MEDICAL GENETICS CLINIC

HANDBOOK

VOLUME 1

CRAFTON AND GENEADVISE PUBL

MEDICAL GENETICS CLINIC HANDBOOK

VOLUME 1

WHOLE GENOME NGS, PRENATAL DIAGNOSIS,

L.Camurri PhD Editor

Proceedings of the Consulting Agreement Service: Personal Genetics in Medicine, Nutritional Genetics, Wellness Genetics, held 2021-2023

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Text, photos, topos: archive L. Camurri

MEDICAL GENETICS CLINIC

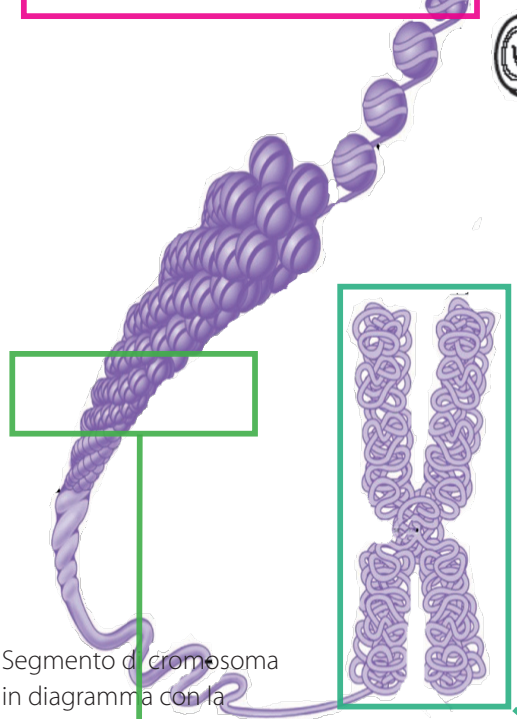
The Medical Genetics Clinic provides comprehensive clinical medical genetics services to prenatal, pediatric and adult patients.

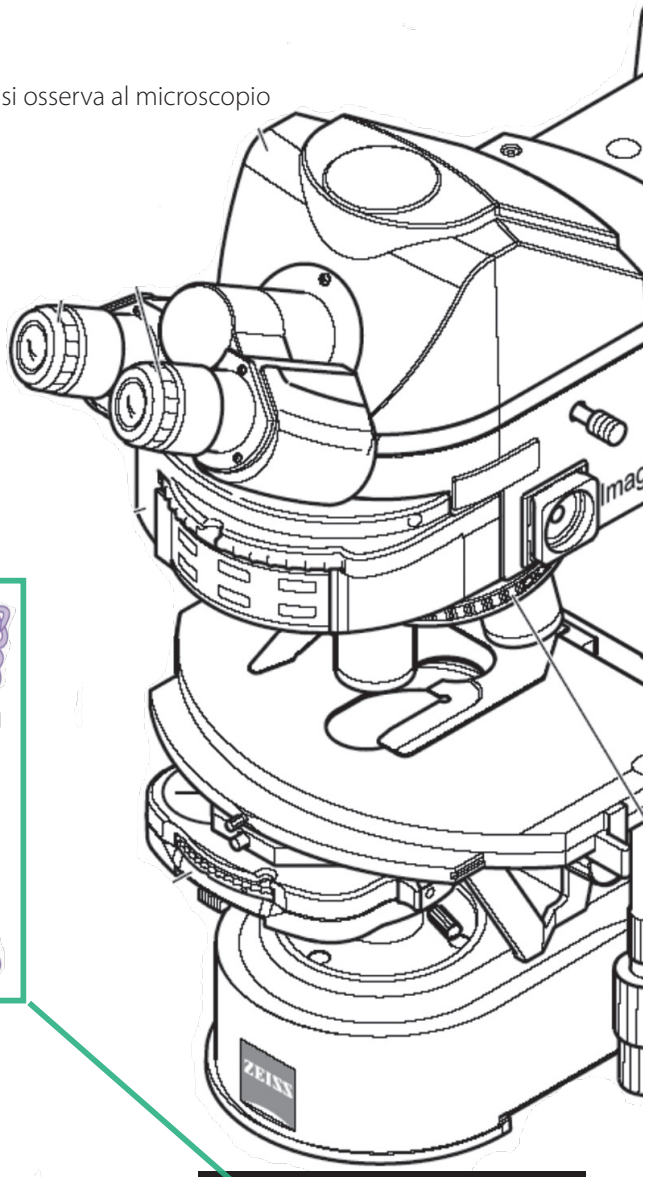
The Clinic contains a team of health care providers who work together to care for patients and their families. Medical geneticists are expert in offering diagnostic services, medical recommendations and treatment options for those who have genetic disorders. Genetic counselors specialize in education and resources about these conditions to patients and their families. Patients with inherited metabolic conditions receive ongoing care from other specialized health care providers in the Clinic including metabolic dietitians.

