I test non invasivi per la diagnosi prenatale

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

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www.unibo.it

Items

- Estensione ad altri cromosomi
- NT e DNA fetale
- Soft marker e DNA fetale
- Microdelezioni – Sindrome di Di George (22q11)
- Pannelli per le malattie mendeliane
- Mancata risposta: ff bassa
- ffcfDNA e patologie ostetriche

SCA

1. Motivi etici (non facile correlazione fra genotipo e fenotipo)
2. Screening meno accurato (90.3% DR FPR 0.23% Gil et al.)
3. Falsi positivi da mosaicismo placentare
4. Mosaicismo materno dell'X (non per il metodo SNPs)
5. SCA della madre non diagnosticate

Altri cromosomi

- Set di anomalie molto variabile e non ben definite (specialmente se diagnosi al 2 trimestre)

- Alcuni cromosomi come il 6, 7, 11, 14, 15, e 20 vanno incontro a disomie uniparentali

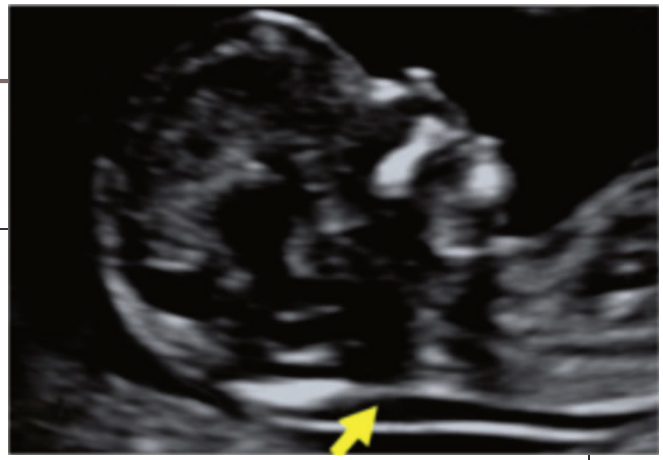
Is nuchal translucency measurement useful when cell-free DNA testing is performed?

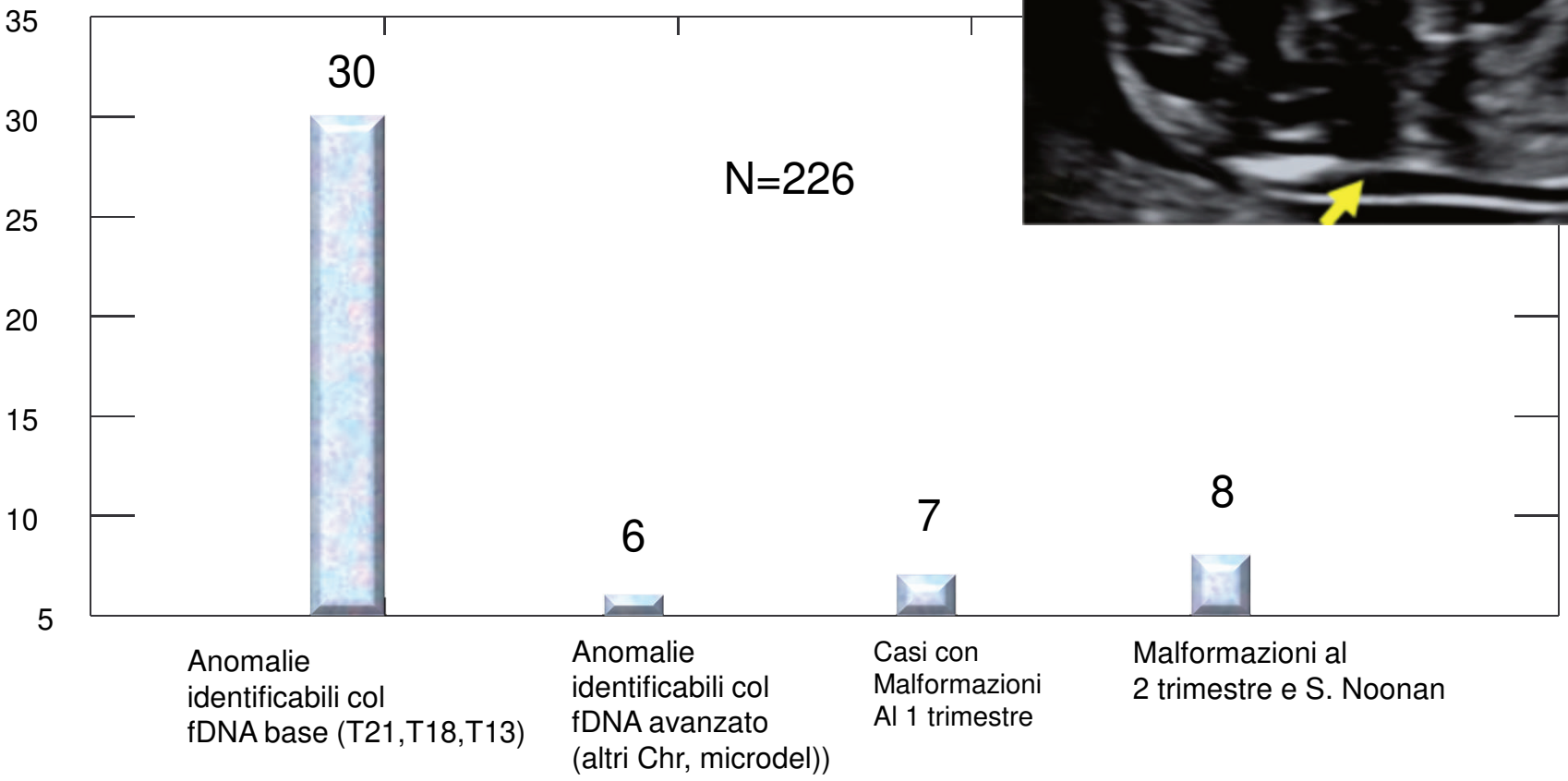
- *Lichtenbelt,15*: When NIPT for trisomies 13, 18, and 21 is offered to all women, NT measurement by itself has a limited added clinical value for the detection of fetal chromosomal anomalies.

