46/47,+2	AS 46,XX	CPM DP-YP T7 GM T2	devam
2	PatUSG	48,+7,+18/47,+18	47,+18	yok	CPM DP-YP	tahliye
3	PatUSG	48,+18,+20/ 47,+18/47,+20	47,+18	F. deri 47,XX,+18	CPM DP-YP	tahliye
4	**PatUSG**	**47,XX,+20**	**45,X/46,XX**	**AS 45,X/46,XX**	**CPM DP-YP T20 YN 45,X**	tahliye
5	**İAY+TG**	**46/47,+18**	**46**	**AS 46**	**CPM DP-YP**	**?**
6	1.TRİ	46/47,+3	46	46	CPM DP-YP	devam
7	PatUSG+ICSI	46/46,t(2q;4q)	46	yok	CPM DP-YP	devam
8	İAY+TG	46/48,+mar,+mar	46	AS 46	CPM DP-YP	devam
9	PatUSG+KOÖ	46,1q-	46	KS N	CPM DP-YP	devam
10	İAY	47,+21	47,+21/48,+21,+10	47+21	CPM HK-YP	tahliye
11	KAÇ	yok	46/47,+10	AS 46	CPM HK-YP	devam
12	1.TRİ+ICSI	46	46/47,+2	yok	CPM HK-YP	devam
13	TG	46	46/47,+2	yok	CPM HK-YP	devam
14	PatUSG+İAY+ ICSI	46	46/46,11q+	AS 46	CPM HK-YP	devam

CVS "DP-Yanlış Pozitif" yani cfDNA "yanlış pozitif olacak"
14/3090 %0,45 yani 1:221

1989-2014/8 ay İTF-TGABD+PREMED

Mozaik X anöploidileri n: 15 15/3090 %0,5 yani 1:206

	Endikasyon	CVS-DP	CVS-HK	Fetal doku	Sonuç	Gebelik akıbeti
1	İAY	yok	45,X	AS 47,XXX	GM	doğdu
2	PatUSG	45,X	45,X/46,XX	yok	GM	tahliye
3	PatUSG	45,X/47,XXX	45,X/47,XXX	yok	GM	tahliye
4	1.TRİ	45,X	45,X/47,XXX	yok	GM	devam
5	1.TRİ	45,X/47,XXX	45,X/47,XXX	AS 45,X/46,XX/47,XXX	GM	tahliye
6	PatUSG	45,X	45,X/46,XXq-	yok	GM	tahliye
7	PatUSG	45,X/46,XX/47,XXX	45,X/46,XX/47,XXX	yok	GM	tahliye
8	1.TRİ	45,X I-FISH XY	45,X/46,XY	AS 45,X/46,XY	GM	?
9	1.TRİ+PatUSG	45,X I-FISH XY	45,X/46,XY I-FISH XY	AS 45,X/46,XY	GM	?
10	**PatUSG**	**46,XX**	**45,X**	**yok**	**GM DP YN**	tahliye
11	**PatUSG**	**47,XXX I-FISH XXX**	**45,X**	**yok**	**GM DP YN**	tahliye
12	**PatUSG**	**46,X,r(X)**	**45,X**	**yok**	**GM DP YN**	tahliye
13	**PatUSG**	**45,X/46,XX**	**46,XX**	**KS 46,XX**	**CPM DP-YP**	devam
14	**İAY+ICSI**	**47,XXY**	**46,XY**	**yok**	**CPM DP-YP**	devam
15	**1.TRİ**	**46,X,r(X)**	**46,XX**	**AS 46,XX**	**CPM DP-YP**	devam