INTRODUCTION

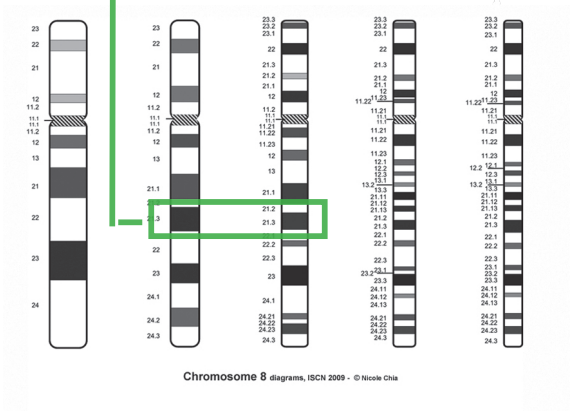
In the last twenty years, genetic research has experienced a true technological revolution thanks to the development of massive DNA sequencing methods, which have reduced the costs and times of genomic analyzes by over 100 thousand times and have exponentially increased their processivity, allowing the large-scale use. The diffusion and improvement of human genome scanning technologies at single base resolution have made it possible to develop application models that guarantee the simultaneous study of different levels of the flow of biological information, through the sequencing of the coding portion of the genome (exome; Whole Exome Sequencing, WES), whole genome sequencing (WGS), the qualitative and quantitative evaluation of the messenger and non-coding RNA populations that characterize cells and tissues (transcriptome), the characterization of epigenetic modifications of the genome that participate in the control of gene expression (epigenome), in particular its methylation profile (methylome). Other important technological innovations in the biomedical field allow the composition of metabolites and proteins (including reversible and non-reversible modifications of proteins) to be systematically characterized, applied to simple and complex systems (metabolome and proteome). The use of these technologies has required the development of new bioinformatics analytical approaches, capable of managing and processing an enormous amount of generated data, as well as tools for archiving the generated data. The transversal application of these technologies, which ranges from the biomedical to the biotechnological field, affects, on a broad spectrum, the theoretical and applied sciences and requires the integration of multidisciplinary knowledge and skills (e.g. medicine, physics, engineering, computer science , robotics, human sciences, ethics). The interdisciplinarity of these approaches makes it necessary to develop a new paradigm, based on the interaction of networks of knowledge, skills and infrastructures, in order to guarantee high levels of application and interpretation of data. The set of these so-called "-omics" technologies allows biological systems to be characterized at very high resolution, and their systematic use will determine the exponential growth of "precision medicine", through the rapid achievement of diagnosis, the understanding of disease mechanisms and the identification of therapeutic approaches based on patient stratification, capable of guaranteeing more effective management. Achieving the diagnosis still represents a significant critical issue for the National Health System (NHS). In recent years, the diagnostic application of exome analysis, which has become a first-line investigation in many clinical conditions, in particular in rare and orphan diseases diagnosed in pediatric age, has made it possible to achieve important results and to obtain a definitive classification in approximately 50% of patients. These data exemplify the need to consider the integrated application of -omics technologies as the only response to the need for diagnosis in patients in whom previously used approaches have not been successful. In addition to reaching the diagnosis, understanding the biological basis of diseases is preliminary to the development of personalized and precision therapies. This objective is particularly important in conditions in which

Sequenza DNA non si osserva al microscopio

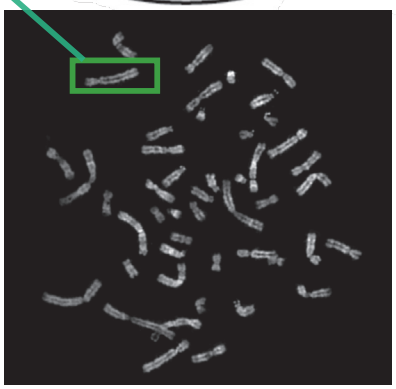




Segmento di cromosoma in diagramma con la dimensione più piccola osservabile di routine al microscopio (10-15 milioni di basi DNA)



Chromosome 8 diagrams, ISCN 2009 - © Nicole Chia



I cromosomi di una cellula al microscopio

different variations in the genome, although underlying the same clinical condition, specifically modify normal cellular processes.

GENOMIC ANALYSIS IN CLINICAL PRACTICE. TRADITIONAL ANALYSES

At the end of the 1950s, karyotype analysis represented the first example of genomic analysis, albeit at very low resolution, transferred into clinical practice. Standard cytogenetic techniques, at an average metaphase resolution (approximately 320 bands per haploid set), allow the identification of chromosomal imbalances of dimensions equal to or greater than 10 megabases (1 Mb = 10 million bases), while current molecular techniques allow either achieve resolution at the single base level (ISCN, 20163). Part of the gap between conventional cytogenetic analysis and single gene analysis has been filled by molecular cytogenetic techniques.

The use of fluorescent molecules has made it possible to standardize fluorescent in situ hybridization (FISH), based on direct binding (combined with a fluorochrome) or indirect binding (through an intermediate molecule incorporated into the probe) with the DNA bases. In this way it was possible to increase the resolution of conventional cytogenetic analysis and identify imbalances below the standard chromosomal resolution (Bishop, 20104).

Comparative genomic hybridization (CGH) analyzes copy number variations (CNVs) on chromosomes, in terms of gain/duplication or loss/deletion. Developed in the early 1990s, this technique is based on quantitative two-color FISH.

Even if CGH substantially improves the resolution of the analysis and therefore increases the possibility of recognizing genomic imbalances, the information gain is still relatively limited (no more than 3 Mb). The development of array-based CGH (array-CGH), in which metaphase chromosomes are replaced by DNA sequences adhered to a support slide, therefore represented significant progress at the end of the 1990s.

Array-CGH has largely replaced chromosomal analysis in clinical practice. Its principle is essentially that of CGH, and consists of a comparative genomic hybridization that uses an array as a substrate instead of metaphases.

Analysis using arrays using polymorphisms of single nucleotides (SNP-array) has more recently allowed resolutions of 5-10 kb to be obtained. In addition to providing information on variations in the number of CNVs, these platforms identify regions of homozygosity and therefore genes potentially related to recessive diseases, mosaic aneuploidies, even when present in low percentages, and uni-parental dismiss.

The first DNA sequencing methodologies date back to the 1970s. The strategy developed by Sanger (19776), based on the enzymatic method of chain terminators and on the electrophoretic migration of the products of the sequencing reaction, is still used today for the sequencing of individual DNA fragments.

This method, which allows you to obtain sequences of up to 800-1000 bases, has been automated to multiply its applicability and facilitate the analysis process. However, the high production costs of each investigation and its relative diagnostic effectiveness do not allow this technique to be applied on a large scale.

“-OMICS” ANALYSIS

Ultrasound Obstet Gynecol 2012; 39: 384–388
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/uog.11092

Microarray application in prenatal diagnosis: a position statement from the cytogenetics working group of the Italian Society of Human Genetics (SIGU), November 2011