- *O'Brien,17*: A positive NT ultrasound scan did not add to the cases of aneuploidy that were detected by cfDNA screening.

- *Langlois,17*: For women with a negative cfDNA screening result, NT measurement has limited clinical utility.

Frequenza delle anomalie genetiche NT>99 centile





Cortesia del prof Borrell University of Barcelona

How to Integrate Cell-Free DNA Screening With Sonographic Markers for Aneuploidy: An Update

Thomas C. Winter[1]
Nancy C. Rose[2]

OBJECTIVE. The sonologist detects a so-called "soft marker" during approximately 10% of routine second-trimester anatomy examinations and is often uncertain about what further management is appropriate. This article will specifically address the management of patients with sonographic markers for six common entities: choroid plexus cysts (CPCs), ventriculomegaly (VM), echogenic intracardiac focus (EIF), urinary tract dilation (UTD), fetal echogenic bowel (FEB), and femoral and humeral shortening. The use of cell-free DNA screening and its relationship to these sonographic findings will be reviewed.

I soft marker come CPC, Foci iperecogeni, pielectasia minima, ventricolomegalia minima sono irrilevanti

Altri come la venticolomegalia moderata, l'intestino iperecogeno, lunghezza femore/omero<2.5 centile e pielectasia sono da considerare per possibili malattie cromosomiche/mendeliane/infettive e nongenetiche (Winter and Rose, 2018)

Society for Maternal-Fetal Medicine
High-risk pregnancy experts

Society for Maternal-Fetal Medicine (SMFM) Consult Series | #42
smfm.org

The role of ultrasound in women who undergo cell-free DNA screening

Society for Maternal-Fetal Medicine (SMFM) with the assistance of Mary E. Norton, MD; Joseph R. Biggio, MD; Jeffrey A. Kuller, MD; Sean C. Blackwell, MD

diagnostic testing should not be recommended to patients solely for the indication of an isolated soft marker in the setting of a negative cell-free DNA screen (GRADE 2B) in women with an isolated soft marker that has no other clinical implications (ie, choroid plexus cyst or echogenic intracardiac focus) and a negative cell-free DNA screen, we recommend describing the finding as not clinically significant or as a normal variant (GRADE 2B);

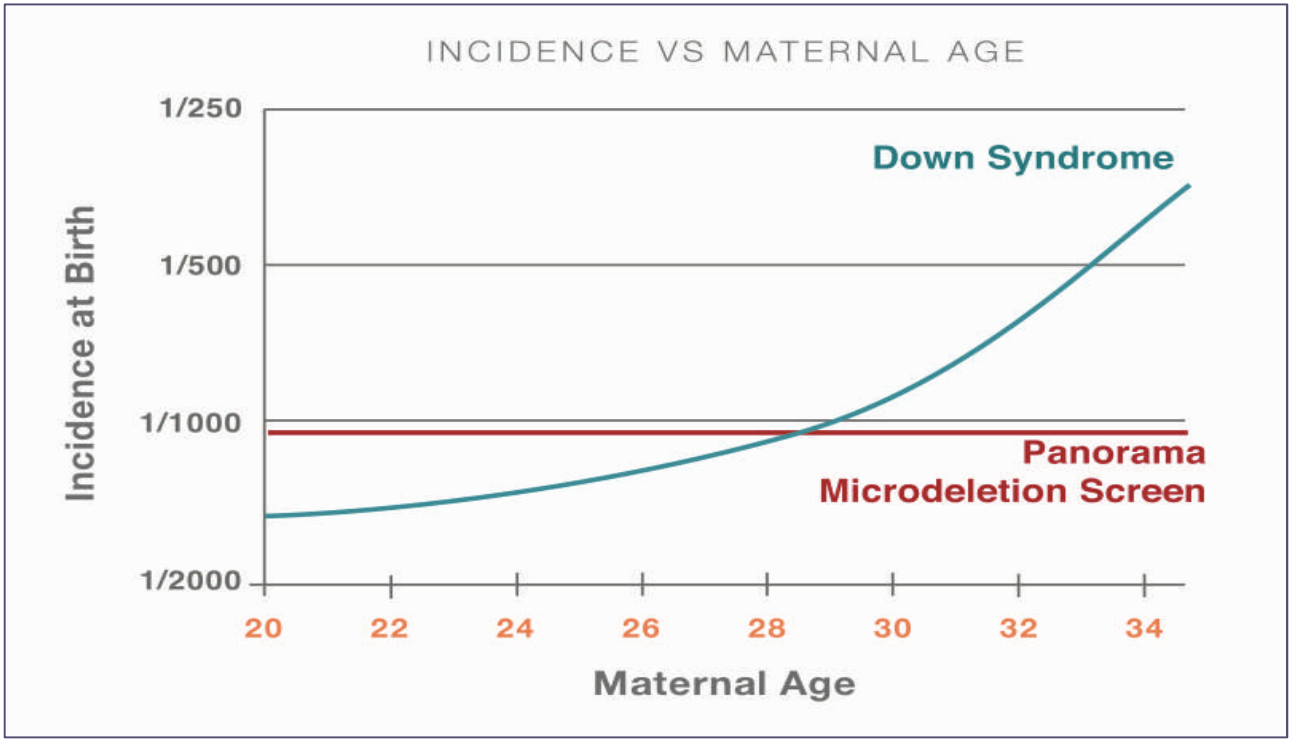
All women in whom a structural abnormality is identified by ultrasound should be offered diagnostic testing with chromosomal microarray (GRADE 1A)