Sonuç; Tüm CVS serisinde tüm kromozom anomalileri için

DP-YN 23/3090 %0,74 ~1:135

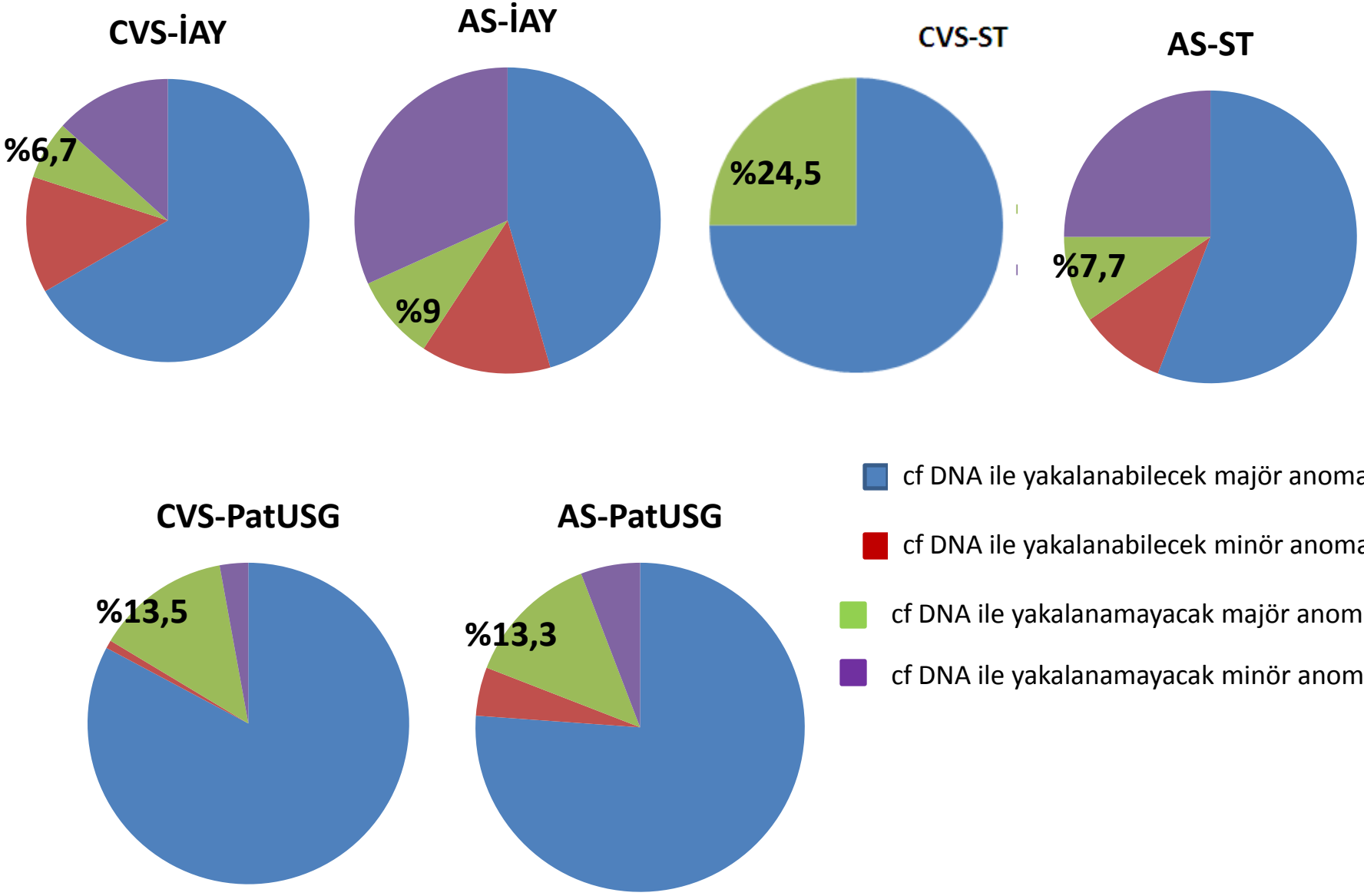
DP-YP 12/3090 %0,39 ~1:256

1989-2011 CVS ve AS serilerinde olguların ana endikasyon gruplarına göre dağılımı ve bu gruplarda saptanan anomali oranları (İTF-TGABD+PREMED)

Girişim endikasyonu	CVS Serisi			AS Serisi		
	Σ n	n ano	ano %	Σ n	n ano	ano %
Parental kro.anomalisi	101	59	58,4	136	126	55,9
Diğer (<1:200)	841	10	1,2	625	6	1
Düşük risk (%1-1,4)	55	4	7,3	956	12	1,3
İleri anne yaşı	335	**16**	**4,8**	10430	**232**	**2,2**
Tarama testi	282	**53**	**18,8**	6749	**186**	**2,8**
Pat-USG	824	**245**	**28,7**	4442	**376**	**8,5**
toplam	2453	387	15,8	22813	889	3,9

CVS serisinin farklı endikasyonlarında saptanan anomalilerde cf-DNA testinin etkinliği (cf-DNA nın %100 yakalama oranı olsaydı)(İTF-TGABD+PREMED, 1989-2011),

		Cf-DNA			Cf-DNA								
					Diğer kromozom anomalileri								
CVS Endikasyon n:2453	Toplam kromozom anomalisi	Sık görülen anöploidiler			**Rezidual Toplam risk**			**Rezidual Dengeli anomaliler**			**Rezidual Dengesiz anomaliler**		
Σn	n	%	n	%	risk	**Σn**	**%**	**risk**	**n**	**%**	**risk**	**n**	**%**	**risk**
Diğer n:841	10	**1,2**	3	0,4	1:250	7	0,8	1:12	5	0,6	1:167	2	0,2	1:500
Düşük risk n:55	4	**7,3**	2	3,6	1:28	2	3,6	1:28	1	1,8	1:56	1	1,8	1:56
İAY n:335	15	**4,5**	12	3,6	1:28	**3**	**0,9**	**1:111**	**2**	**0,6**	**1:167**	**1**	**0,3**	**1:333**
ST n:282	53	**18,8**	39	13,8	1:7	**14**	**5**	**1:20**	**1**	**0,4**	**1:250**	**13**	**4,6**	**1:22**
P-USG n:824	244	**29,6**	204	24,8	1:4	**40**	**4,9**	**1:20**	**7**	**0,9**	**1:111**	**33**	**4**	**1:25**
KAE n:101	59	**58,4**	8	7,9	1:13	**51**	**50,5**	**1:2**	**35**	**34,7**	**1:3**	**16**	**15,8**	**1:6**


CVS-İAY
%6,7
AS-İAY
%9
CVS-ST
%24,5
AS-ST
%7,7
CVS-PatUSG
%13,5
AS-PatUSG
%13,3
cf DNA ile yakalanabilecek majör anomaliler
cf DNA ile yakalanabilecek minör anomaliler
cf DNA ile yakalanamayacak majör anomaliler
cf DNA ile yakalanamayacak minör anomaliler

"cf-DNA Tarama Testi"

1. Test öncesi genetik danışma önemlidir.

2. Aile öyküsü alınmalı ve gebelikte prenatal tanısı mümkün diğer genetik hastalıklar için risk olup olmadığı belirlenmelidir.

3. cf-DNA testi rutin prenatal laboratuvar uygulamalarının bir parçası olmamalı, test hasta seçimine bırakılmalıdır.

4. Eğer fetal USG de herhangi bir anomali saptanırsa invaziv prenatal tanı önerilmelidir.

5. cf-DNA testi sonucu "**pozitiv**" ise genetik danışma ve konfirmasyon amacı ile invaziv prenatal tanı önerilmelidir.

6. cf-DNA test sonucu "**normal" ise** genetik danışma daha da önemli, sonucun incelenen anomiler için "**yanlış negatif"** olabileceği, olası "**diğer kromozom"** anomalilerinin araştırılmadığı anlatılmalı ve USG ile takip önerilmelidir.

7. cf-DNA testi, CVS veya amniosentezin tanısının güvenilirliğini ve doğruluğunu karşılayamaz.