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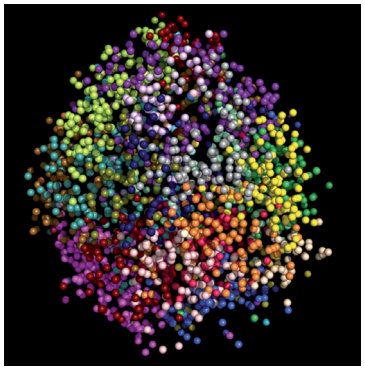
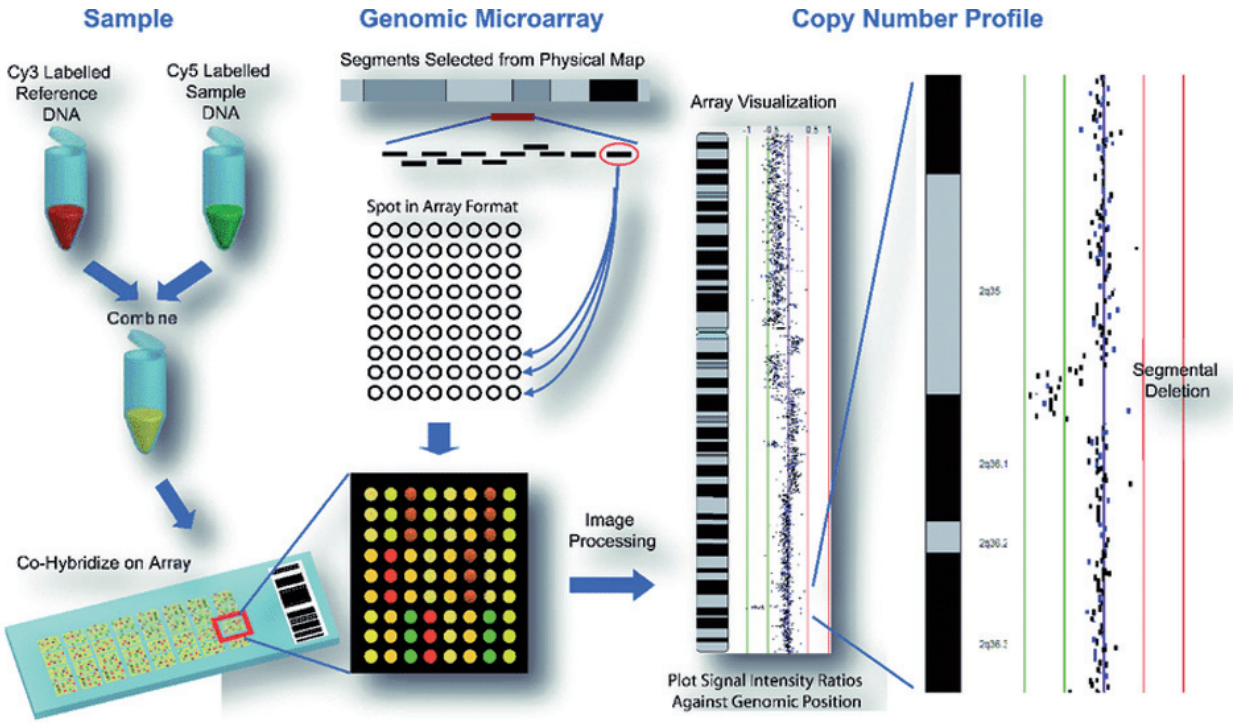


ARRAY-CGH



Nucleo di cellula. Ogni colore un cromosoma, ogni sfera la collocazione di un gene.
(Takei et al. Science/Nature 2022)

Lo schema della ibridazione con a-CGH, Comparative Genomic Hybridization

Highly parallel sequencing or second generation sequencing (so-called Next Generation Sequencing - NGS), introduced into clinical practice about ten years ago, has the ability to sequence many DNA fragments simultaneously. This technology makes it possible to analyze, at a relatively low effective production cost, millions of DNA sequences in each single test and, thanks to the possibility of subsequently automatically analyzing the raw sequencing data, to acquire an enormous amount of information on the individual genome. In this way it is possible to sequence an entire genome in a few days, an analysis that, with traditional techniques, would take years.

The parallel development of bioinformatics tools, necessary in the management and analysis of sequencing data, allows previously unthinkable cognitive objectives to be achieved. In particular, it has become possible to develop faster and more efficient diagnostic tests and identify, more effectively, numerous new disease genes. Most genetic diseases are heterogeneous, meaning they can be caused by mutations of different genes in different patients. For a long time, their molecular characterization used the "gene-by-gene" sequencing approach, an extremely time-consuming and expensive strategy.

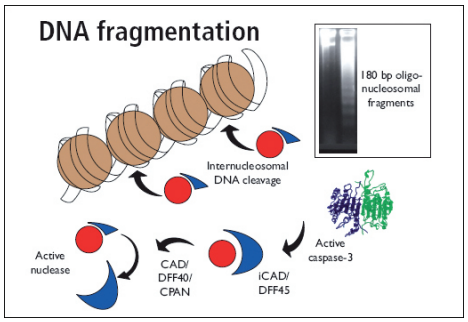
Second generation sequencing techniques allow us to overcome these limitations and many laboratories currently use them for the molecular characterization of diseases. This approach is particularly efficient in the case of rare and orphan diagnosed diseases.

A first parallel sequencing method is based on the enrichment of specific genomic regions (those in which the disease genes are located) and on their massive parallel sequencing, analyzing several patients simultaneously. Using NGS techniques it is possible to test up to 96 samples simultaneously, each for the panel of disease genes responsible for the suspected condition at a clinical level, obtaining data that can be analyzed in a few days. These techniques have therefore revolutionized genetic testing protocols, as they allow diagnostic results to be obtained quickly, containing costs of roduction of the test and maintaining high quality of the results. Furthermore, they have had notable clinical implications, both in the case of highly heterogeneous diseases and in those associated with a nuanced phenotype or one devoid of pathognomonic characteristics, in which it can be problematic to hypothesize a clinical diagnosis. In fact, the simultaneous analysis of all genes potentially associated with the disease in question reduces the time necessary to identify the molecular defect, to the benefit of genetic counseling and patient care.

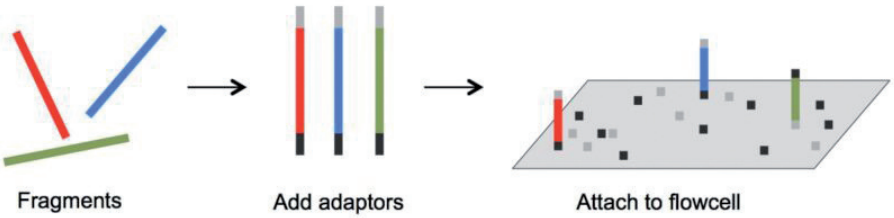
Although whole genome sequencing is in principle the strategy of excellence for the study of interindividual genetic variability, it still presents some issues that limit its large-scale application, in particular the computational capabilities required by the analysis, l archiving of the enormous mass of data produced, their interpretation and the higher management cost. For these reasons, second generation sequencing techniques are now often used for exome sequencing. With this approach, based on the enrichment of genomic fragments that refer to gene sequences coding for proteins and for selected subclasses of RNA that have a regulatory function (e.g. microRNA), it is possible to limit the analysis to 1-2% of the genome, thus excluding non-coding regions and, consequently, losing information that can impact gene expression.