1A: Strong recommendation,high-quality evidence
1B; Strong recommendation, moderate-quality evidence
2B: Weak recommendation, moderate-quality evidence

NT e soft Marker vs cff-DNA

- Per la diagnosi delle Trisomie più comuni i soft marker non hanno alcuna utilità
- Nessun LR è in grado di riportare ad un valore di rischio >1:300 un DNA fetale negativo
- Non è corretto utilizzare marker meno sensibili solo sulla base temporale degli eventi
- NT>99 può essere utile ad identificare patologie rare cromosomiche, subcromosomiche e mendeliane
- Alcuni soft marker se oltre il valore minimo (VM, DCP) possono essere utili per cariotipo molecolare
- In un sospetto di quadro sindromico è raccomandabile un cariotipo molecolare

Microdelezioni



[1]Snijders, et al. *Ultrasound Obstet Gynecol 1999;13:167–170.*
[2]Combined prevalence using higher end of published ranges from Gross et. al., *Prenatal Diagnosis 2011; 39, 259-266;* and www.genetests.org.
Total prevalence may range from 1/1071 - 1/2206.

Condition	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value	Negative Predictive Value
22q11.2 deletion syndrome[7,8,9]	95.7% (CI 85.5-99.5)	>99 (CI 98.6-99.9)	20%**	99.97-99.99%***
1p36 deletion syndrome[7,8]	>99% (CI 2.5-100)	>99% (CI 99.1-100)	7-17%***	99.98-99.99%***
Angelman syndrome[7,8]	95.5% (CI 77.2-99.9)	>99% (CI 99.1-100)	4%	>99.99%
Cri-du-chat syndrome[7,8]	>99% (CI 85.8-100)	>99% (CI 99.1-100)	2-5%***	>99.99%
Prader-Willi syndrome[7,8]	93.8% (CI 69.8-99.8)	>99% (CI 99.1-100)	5%	>99.99%

* Ongoing clinical follow-up is performed to ensure the NPV does not fall below the quoted value but follow up is not obtained for all low risk calls.

** PPV for 22q11.2 deletion syndrome in published studies was 20% when no ultrasound anomalies were seen and was up to 100% when ultrasound anomalies were seen prior to testing.

*** Dependent upon fetal fraction, see Panorama Risk score on report for accurrate PPV/NPV for a specific patient.

For additional information, please visit: ***www.natera.com/panorama-test/test-specs***

MaterniT GENOME Lab Report

Sequenom Laboratories
3595 John Hopkins Court, San Diego, CA 92121
CLIA #: 05D2015356 CAP #: 7527138
Lab Director: Phillip Cacheris, MD, PhD
Tel: 877.821.7266

FINAL REPORT

Performance Characteristics

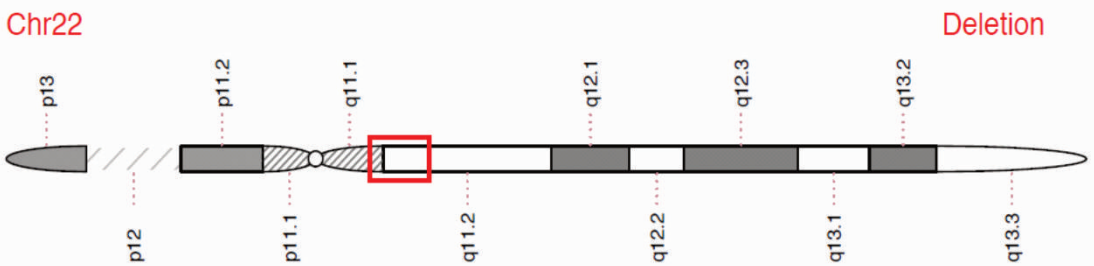
Region (associated syndrome)	Size Range (Mb)*	Median Size (Mb)*	Reportable Fetal Fraction	Estimated Sensitivity**	Estimated Specificity
Genome-wide	NA	NA	≥ 4%	96% (61–> 99%)	> 99.9%
22q11.2 (DiGeorge)	0.8–3.6	2.6	≥ 4%	> 74% (17–94%)	> 99.9%
15q11.2 (Prader-Willi & Angelman)	1.2–15.8	5.1	≥ 4%	> 59% (16–74%)	> 99.9%
11q23 (Jacobsen)	1.3–15.7	9	≥ 4%	> 87% (57–> 99%)	> 99.9%
8q24.11-q24.13 (Langer-Giedion)	7.6–8.8	7.9	≥ 4%	> 97% (80–> 99%)	> 99.9%
5p15.3 (Cri du Chat)	1.5–17.8	6	≥ 4%	> 83% (48–96%)	> 99.9%
4p16.3 (Wolf-Hirschhorn)	1.1–17.3	4.2	≥ 4%	> 73% (37–91%)	> 99.9%
1p36 (1p36 deletion syndrome)	1.6–13.3	3.8	≥ 4%	> 51% (13–81%)	> 99.9%

** Sensitivity estimated across the observed size distribution of each syndrome [per ISCA database nstd37] and across the range of fetal fractions
observed in routine clinical NIPT. Figures in parentheses indicate upper and lower estimates for sensitivity at the lowest reportable fetal fraction (4%)
and at fetal fraction ≥20%, respectively. Actual sensitivity can also be influenced by other factors such as the size of the event, total sequence counts,
amplification bias, or sequence bias.