……coming soon …….

www.ScienceTranslationalMedicine.org 6 June 2012 Vol 4 Issue 137 137ra76

RESEARCH ARTICLE

GENOMICS

Noninvasive Whole-Genome Sequencing of a Human Fetus

Jacob O. Kitzman,[1]* Matthew W. Snyder,[1] Mario Ventura,[1,2] Alexandra P. Lewis,[1] Ruolan Qiu,[1] LaVone E. Simmons,[3] Hilary S. Gammill,[3,4] Craig E. Rubens,[5,6] Donna A. Santillan,[7] Jeffrey C. Murray,[8] Holly K. Tabor,[5,9] Michael J. Bamshad,[1,5] Evan E. Eichler,[1,10] Jay Shendure[1]*

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Non-invasive prenatal measurement of the fetal genome

H. Christina Fan[1]†*, Wei Gu[1]*, Jianbin Wang[1], Yair J. Blumenfeld[2], Yasser Y. El-Sayed[2] & Stephen R. Quake[1,3,4]

Ayrıca Türkiye özelinde;

1. Bugüne kadarki uygulamalar nasıl yürütüldü?
2. Genetik "danışma" veriliyor mu?
3. Sonuçlar nasıl ve bunlar kayıt altında mı?
4. Ekstra bir ücret ile yapılan bu tıbbi testte "**sorumluluk**" kimde?
5. Cinsiyet tanısının kötüye kullanımı nasıl kontrol edilecek?
6. Genetik materyalin topluca yurtdışına gönderilmesi için Sağlık Bakanlığının izni gerek
7. cf-DNA testinin yeri nerededir?

Prenatal Tanı?

CVS (n:2 453) ve AS (n:23 338) serilerinde saptanan anomaliler içinde sık görülen 5 anöploidinin oranı ve cf-DNA testinin (hassasiyeti %100 kabul edildiğinde) bu anomalileri hipotetik yakalama oranı ve rezidual riskler (PRETAM+PREMED, 1989-2011)

İnvaziv yöntem, endikasyon, saptanan kromozom anomalileri, ve % leri		cf-DNA testi ile yakalanabilecek anomaliler		cf-DNA testi ile yakalanamayan anomaliler		Endikasyon grublarındaki rezidual risk
		n	%	n	%	%
CVS-ileri anne yaşı 335'de 15 anomali	**%4,5**	12	66,8	3	33,2	%0,9 1:111
AS- ileri anne yaşı 10 430'da 233 anomali	**%2,2**	135	57,9	98	42,1	%0,9 1:111
CVS- Tarama testi + 282'de 53 anomali	**%18,8**	39	73,6	14	26,4	%5 1:20
AS- Tarama testi + 6749'da 185 anomali	**%2,7**	120	64,9	65	35,1	%1 1:100
CVS- Patolojik USG 824'de 244 anomali	**%29,6**	199	81,6	45	18,4	%5,5 1:18
AS- Patolojik USG 4442'de 378 anomali	**%8,5**	298	78,8	80	21,2	%1,8 1:56

Comparison of NIPT Technology Claims

Sensitivity False Positive Rate	Sequenom MaterniT21 plus	Verinata Verifi	Ariosa Harmony	Natera Panorama™
Methodology	**MPSS**	**MPSS with SAFeR**	**Targeted Sequencing with FORTE**	**Targeted Sequencing with SNPs**
Trisomy 21 (Down Syndrome)	**>99.9% 0.2%**	**>99.9% 0.2%**	**>99% <0.1%**	**>99% 0%**
Trisomy 18 (Edwards Syndrome)	**>99.9% 0.3%**	**97.4% 0.4%**	**>98% <0.1%**	**>99% 0%**
Trisomy 13 (Patau Syndrome)	**91.7% 0.9%**	**87.5% 0.1%**	**80% <0.1%**	**>99% 0%**
45,X (Monosomy X)	Not evaluated	**95.0% 1.0%**	Not evaluated	**>99% 0%**

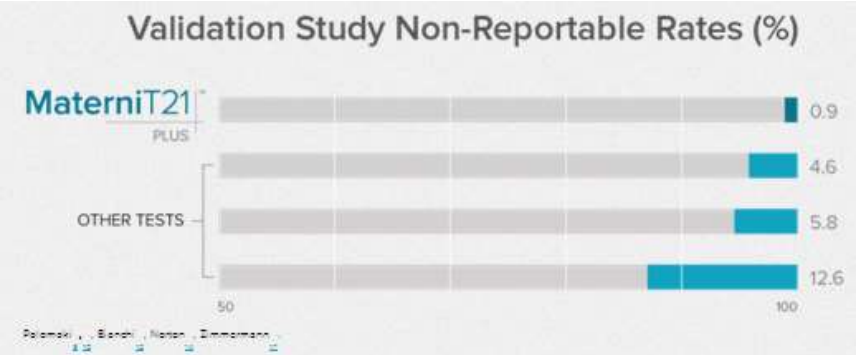
(1) *Zimmermann et al. Prenat Diag 2012 (2) Natera Internal Data (3) Rabinowitz et al. Presented at ASHG 2012*

(2) *Downloaded on February 5, 2013 http://www.sequenomcmm.com/Home/Health-Care-Professionals/Trisomy-21/Performance-Data*

(3) *Downloaded on February 5, 2013 http://www.verinata.com/providers/provider-overview/*