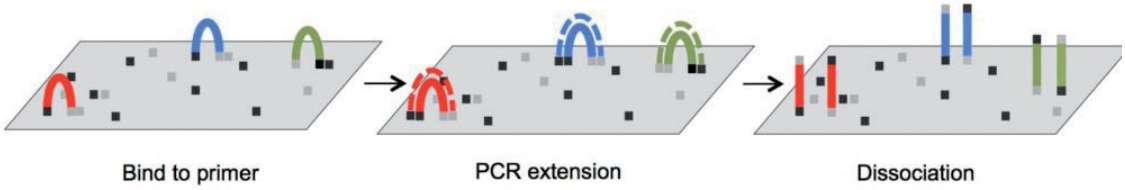
Current knowledge on the genetic causes of Mendelian diseases suggests that most of

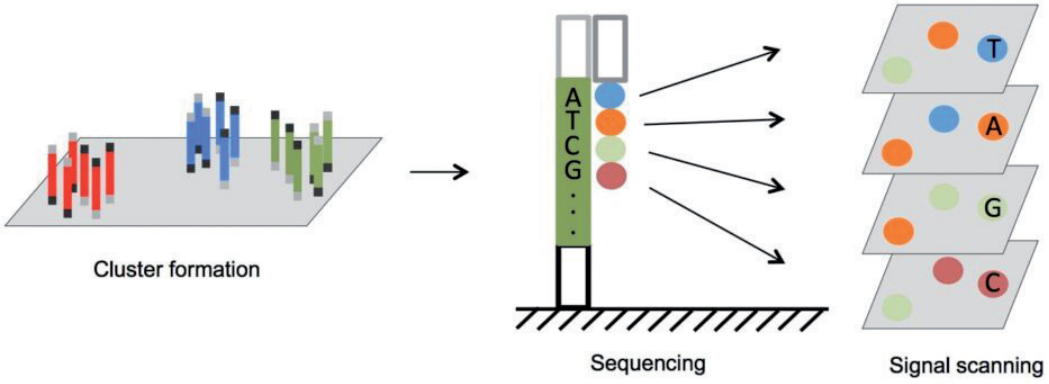
NGS NEXT GENERATION SEQUENCING



Frammentazione del DNA







their mutations consist of changes in the coding sequence of a gene or an abnormality in transcript processing. Therefore, the exome is an enriched portion of the genome, in which it is useful to search for mutations with potential clinical impact. It follows that its sequencing is fundamental in the diagnosis of rare diseases and in understanding the molecular basis of many Mendelian pathologies, as documented by the diagnoses obtained in recent years on large cohorts of undiagnosed patients and the hundreds of disease genes identified with this technique.

A complementary approach to exome or genome sequencing is the sequencing of the transcriptome, i.e. the RNAs transcribed from a homogeneous or heterogeneous population of cells. In principle, the transcriptome is able to characterize the entire expression profile of the biological sample, in quantitative and qualitative terms, and allows to identify both the molecular events with clinical impact that affect the coding regions, and those that, involving the regulatory regions determine changes in the processing of transcripts or in their stability. This analysis also allows the identification of structural rearrangements of the genome with a quantitative or qualitative impact at the messenger RNA level. Its limit is linked to the availability of the tissue to be analyzed. The areas in which it can be effectively applied are those of muscular diseases and, probably, syndromic conditions.

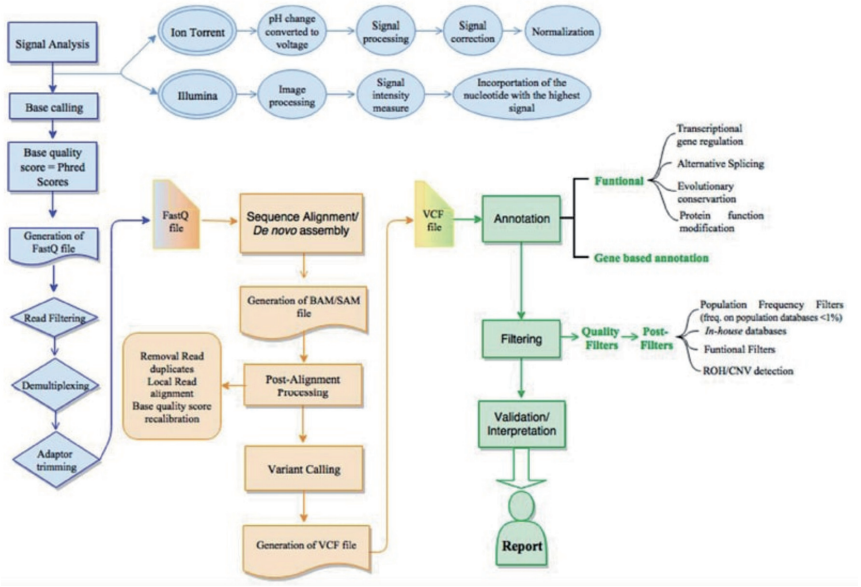
Regardless of the specific type of sequencing used (exome, genome or transcriptome), the analysis and interpretation of sequencing data require numerous bioinformatic tools for the processing of the sequences obtained and the annotation, filtering and prioritization of the identified variants . Sequencing platforms generate a huge amount of raw data, which is converted into nucleotide sequences using computational tools. The generated files are usually found in a format that contains, in addition to the reading of the nucleotide sequences, quality scores associated with each base read. The sequenced single base resolution requires the analysis of the files using a complex bioinformatic workflow which allows, in a first phase, to align the sequences produced to the reference genome and, subsequently, to identify and functionally annotate the variants that characterize it.