Test Result	**Positive** **Loss of chromosome 22(q11.2) material**

Laboratory Director's Comments

A loss of chromosome 22 material was observed. It is estimated to be 2.6 Mb in size and is suggestive of a deletion in the region 22q11.2, which is associated with DiGeorge syndrome. Genetic counseling and clinical correlation are recommended. Confirmatory testing is required if fetal confirmation and clinical interpretation of the suspected event are desired. Please refer to the "Performance" and "Limitations of the Test" sections of this laboratory report for additional information.



Description: An approximate 2.6 Mb loss of chromosome 22 material was observed, suggestive of a deletion in the region q11.2, associated with DiGeorge syndrome.

CONCLUSIONE

ALTO RISCHIO per delezione 22q11.2 (Sindrome di DiGeorge)

Si consiglia consulenza genetica. Si segnala che l'esame svolto è un test di screening, pertanto per una diagnosi definitiva è necessario eseguire test diagnostici con metodica microarray su villi coriali o liquido amniotico.

ESITO DEL TEST

Sesso del feto: Femmina

Frazione fetale: 7,2 %

Microdelezione testata [1]	Rischio a priori [2,4]	Rischio Panorama [3]	Risultato
22q11.2 (DiGeorge)	**1/2000**	**1/19**	**Alto rischio**
1p36	1/5000	1/12400	Basso rischio
Angelman	1/12000	1/16600	Basso rischio
Cri-du-chat	1/20000	1/57100	Basso rischio
Prader-Willi	1/10000	1/13800	Basso rischio

harmony®
PRENATAL TEST

Toma
Il tuo futuro, oggi
Istituto di Genetica Umana dal 1979

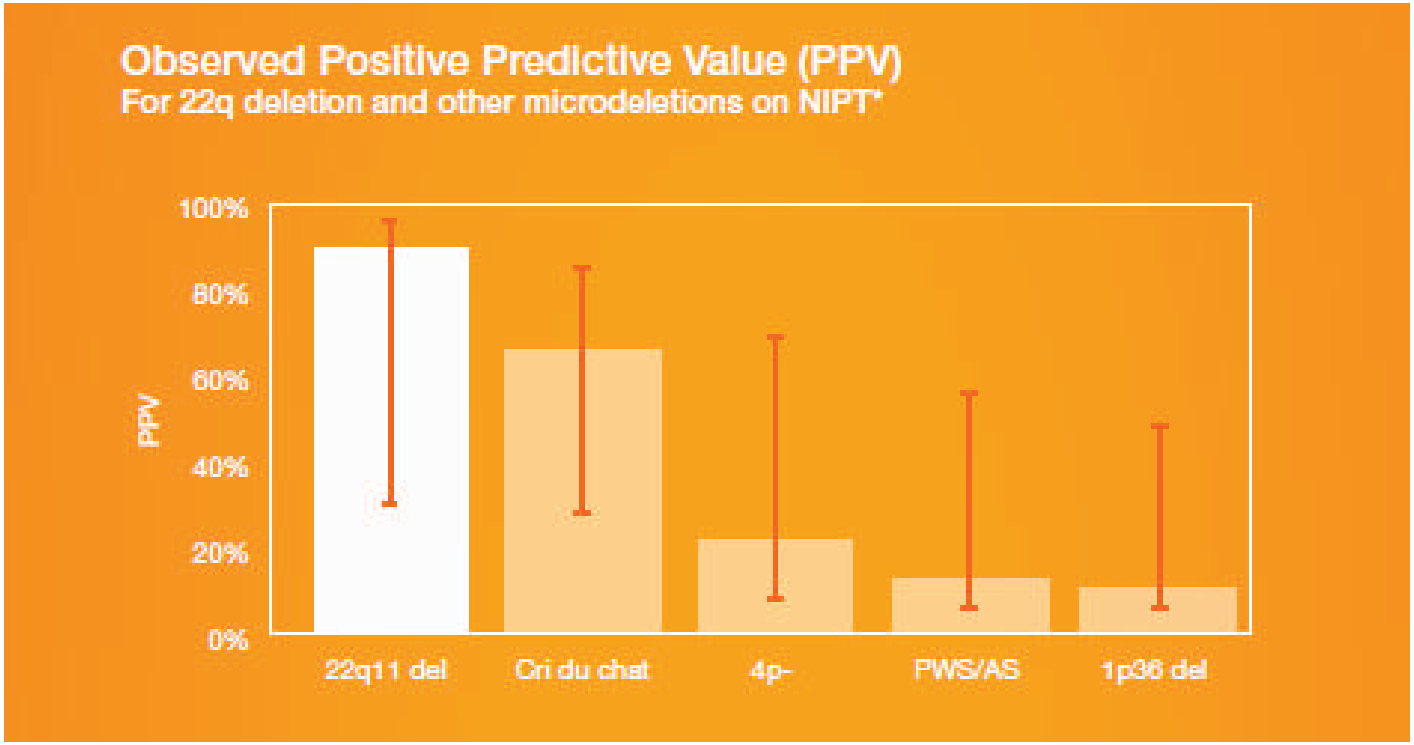
Screening della microdelezione 22q11.2 col test Harmony