(4) *Downloaded on February 5, 2013 http://www.ariosadx.com*

Sequenom – LifeCodexx – Materni21 plus



MaterniT21 PLUS Test Validation

CLINICAL VALIDATION STUDY	POSITIVE RESULTS	SENSITIVITY	SPECIFICITY
1,696 SAMPLES DNA Sequencing of Maternal Plasma to Detect Down syndrome: An International Clinical Validation Study. Genet Med. 2011;13(11):913-920.	210 OF 212 Trisomy 21	99.1%	99.9%
1,988 SAMPLES DNA Sequencing of Maternal Plasma Reliably Identifies Trisomy 18 and Trisomy 13, as well as Down syndrome: An International Collaborative Study. Genet Med. 2012;14(3):296-305.	59 OF 59 Trisomy 18	>99.9%	99.6%
	11 OF 12 Trisomy 13	91.7%	99.7%
2,015 SAMPLES DNA Sequencing of Maternal Plasma to Identify Down syndrome and Other Trisomies in Multiple Gestations. Prenat Diagn. 2012;32(8):730-734.	8 OF 8 7 OF Trisomy 21 1 OF Trisomy 13	DETECTION RATE: >99.9%	
Y CHROMOSOME			
2,107 SAMPLES Accuracy of noninvasive prenatal fetal sex determination. Poster presented at American Society of Human Genetics Meeting (ASHG); 2012; San Francisco.		ACCURACY: 99.4%	
COMBINED SEX ANEUPLOIDIES	**POSITIVE RESULTS**	**SENSITIVITY**	**SPECIFICITY**
420 SAMPLES Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn. 2013;33(6):591-597.	25 OF 26	96.2%	99.7%

Trizomiler; 21, 18, 13, X and Y ve 16 ve 22
Mikrodeletions;
del 22q11.2 (DiGeorge s.),
5p- (Cri-du-chat s.),
del 15q11.2 (Prader-Willi/Angelman s.,
del 1p36

Verinata - Verifi

Test Performance[1]

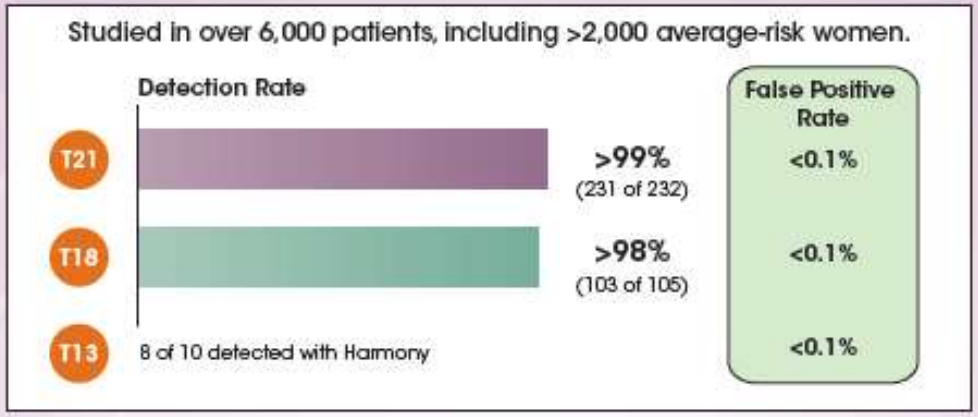
As sequencing technology rapidly evolves, our research team has analyzed and implemented several changes to the testing procedure that yield enhanced test performance.

	N	Sensitivity	95% CI	Specificity	95% CI
21	500	>99.9% (90/90)	96-100.0	99.8% (409/410)	98.7-100.0
18	501	97.4% (37/38)	86.2-99.9	99.6% (461/463)	98.5-100.0
13	501	87.5% (14/16)	61.7-98.5	>99.9% (485/485)	99.2-100.0
Both 'Aneuploidy Detected' and 'Aneuploidy Suspected (Borderline Value)' results were included for performance calculation.					
MX	508	95.0% (19/20)	75.1-99.9	99.0% (483/488)	97.6-99.7

The verifi® test now includes an option for the most common sex aneuploidies, providing information previously known only through invasive results.

	N	Sensitivity	95% CI	Specificity	95% CI	Accuracy	95% CI
XX	508	97.6% (243/249)	94.8-99.1	99.2% (257/259)	97.2-99.9	98.4%	96.9-99.3
XY	508	99.1% (227/229)	96.9-99.9	98.9% (276/279)	96.9-99.8	99.0%	97.7-99.7
XXX, XXY, XYY	Limited data of these more rare aneuploidies preclude performance calculations.						

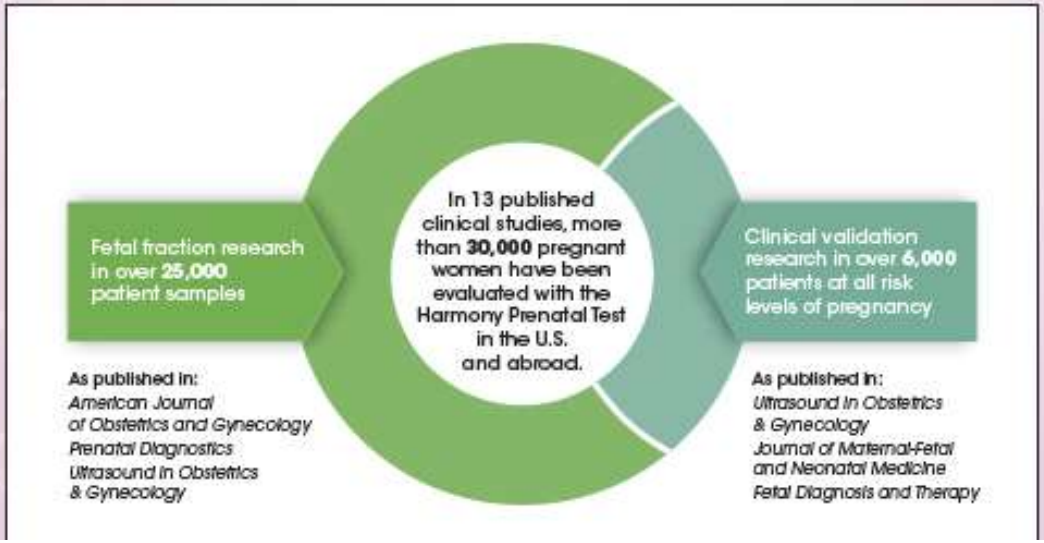
ARIOSA – Harmony test



Detection and false positive rates calculated based on risk cut-off of 1/100 (1%)[4]

- Extremely low cumulative false positive rate
- Optional X,Y sex chromosome analysis with 99% accuracy for fetal sex (95% CI: 95-100)[8]

Exceptional clinical validation, laboratory performance, and ongoing research[1-6, 9-12]



Chromosome-selective sequencing of maternal plasma cell–free DNA for first-trimester detection of trisomy 21 and trisomy 18

Ghalia Ashoor, MD; Argyro Syngelaki, RM; Marion Wagner, MD; Cahit Birdir, MD; Kypros H. Nicolaides, MD

OBJECTIVE: The purpose of this study was to assess the prenatal detection rate of trisomy 21 and 18 and the false-positive rate by chromosome-selective sequencing of maternal plasma cell–free DNA.