The alignment phase is performed with computational systems that compare each of the sequences produced with the reference genome, allowing their correct positioning. To ensure the reliability of these systems and obtain a global assessment of sequencing efficiency, several quality parameters are usually applied. Among them, particularly relevant are coverage, i.e. the percentage of target genomic sequences read by sequencing, and depth, i.e. the number of reads referring to a specific base of the genomic sequence of interest. The next step in the bioinformatics approach is “variant calling,” which identifies locations where the aligned sequences differ from the reference sequences.

The list obtained is subsequently annotated: all the information available in the literature and databases is associated with each variant. The variants obtained can be processed with heuristic prioritization and filtering methods, in order to reduce the high number of variants and select those with functional significance. Typically, in the first phase, high allele frequency variants in the general population are eliminated, which are assumed not to have a pathological impact on a phenotype classified as rare. For this purpose,

NEXT GENERATION SEQ. WORKFLOW

Primary Analysis	Secondary Analysis	Tertiary Analysis



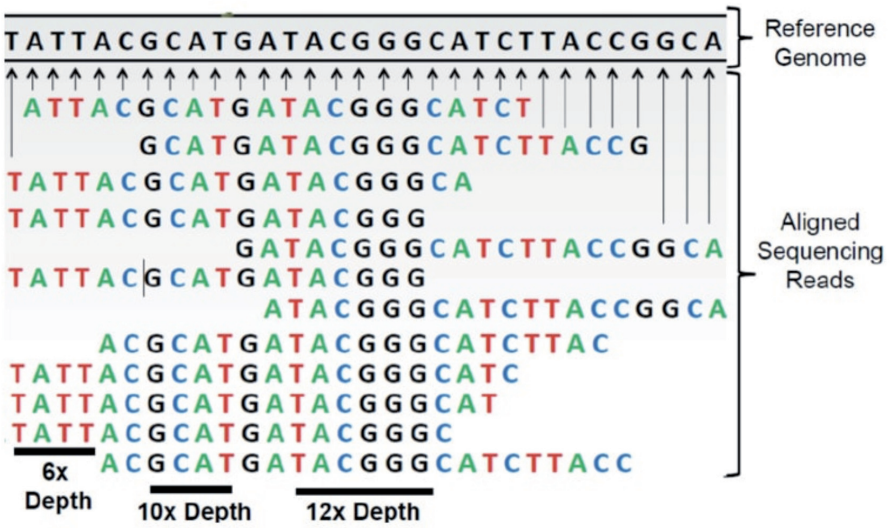


Figure 3: Visualisation of reads aligned to a reference genome

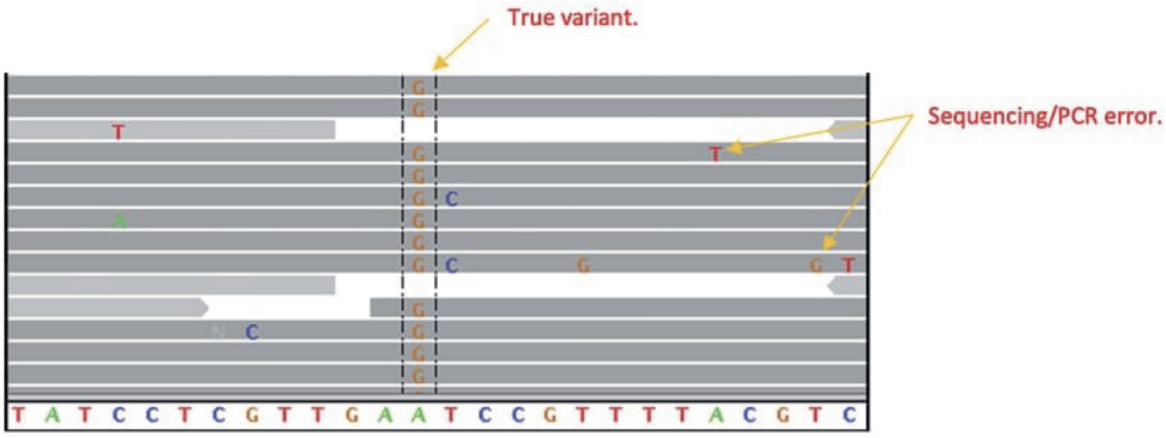


Figure 4: Reads aligned to a reference showing a G nucleotide variant that was detected which differs to the A nucleotide of the reference. Variants or two reads are likely to be PCR or sequencing error and not true variants

public and in-house databases are used, which allow the identification of variants that have low frequency in the population or that have not been previously identified. In a second phase, the available information on each variant and its gene is collected and evaluated, in order to prioritize the former based on their predicted effect, and the genes based on their biological relevance (e.g. expression, function), with respect to the phenotype of interest. Different tools are used for the annotation and functional prediction of variants, each of which has strengths and weaknesses. For this reason, a prioritization strategy capable of exploiting multiple prediction tools is generally implemented.

The last phase of the analysis is the interpretation of the data, which cannot be automated, at least with current tools, and requires particular attention and specific experience and knowledge. This phase is strongly influenced by the detail of the clinical information collected on the patient and may require re-evaluation. -omics analyses, even in the case of negativity, offer the advantage of being able to be re-evaluated over time. Given that the interpretation is strictly dependent on clinical data (a child's phenotype can evolve and define itself over time) and on the knowledge of the genes and variants available at the time of the analysis, the data from an exome or from any other genomic approach can be reworked over time.

Exome sequencing has proven to be particularly efficient in the diagnostic field and is used as a first-line test in large international centers. Recent studies agree on a detection rate of approximately 30-50% in patients affected by genetic diseases without diagnosis or with an uncertain diagnosis.

However, success in achieving a molecular diagnosis through exome sequencing can vary considerably based on patient characteristics disease under examination (e.g. a condition that can be classified in a specific group of clinical conditions, such as spastic paraplegia, retinopathy, osteochondrodysplasia, etc.) and the sequencing strategy used (analysis of the proband only compared to the analysis of the family unit).

The clinical use of genome sequencing theoretically offers the greatest resolution and information. While on the one hand, the diagnostic yield of whole genome sequencing would appear to be only slightly higher than that of exome sequencing (gain of information approximately 10% in exome negative cases), this analysis also allows the identification of the presence of structural rearrangements (e.g. CNVs, translocations, inversions), which can only occasionally be detected with exome sequencing. However, it should be underlined that the potential of WGS is not yet fully known and it is therefore desirable that it can begin to be used in centers with documented expertise, with dedicated investments.