Delezione 22q11.2

	Sensibilità	Rischio di falso positivo
Interne alla regione tipica da 3Mb*	75%[3-4]	0.5%[3-4]

*Include anche le piccole microdelezioni atipiche localizzate internamente alla regione tipica da 3Mb

Manca la Sensibilità e i FP



22q11: Sequenom vs. Natera

Sequenom

- Frazione fetale più alta (>4%)
- Size media 2.6 Mb
- Sensibilità del 17% se la frazione fetale è >4%
- Sensibilità dell'94% per frazione fetale >=20%
- VPP Non riportato

Natera

- Frazione fetale più bassa (2.8%)
- 672 SNPs targeting fino a md >2.91 Mb nella regione 22q11.2 (87%) di tutti i casi di 22q cases.
- Non validato per delezioni più piccole, anche se occasionalmente si trovano 1.2Mb.
- Alta sensibilità (99%)
- VPP 20%

Quante S. di DiGeorge ??????

Cytocell an OGT company aquarius

Instructions For Use
REF: LPU 015-S / LPU 015

DiGeorge II (10p14) Probe

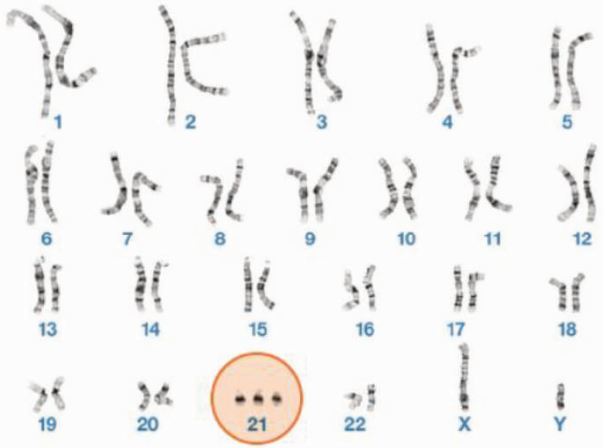
Probe Information

DiGeorge syndrome[1], and a variety of congenital malformation syndromes including Velocardiofacial syndrome (VCFS)[2], share the deletion of chromosome 22 at 22q11.2[2,3,4,5]. These chromosome 22 deletions are collectively coined CATCH22, a mnemonic that covers the clinical findings of Cardiac abnormality, Abnormal facies, Thymic aplasia, Cleft palate and Hypocalaemia/Hyperthyroidism due to a chromosome 22 deletion. In DiGeorge syndrome, however, cases have also been found in which patients have a deletion on chromosome 10p13.4 (DGS2) instead of chromosome 22[6, 7,8]. The deletion of the DGS2 locus on 10p may be 50 times less frequent than that of the DGS1 locus on 22q and has been estimated to occur in 1 in 200,000 live births[9]. A gene called BRUNOL3 (now named CELF2) has been identified within the 300kb minimally deleted region of DGS2 and is postulated to be involved in the DGS2 deletion[10]. BRUNOL3 is a candidate gene for the heart defect and thymus hypoplasia/aplasia associated with partial monosomy 10p[10] and may be involved in atrial septal defects (ASDs), a common cardiac anomaly associated with DGS2[11].

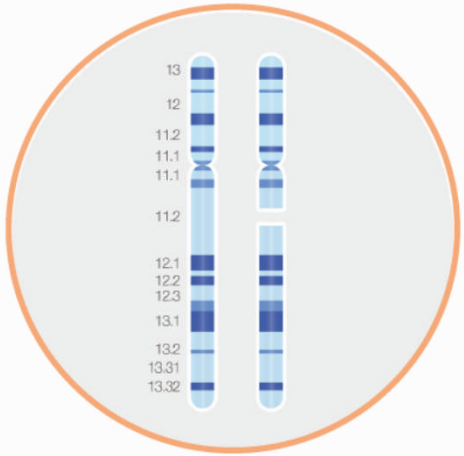
Evolution of NIPT

Now can provide more comprehensive coverage

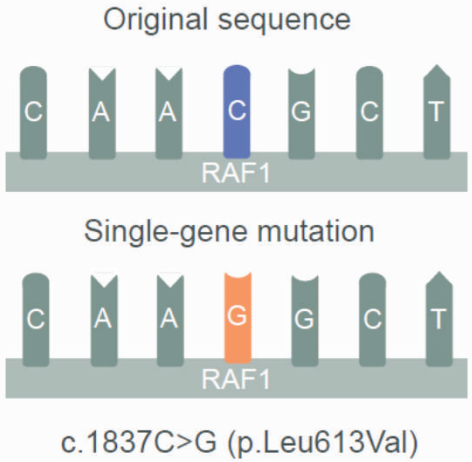
Aneuploidies
like trisomy 21



Microdeletions
like 22q



Single-gene disorders
like Noonan syndrome



c.1837C>G (p.Leu613Val)

natera
Conceive. Deliver.