STUDY DESIGN: Nested case-control study of cell-free DNA was examined in plasma that was obtained at 11-13 weeks before chorionic villous sampling from 300 euploid pregnancies, 50 pregnancies with trisomy 21, and 50 pregnancies with trisomy 18. Laboratory personnel were blinded to fetal karyotype.

RESULTS: Risk scores for trisomy 21 and 18 were given for 397 of the 400 samples that were analyzed. In all 50 cases of trisomy 21, the risk score for trisomy 21 was ≥99%, and the risk score for trisomy 18 was ≤0.01%. In all 50 cases of trisomy 18, the risk score for trisomy 21 was ≤0.01%, and the risk score for trisomy 18 was ≥99% in 47 cases, 98.8% in 1 case, 88.5% in 1 case, and 0.11% in 1 case. In 3 of the 300 euploid pregnancies (1%), no risk score was provided, because there was failed amplification and sequencing. In the remaining 297 cases, the risk score for trisomy 21 was ≤0.01%, and the risk score for trisomy 18 was ≤0.01% in 295 cases, 0.04% in 1 case, and 0.23% in 1 case. Therefore, the sensitivity for detecting trisomy 21 was 100% (50/50 cases); the sensitivity for trisomy 18 was 98% (49/50 cases), and the specificity was 100% (297/297 cases).

CONCLUSION: In this study, chromosome-selective sequencing of cell-free DNA separated all cases of trisomy 21 and 98% of trisomy 18 from euploid pregnancies.

Key words: first trimester, trisomy 18, trisomy 21

HARMONY 400 kan örneği

300 öploid 3 örnekte risk skoru belirlenememiş.

297 örnekte risk değeri < 0,01% specificity %100

50 T21 50/50 risk değeri ≥ 99% sensitivity %100

50 T18 47/50 risk değeri ≥ 99%

1/50 risk değeri ≥ 98,8%

1/50 risk değeri ≥ 88,5%

1/50 risk değeri 0,11%

Toplam 49/50 sensitivity %98

FNR %2

Ultrasound Obstet Gynecol 2013; **41**: 21–25
Published online 23 November 2012 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.12299

Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method

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*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Ariosa Diagnostics, Inc., San Jose, CA, USA; ‡Department of Fetal Medicine, University College London Hospital, London, UK

2002 örnekten 53 ünde sonuç alınamamış, 1949 örnekte risk skoru alınmış.

10 T13	risk skoru	> 99%	8/10	sensitivity	80.0%
				FNR 20%	
1939 öploid	risk skoru	< 0.01%	1937/1939	specificity	99.9%
		0.79%	1/1939		
		> 99%	1/1939	FPR 0.05% (95% CI,0.0-0.3%)	

PRENATAL DIAGNOSIS
Prenat Diagn 2010; **30**: 702–703.
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.2516

30th Anniversary Issue of Prenatal Diagnosis

HORIZON SCANNING

Noninvasive prenatal diagnosis in 2020

Y. M. Dennis Lo[1,2]*

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make even such an application practically feasible. Furthermore, the rapid development in targeted sequencing, in which only selected parts of the genome are analyzed (Mamanova *et al.*, 2010), would further reduce the costs of this approach. Thus, I would predict that by 2020, many groups would routinely be using massively parallel sequencing technology for the NIPD of multiple monogenic diseases from maternal plasma. Furthermore, as discussed above, the same tests would also provide diagnostic information on fetal chromosomal aneuploidies. Indeed, I would predict that by 2020, the sequencing of the complete fetal genome from maternal plasma would already have been achieved by a number of groups. These developments would likely lead to a drastic reduction in the use of invasive techniques, such as amniocentesis and chorionic villus sampling, at least in the more developed regions of the world. I would foresee that in 2020, there would be multiple articles in *Prenatal Diagnosis* debating about the ethical and social implications of the information explosion from NIPD.

Original Paper

Fetal Diagnosis and Therapy

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DOI: 10.1159/000358326

Received
Accepted
Published

Analysis of Cell-Free DNA in Maternal Blood in Screening for Aneuploidies: Meta-Analysis

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[a]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, and [b]Fetal Medicine Unit, Medway Maritime Hospital, Gillingham, UK

Abstract

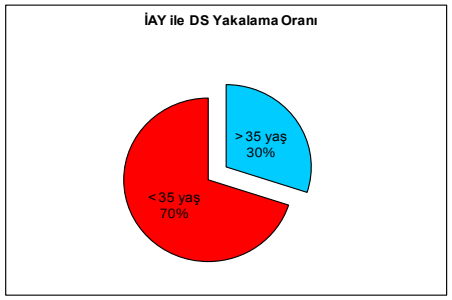
Objective: To review clinical validation or implementation studies of maternal blood cell-free (cf) DNA analysis in screening for aneuploidies and to explore the potential use of this method in clinical practice. ***Methods:*** Searches of PubMed and MEDLINE were performed to identify all peer-reviewed articles on cfDNA testing in screening for aneuploidies between 2011, when the first such study was published, and 20 December 2013. ***Results:*** Weighted pooled detection rates (DR) and false-positive rates (FPR) in singleton pregnancies were 99.0% (95% CI 98.2–99.6) and 0.08% (95% CI 0.03–0.14), respectively, for trisomy 21; 96.8% (95% CI 94.5–98.4) and 0.15% (95% CI 0.08–0.25) for trisomy 18; 92.1% (95% CI 85.9–96.7) and 0.20% (95% CI 0.04–0.46) for trisomy 13; 88.6% (95% CI 83.0–93.1) and 0.12% (95% CI 0.05–0.24) for monosomy X, and 93.8% (95% CI 85.9–98.7) and 0.12% (95% CI 0.02–0.28) for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR was 94.4% (95% 74.2–99.0) and the FPR was 0% (95% CI 0.00–1.84) for trisomy 21. ***Conclusion:*** An analysis of cfDNA in maternal blood provides effective screening for trisomies.