The possibility of integrating genomic data obtained with complementary approaches (e.g. transcriptome associated with genome sequencing) paradigmatically illustrates the extraordinary potential of -omics technologies in clinical practice.

An aspect of particular relevance, relating to the use of new genomic sequencing technologies, concerns the identification of sequence variations in disease genes not correlated with the clinical picture that required molecular assessment (so-called incidental findings). This aspect is important considering the high resolving power of

these analyzes and the potential ethical implications.
There are specific indications on whether or not to communicate these results to patients who specifically request them, and this underlines the importance of activating a pre- and post-test genetic counseling process, managed by specialists who are familiar with the interpretation of genomic data, as well as to use dedicated consent forms, which take into consideration all possible options from users. Available data indicate that the error rate in second generation sequencing is very low, but not negligible, and is strictly dependent on the type of variation (single nucleotide change vs multi-base insertion/ deletion) and its sequence context. For this reason it is essential to always validate the selected variants with Sanger sequencing, or other techniques, although it still represents the reference sequencing technique today.

RARE DISEASES
Rare diseases offer a paradigmatic model of large-scale development of a new concept of diagnostic activity, based on the introduction of innovative analytical tools that transfer -omics techniques into clinical practice.
This is a heterogeneous group of conditions which affect, in approximately 60% of cases, the pediatric range and which, in approximately 80% of cases, have a genetic cause or a large genetic component.
Their overall number represents a health problem of great social impact (probably around one million people affected in Italy, excluding rare tumors).
Despite the peculiarity of the approximately 8,000 rare diseases identified to date, patients affected by these conditions and their families share common healthcare and social needs, such as the uncertainty of the diagnosis, which pushes them towards a true diagnostic odyssey punctuated by repeated visits , expensive and varied investigations and analyses. Difficulties in diagnosis are, among other things, justified by the fact that approximately 85% of known rare diseases have a frequency of less than one affected person per million people (Wakap, 20208).
For this reason, approximately half of patients do not obtain the diagnosis, 25% reach it in a period between 5 and 30 years, during which approximately 40% of them receive incorrect diagnoses and inappropriate treatments or even unnecessary surgical interventions (EURORDIS, 20099; Molster, 201610).
This scenario has been significantly revolutionized by the availability of second generation sequencing techniques. One of the first studies carried out in the USA at the Baylor College of Medicine, relating to 2000 patients, reported a diagnostic resolution in 25% of patients, with percentages varying between 20%, in the case of subjects who did not present neurological symptoms, and 36% in those suffering from neurological problems (ataxia, movement disorders) (Yang, 201411).
In the Canadian national FORGE project, WES analysis of 362 families allowed us to characterize the molecular defect in 188 (51.7%), including 105 (29%) with mutations in known disease genes and 83 in new disease genes. A possible mutation was identified in 28 families patogenetica in a new disease gene (Sawyer, 201612).

PRENATAL DIAGNOSIS.

Prenatal exome. Prenatal diagnosis includes instrumental and laboratory investigations carried out during pregnancy aimed at monitoring the health of the embryo/fetus, starting from the early stages of development up to the moments preceding birth. Prenatal genetic diagnosis, depending on the time and clinical indication, uses a series of different investigations (karyotyping, array-CGH, molecular analyses).

Their appropriateness is evaluated based on the usefulness of the result for the management of pregnancy and not only on their diagnostic yield. In this sense, for a rational use of NHS resources, the indiscriminate use of predictive genetic investigations must be discouraged in pregnancies that are not at risk. NGS techniques, particularly WES, have changed the diagnostic approach of genetic diseases.

Considering that the primary objective of prenatal genetic analysis is to provide explanations regarding the finding of fetal pathologies and their clinical management, it is necessary that clear results emerge from genomic analyzes and therefore such as not to create interpretative doubts.

For this reason, it is important that the analysis is carried out on the trio (fetus + parents) and that the most complete clinical data possible is available (ultrasound scans, family history, any autopsy findings in the event of pregnancy termination).

The limitation of objective data on the fetus and the lack of knowledge of the prenatal phenotype of rare diseases, which are mostly diagnosed after birth, often makes it difficult to interpret the results of genomic analyses.

For this reason, only the variants classified as pathogenetic or probably pathogenetic must be reported on the report, reporting variants of uncertain or unknown significance (Variations Of Uncertain Significance - VOUS) only if associated with a known clinical picture. It is therefore important to provide pre-test and post-test consultancy and the management of the entire diagnostic process at accredited laboratories, which can make use of professionals with specific clinical and laboratory skills.

A critical issue is that of time, given that the prenatal diagnosis must be reported within a short time frame, while the analysis of the exome requires relatively long times, which include, among other things, those necessary to confirm with alternative methods any pathogenetic variants identified. However, with optimal organization, it is possible to obtain results within about ten days, but it should not be forgotten that WES, similarly to other genetic investigations, has limitations, as it does not identify genomic imbalances (CNV), small intragenic rearrangements and triplet expansion mutations.

There are still few scientific works relating to the use of WES in prenatal diagnosis, which however have only focused on some aspects, especially the diagnostic yield, in the absence of follow-up data or cost-effectiveness analyses.

All recent studies have involved selected case series, in particular pregnancies with ultrasound-evident defects, with normal karyotype and array-CGH. Best et al. (2018) performed a meta-analysis of 31 studies, reporting a diagnostic yield between 6.2 and 80%. The highest diagnostic yields were obtained for investigations carried out on trios and on fetuses with multiple anomalies or with ultrasound findings attributable to

POSTNATAL GENETIC DIAGNOSIS

CHROMOSOME AND GENETIC STUDY CONSTITUTIONAL DISORDERS

PRECONCEPTIONAL SURVEY IN PHISIOLOGICAL OR MEDICAL REPRODUCTION.

Conventional Karyotype (diagnosis), genetic panel of variants predisposing to Trombophilia (not diagnosis)

Phisiological Reproduction Procedures, normal phenotype, spontaneous abortions

KARYOTYPE TECHNICAL INDICATIONS.