Noninvasive prenatal diagnosis for single gene disorders

Stephanie Allen, Elizabeth Young, and Benjamin Bowns

Table 1. Techniques for analysis of cell-free fetal DNA for noninvasive prenatal diagnosis of single gene disorders

Technique	Disorders	Reference	Number of reports
Haplotype-based NGS/relative haplotype dosage	Duchenne/Becker muscular dystrophy, spinal muscular atrophy, congenital adrenal hyperplasia, *GJB2*-associated hearing impairment, Gaucher disease	[4–6,7▪,8▪,9–12]	9
Droplet digital PCR	CF, neonatal diabetes and achondroplasia	[13–15,16▪]	4
Minisequencing	Achondroplasia	[15]	1
Bespoke/targeted NGS	Achondroplasia, thanatophoric dysplasia and lethal skeletal dysplasia	[17,18▪]	2
COLD-PCR and microarray	CF, beta thalassemia	[19]	1
Fragment analysis	Huntington disease	[20]	1
Allele-specific real-time PCR	β-thalassemia	[21,22]	2
cSMART	Wilson disease	[23]	1

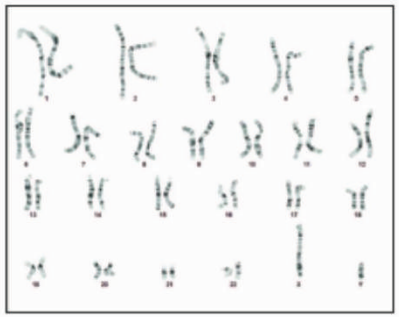
Articles within the review period reporting NIPD of SGDs by analysis of cell-free fetal DNA. These are categorized into methods used for testing and disorders tested. *Source:* original. CF, cystic fibrosis; COLD, coamplification at lower denaturation temperature; cSMART, ciruclating single-molecule amplification and resequencing technology; NGS, next-generation sequencing; NIPD, noninvasive prenatal diagnosis; SGD, single gene disorder.

Single Gene Mutations
NOT Detected by Routine Karyotype or Microarray

Karyotype

>7Mb Resolution

Can detect whole chromosome aneuploidy, large deletions and duplications



Microarray

>1Mb genome resolution, even smaller for critical regions

Can detect microdeletions and microduplications

Conditions screened

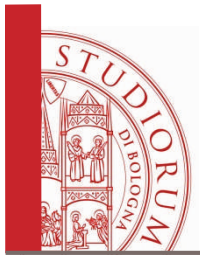
Syndromic Disorders	
Condition	**Gene**
Alagille Syndrome	JAG1
CHARGE syndrome	CHD7
Cornelia de Lange syndrome 1	NIPBL
Cornelia de Lange syndrome 2	SMC1A
Cornelia de Lange syndrome 3	SMC3
Cornelia de Lange syndrome 4	RAD21
Cornelia de Lange syndrome 5	HDAC8
Epileptic encephalopathy, early infantile, 2	CDKL5
Intellectual disability	SYNGAP1
Rett syndrome	MECP2
Sotos syndrome 1	NSD1
Tuberous sclerosis 1	TSC1
Tuberous sclerosis 2	TSC2

Craniosynostosis Syndromes	
Condition	**Gene**
Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis	FGFR2
Apert syndrome	
Crouzon syndrome	
Jackson-Weiss syndrome	
Pfeiffer syndrome type 1/2/3	

Skeletal Disorders	
Condition	**Gene**
Achondroplasia	FGFR2
CATSHL syndrome	
Crouzon syndrome with acanthosis nigricans	
Hypochondroplasia	
Muenke syndrome	
Thanatophoric dysplasia, type I	
Thanatophoric dysplasia, type II	
Ehlers-Danlos syndrome, classic	COL1A1
Ehlers-Danlos syndrome, type VIIA	
Osteogenesis imperfecta, type I	
Osteogenesis imperfecta, type II	
Osteogenesis imperfecta, type III	
Osteogenesis imperfecta, type IV	
Ehlers-Danlos syndrome, cardiac valvular form	COL1A2
Ehlers-Danlos syndrome, type VIIB	
Osteogenesis imperfecta, type II	
Osteogenesis imperfecta, type III	
Osteogenesis imperfecta, type IV	

Noonan Spectrum Disorders	
Condition	**Gene**
Cardiofaciocutaneous syndrome 1	BRAF
Cardiofaciocutaneous syndrome 3	MAP2K1
Cardiofaciocutaneous syndrome 4	MAP2K2
Costello syndrome / Noonan syndrome	HRAS
Noonan syndrome 1 / LEOPARD syndrome/cancers	PTPN11
Noonan syndrome 4	SOS1
Noonan syndrome 5 / LEOPARD syndrome 2	RAF1
Noonan syndrome 6 / cancers	NRAS
Noonan syndrome 8	RIT1
Noonan syndrome 9	SOS2
Noonan syndrome/cancers	KRAS
Noonan syndrome-like disorder with loose anagen hair	SHOC2
Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia (NSLL)	CBL

Disorder	Clinical actions
Osteogenesis imperfecta	• Labor and delivery management to avoid fractures • Neonatal care • Early recognition and treatment of fractures
Achondroplasia	• Labor and delivery management • Monitor for spinal stenosis • Early sleep studies to reduce probability of SIDS
Noonan syndrome	• Fetal echocardiogram • Labor and delivery management • Early assessment for learning differences
Craniosynostosis	• Fetal MRI • Avoid instrumented delivery • Corrective surgery • Early medical and behavioral interventions