Konjenital Malformasyonlar

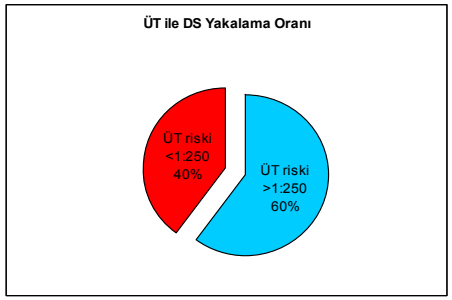
Tanı → USG

- Yeni doğan sıklığı %2-4
- Etiyolojik faktörler;
  - Kromozom anomalileri
  - Tek gen hastalıkları
  - Multifaktöriyel hastalıklar
  - Maternal faktörler
  - Deformasyonlar

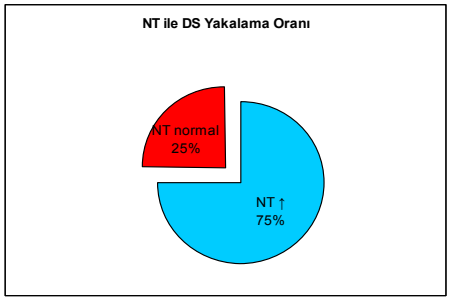
TARAMA Testleri



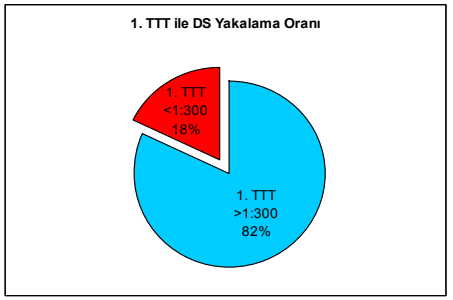
İleri anne yaşı



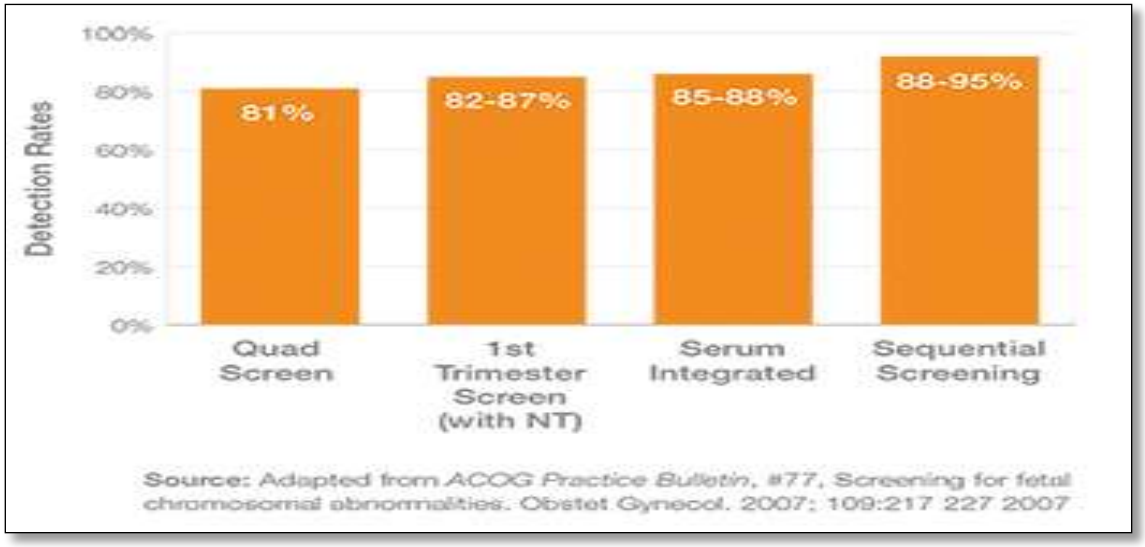
Üçlü test/
Dörtlü test



USG; NT ve
diğer markerler



11-14 hafta tarama



MS- cell free DNA/RNA analizleri ile anöploidi taraması

1989-2014/8 ay İTF-TGABD+PREMED
nonmozaik CVS – normal/moz AS karyotip (5/3090 - %0,16, 1:625)

	Endikasyon	CVS-DP	CVS-HK	Fetal doku	Sonuç	Gebelik akıbeti
1	1.TRİ	47,+13	47,+13	AS 46 I-FISH mos	GM - AS YN	USG N tahliye
2	PatUSG+1.TRİ	47,+16	47,+16	AS 46 I-FISH mos	GM - AS YN	tahliye
3	1.TRİ	47,+16	47,+16	AS 46	GM - AS YN	USG; IUGG+oligohidr.+ HI tahliye
4	1.TRİ+İAY	I-FISH +16	47,+16	AS 46	GM / CPM YP/UPD	USG N gebelik devam
5	PatUSG	yok	47,+16	AS 46 I-FISH N	CPM YP/UPD/GM?	Diafragma hernisi nedeniyle tahliye

Konfirmasyon için AS'da I-FISH sonuçları ÇOK ÖNEMLİ !!!