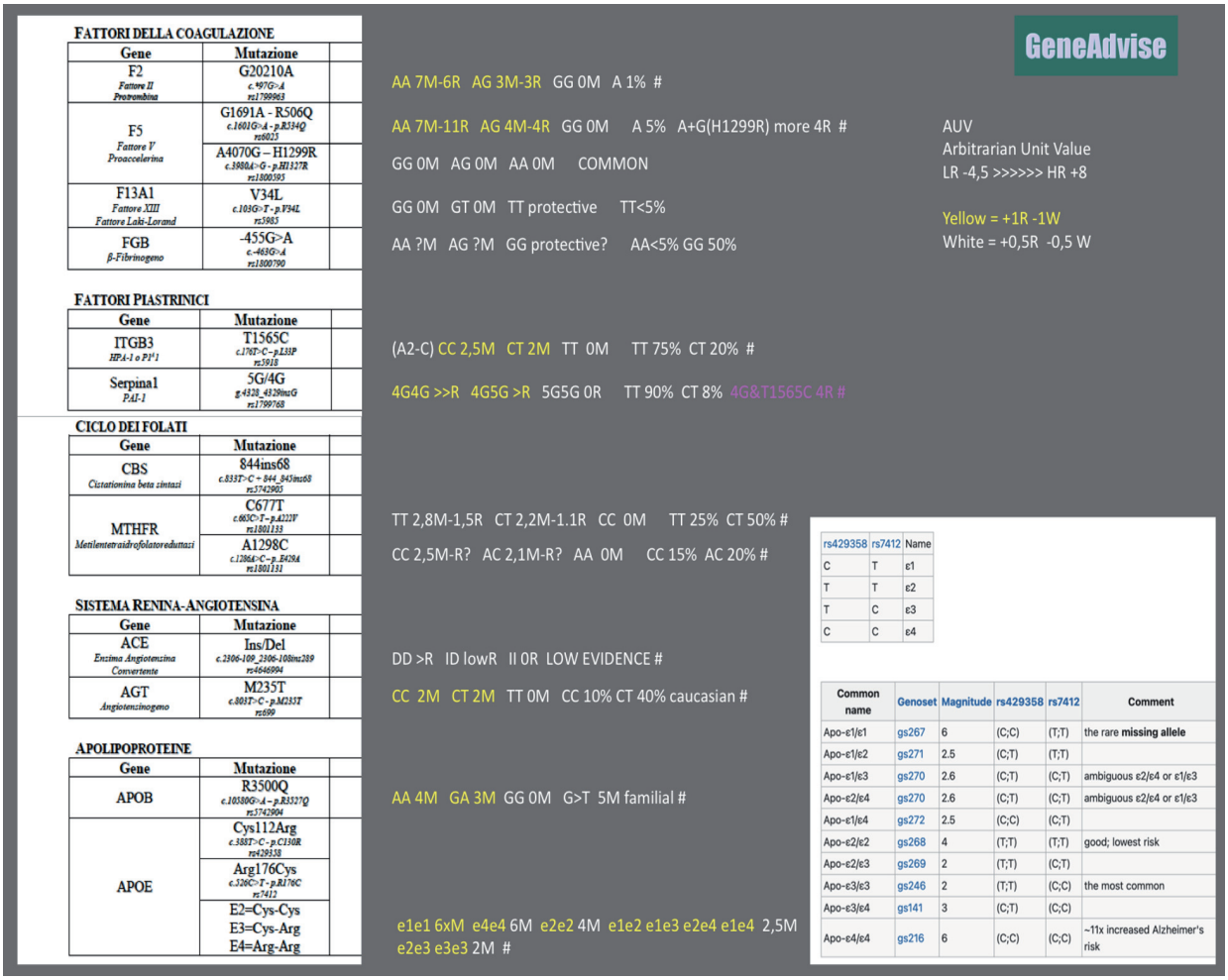
Cell cultures Cytogenetic investigations can be performed on different tissues using short/medium/long cell cultures term Chromosome banding. The karyotype must be analyzed with banding technique (G, Q, R), with the exception of chromosomal instability syndromes and in the search for aberrations induced by clastogenic agents. Differential staining techniques must be available for specific regions chromosomal (C, Nor, Da-DAPI).

The banding resolution level must be reported in the documentation laboratory and in the report. The International System for Chromosome Nomenclature (ISCN, current edition) defines 5 chromosome banding resolution levels (300,400,550,700,850), to be used as reference. The level of resolution of the banding of a metaphase can sometimes be obtained directly from the data image analysis systems. The level of resolution must be related to the diagnostic question and the type of tissue studied: the number of 300 bands is the minimum recommended level for chorionic villus analysis with the method direct; 400 bands is the minimum level in postnatal diagnosis.

The karyotype is not the investigation of choice for the identification of structural anomalies in cases of mental retardation, congenital defects, dysmorphism, the array-CGH/SNP technique being preferable. The Karyotyping is not the investigation of choice for the identification of structural anomalies in cases of suspected microdeletion syndrome, FISH analysis with selected probes being preferable based on clinical suspicion.

When it is not possible to obtain the recommended resolution in relation to the question diagnostic, in the absence of chromosomal anomalies, genetic counseling is indicated if necessary execution of the array-CGH/SNP technique or repetition of the exam, in the presence of a clear clinical indication.

TROMBOPHILIA PREDISPOSING VARIANTS



GENE- PERSONA
PREDICTIVE AND FUNCTIONAL MARKERS
GENETIC PREDISPOSITION
TROMBOPHILIA

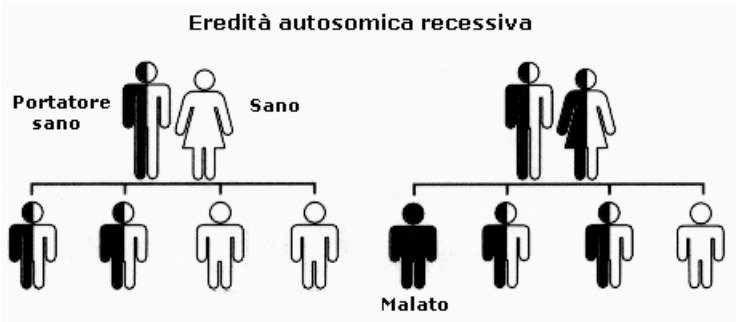
The test analyzes the genetic polymorphisms predisposing to the indicated functions, the technical evaluation is performed on the panel of genes considered valid and informative to the best of today's scientific knowledge. This evaluation aims to obtain a summary value where the degree of predisposition enhances the population prevalence and frequency data, the Magnitude data. The indications do not depend on the subject's medical history and are not a medical tool. The indications are a contribution to the optimization of the functional picture of the subject available to the phisician.