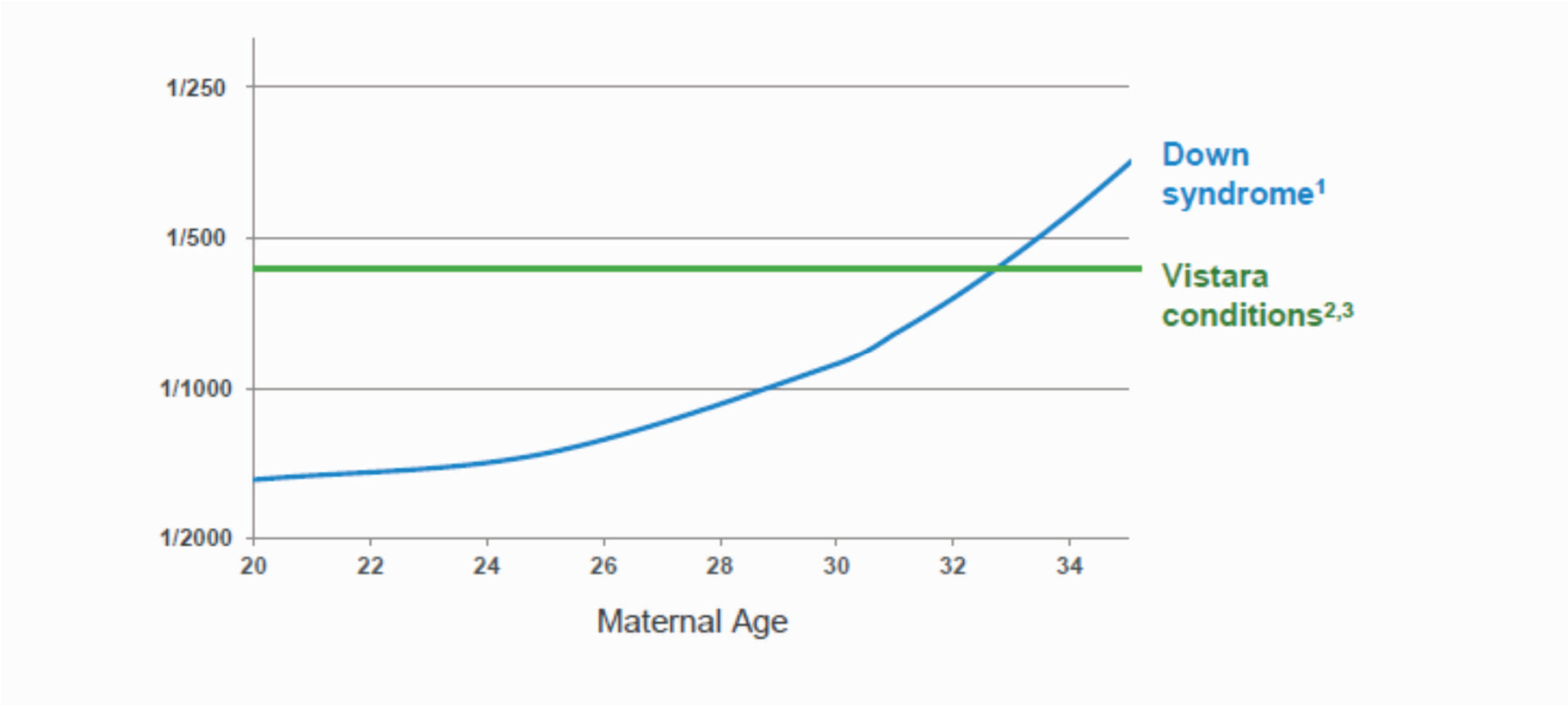
GeneSafe™ DE NOVO

identifies fetal conditions that could be **missed by traditional prenatal screening.**

GENE	SYNDROMIC DISORDERS
JAG1	Alagille syndrome
CHD7	CHARGE syndrome
HDAC8	Cornelia de Lange syndrome 5
NIPBL	Cornelia de Lange syndrome 1
MECP2	Rett syndrome
NSD1	Sotos syndrome 1
ASXL1	Bohring-Opitz syndrome
SETBP1	Schinzel-Giedion syndrome
SIX3	Holoprosencephaly
	NOONAN SYNDROMES
BRAF	Cardiofaciocutaneous syndrome 1
CBL	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia (NSLL)
KRAS	Noonan syndrome/cancers
MAP2K1	Cardiofaciocutaneous syndrome 3
MAP2K2	Cardiofaciocutaneous syndrome 4
NRAS	Noonan syndrome 6/cancers
PTPN11	Noonan syndrome 1/ LEOPARD syndrome/cancers
PTPN11	Juvenile myelomonocytic leukemia (JMML)
RAF1	Noonan syndrome 5/ LEOPARD syndrome 2
RIT1	Noonan syndrome 8
SHOC2	Noonan syndrome-like disorder with loose anagen hair
SOS1	Noonan syndrome 4

GENE	SKELETAL DISORDERS
COL2A1	Achondrogenesis, type II or hypochondrogenesis
FGFR3	Achondroplasia
	CATSHL syndrome
	Crouzon syndrome with acanthosis nigricans
	Hypochondroplasia
	Muenke syndrome
	Thanatophoric dysplasia, type I
	Thanatophoric dysplasia, type II
COL1A1	Ehlers-Danlos syndrome, classic
	Ehlers-Danlos syndrome, type VIIA
	Osteogenesis imperfecta, type I
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV
COL1A2	Ehlers-Danlos syndrome, cardiac valvular form
	Ehlers-Danlos syndrome, type VIIB
	Osteogenesis imperfecta, type II
	Osteogenesis imperfecta, type III
	Osteogenesis imperfecta, type IV
	CRANIOSYNOSTOSIS SYNDROMES
FGFR2	Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis
	Apert syndrome
	Crouzon syndrome
	Jackson-Weiss syndrome
	Pfeiffer syndrome type 1
	Pfeiffer syndrome type 2
	Pfeiffer syndrome type 3