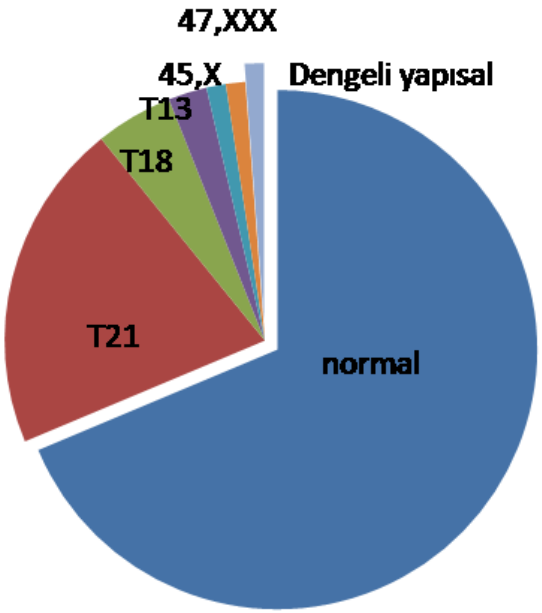
CVS Serisi 2010-2014/8

izole NT-CVS

toplam 83	n	%
normal	57	68,7
T21	17	**20,5**
T18	4	**4,8**
T13	2	**2,4**
45,X	1	**1,2**
47,XXX	1	1,2
dengeli yapısal	1	**1,2**

28,9%

31,3%



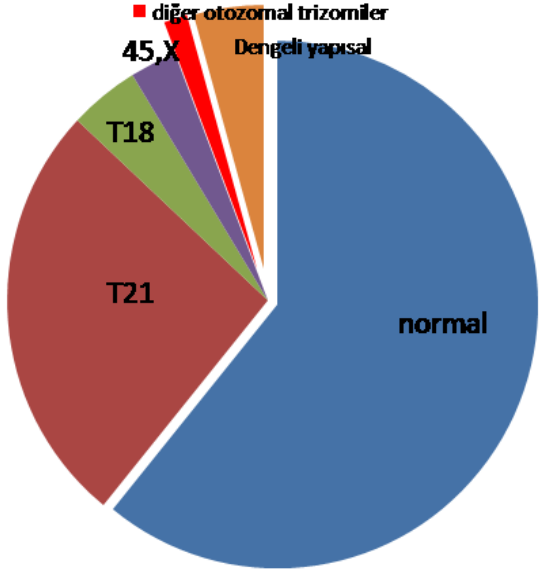
NT+1.TTT-CVS

toplam 69	n	%
normal	42	60,1
T21	18	**26,1**
T18	3	**4,3**
45,X	2	**2,9**
diğer otozomal trizomiler	1	**1,5**
dengeli yapısal	3	**4,3**

33,3%

39,1%

5,8%



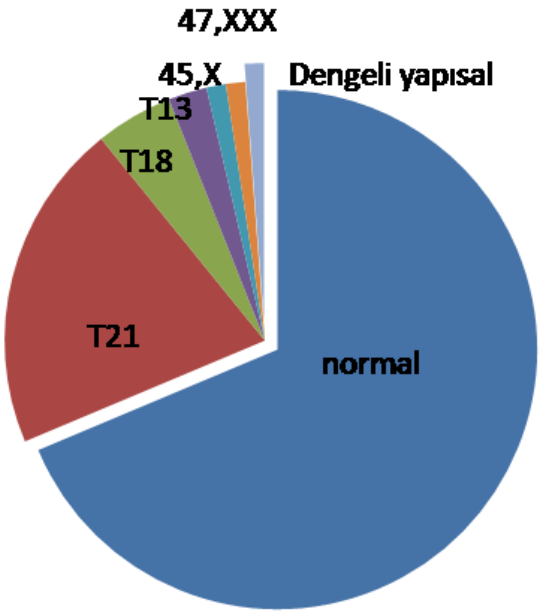
CVS Serisi 2010-2014/8

izole NT-CVS

toplam 83	n	%
normal	57	68,7
T21	17	**20,5**
T18	4	**4,8**
T13	2	**2,4**
45,X	1	**1,2**
47,XXX	1	1,2
dengeli yapısal	1	**1,2**

28,9%

31,3%



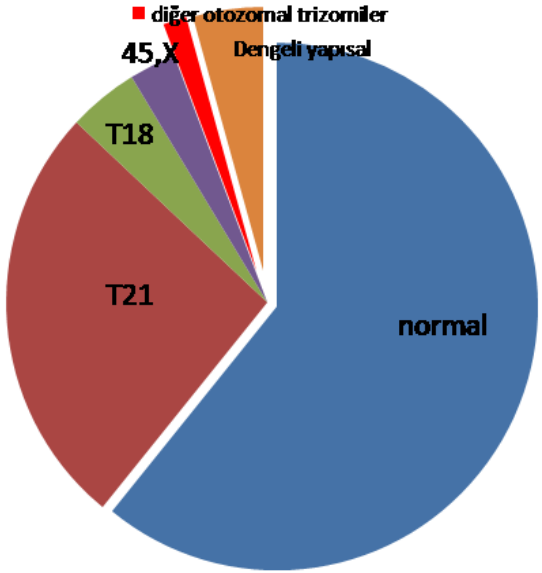
NT+1.TTT-CVS

toplam 69	n	%
normal	42	60,1
T21	18	**26,1**
T18	3	**4,3**
45,X	2	**2,9**
diğer otozomal trizomiler	1	**1,5**
dengeli yapısal	3	**4,3**