GeneSafe™ detects de novo mutations in 25 genes causing 44 different genetic disorders. The genetic conditions screened by this innovative test often occur in the absence of a family history of the condition. This is a paradigm shift in prenatal screening. GeneSafe™ screens for de novo mutations that cannot be detected by standard carrier screening, as these mutations are not present on the parents. The genetic disorders screened by GeneSafe™ can cause **skeletal dysplasias, cardiac defects,** [1-2-3] **multiple congenital anomalies,** [4-5] **autism,** [6] **epilepsy** [7] **and/or intellectual disability.** [8-9]


1/250
1/500
1/1000
1/2000
20
22
24
26
28
30
32
34
Maternal Age
Down syndrome[1]
Vistara conditions[2,3]

Consider Vistara for the following indications:

Advanced paternal age

Women who want to know "more"

Ultrasound anomalies, such as shortened long bones and increased NT

Adjunct to CVS and amniocentesis

GeneSafe™ INHERITED

allows detection of common inherited genetic disorders in the fetus

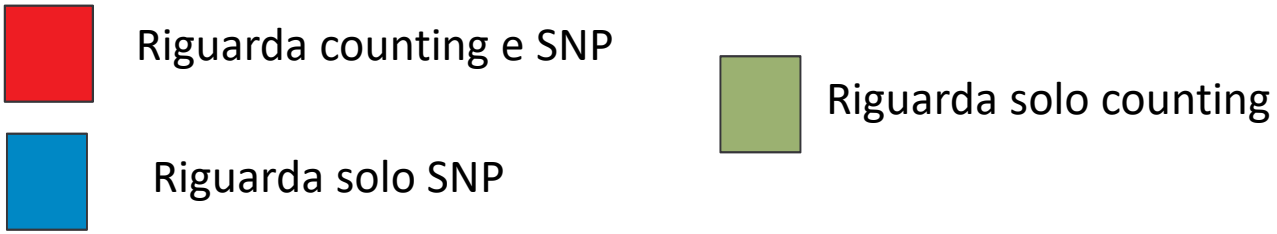
GENE	GENETIC DISORDER
CFTR	Cystic Fibrosis
CX26 (GJB2)	Deafness autosomal recessive type 1A
CX30 (GJB6)	Deafness autosomal recessive type 1B
HBB	Thalassemia-Beta
HBB	Sickle cell anemia

Conclusioni

- Quadro in rapida evoluzione
- Competenza di genetica clinica e molecolare
- Alta aspettativa delle pazienti (spesso inaccettabile la performance del test combinato)
- Offerta «privata» deve essere «provata» da pubblicazioni su casistiche prospettiche
- La diagnosi invasiva del futuro ha un nuovo ruolo molto più mirato e di alta competenza

Cause di Falsi positivi

- Mosaicismo confinato alla placenta I e III
- Trapianti d'organo (per il sesso)
- Donne politrasfuse
- Small copy number variant (CNV) materne (17% di tutti i FP)
- Patologie oncologiche materne
- Mosaicismo X materno (10 circa di tutti i NIPT positivi per SCA)
- Discordanza fra T21 al NIPT e presenza di UPD Chr21 alla amnio (Pan et al)
- Lo screening per le microdelezioni ha un alto tasso di FP
- Eparina (più frammenti con CG) : solo per il counting non normalizzato (z-score) falso positivo per la T18



Cause Falsi negativi

- Mosaicismo tipo V
- Mosaicismo sotto il 30% non sembra essere visto al NIPT (dubbi con la correlazione clinica). Equilibrio fra ff e % di mosaicismo
- Triploidia (counting)
- No call (cut-off ?? 4% in Norton et al 4.7%) Diverso rate di no call fra SNP e Counting) . La ripetizione del test ha un rate di successo del 60-70%
- FF basse sembrano associate ad un maggior rate di aneuploidia
- No call rate e pazienti politrasfuse per SNP (?)
- Presenza contemporanea di duplicazioni e delezioni a carico dello stesso Chr (falsa normalizzazione) che produce uno z-score entro i limiti ma che nel feto è clinicamente rilevante (descritto per il Chr13 da Buysse et al)
- Problemi tecnici (variabilità intercromosomica nel contenuto delle basi CG)
- Eparina (più frammenti con CG) : solo per il counting non normalizzato (z-score) falso negativo per la T13 e T21

Riguarda counting e SNP

Riguarda solo SNP

Riguarda solo counting

La ´no call´ o mancata risposta (test nullo)

- No call (cut-off ?? 4% in Norton et al 4.7%)
- La ripetizione del test ha un rate di successo del 60-70%
- Maggior tasso di no call
- FF basse sembrano associate ad un maggior rate di aneuploidia
- No call rate e pazienti politrasfuse per SNP
- In caso di alta percentuale di omozigosi fra genitori gli SNP informativi calano drasticamente tanto da non discriminare correttamente il feto dalla madre
- Vanishing twin

Riguarda counting e SNP

Riguarda solo SNP

Falsi positivi e negativi vantaggiosi

- Per Verinata che normalizza sul Chr 9 alcuni FP per T21 possono essere invece positivi alla diagnosi invasiva per delezione del Chr 9
- Mosaicismo tipo V non associati a patologie clinicamente rilevanti (DNA neg, CVS pos, feto pos)
- Mosaicismo di tipo II (DNA neg, CVS pos, feto neg)
- Alcune microdelezioni non rilevate che però non hanno conseguenze cliniche