33,3%

39,1%

5,8%



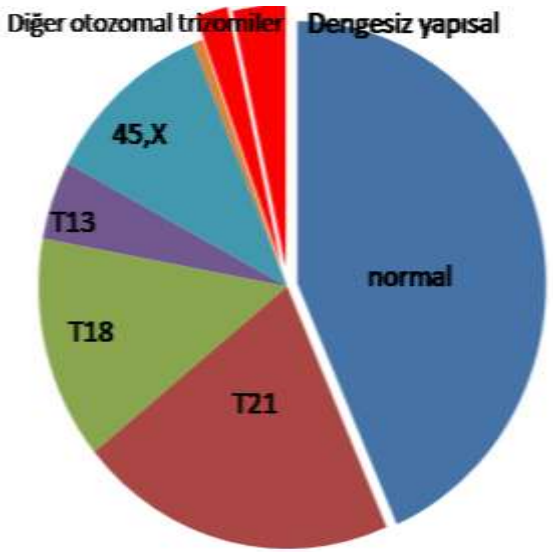
NT+multiple anomaliler-CVS

toplam 145	n	%
normal	63	43,4
T21	**30**	**20,7**
T18	**20**	**13,8**
T13	**7**	**4,8**
45,X	**16**	**11**
47,XYY	1	0,7
diğer otozomal trizomiler	3	**2,1**
dengesiz yapısal	5	**3,5**

51%

56,6%

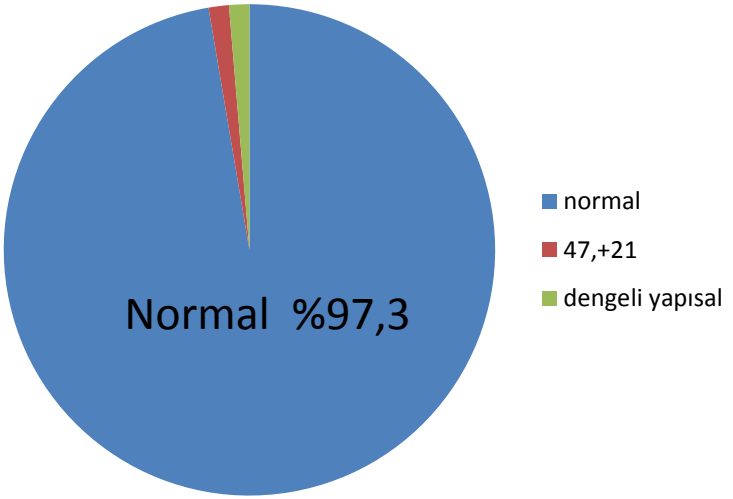
5,6%



AS Serisi 2010-2014/8

İzole Ense Plisi artışı-AS

toplam 75	n	%
abnormal	**2**	**2,7**
normal	73	97,3
47,+21	**1**	**1,3**
dengeli yapısal	1	**1,3**



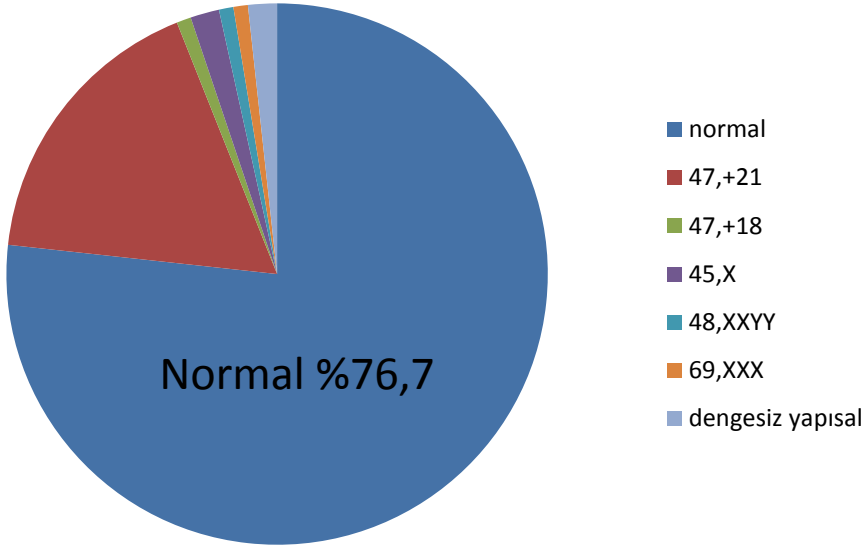
EP+ multiple anomaliler-AS

toplam 116	n	%
abnormal	**27**	**23,3**
normal	89	76,7
47,+21	**20**	**17,2**
47,+18	**1**	**0,9**
45,X	**2**	**1,7**
48,XXYY	1	0,9
69,XXX	1	**0,9**
dengesiz yapısal	2	**1,7**

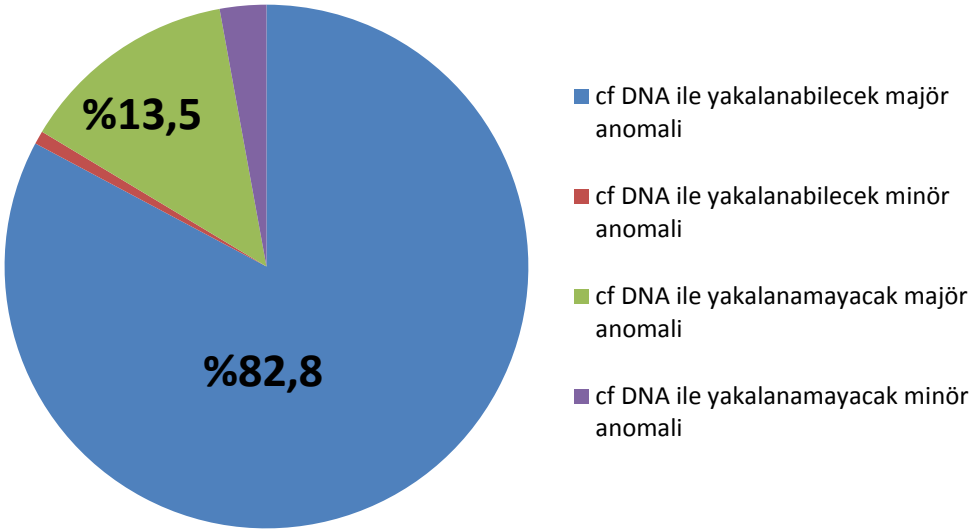
19,8%

23,3%

2,6%



CVS-PatUSG



AS-PatUSG