The allelic component of the analyzed variants is expressed with an arbitrary value of predisposition to the function of the variants themselves, considering the population frequency and the Magnitude. The scheme goes from a MINIMUM PREDISPOSITION value of -4.5 to a MAXIMUM value of +8. The value 0 is the equilibrium point

Results: Arbitrary predisposition value: -0.5 The panel of variants analyzed is within a profile common to the general population

Conventional Karyotype, genetic panel of variants for Cystic Fibrosis, Spinal Muscolar Atrophy, FRAXA mental retardation.

Medical Reproduction, Infertility. Screening for AR and X linked mendelian disorders in phisiological Reproduction, normal phenotype.

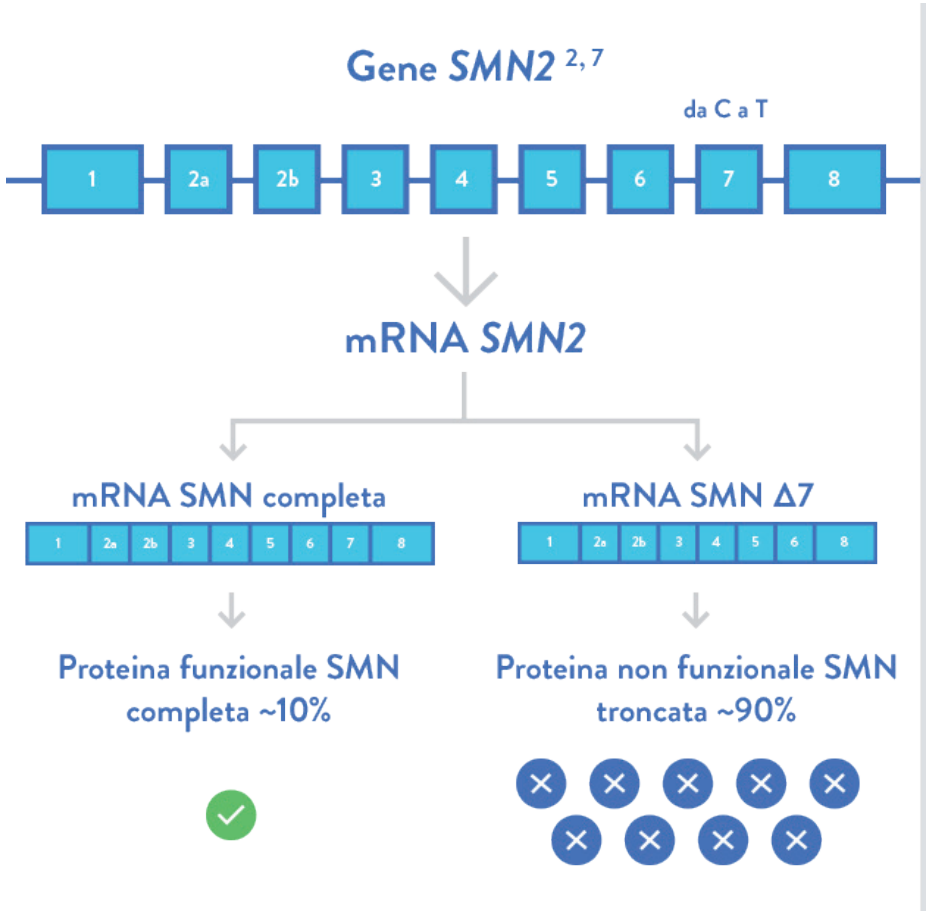


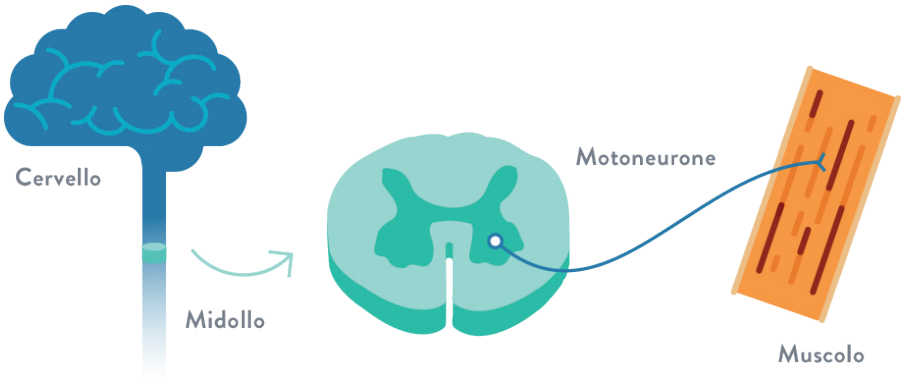
Spinal Muscular Atrophy (SMA) is a neuromuscular disease with death of the nerve cells in the spinal cord that give the muscles the movement command; it is very disabling and with reduced life expectancy.

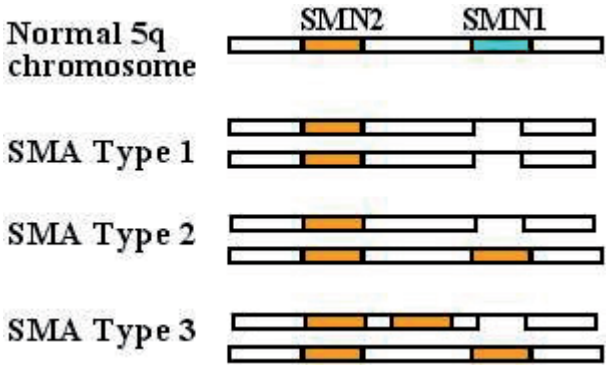
SMA affects approximately 1:10,000 births and is the most common genetic cause of infant death.
One healthy carrier for every 50 people.
Two healthy carriers, in each pregnancy, have a 25% chance of transmitting the disease to each of their children.

Method: AmplideX® SMA Plus Kit according to the manufacturer's instructions. The kit is based on amplification gene analysis (PCR) and capillary electrophoresis (CE) on SeqStudio Flex Genetic Analyzers (Thermo Fisher Scientific), to obtain the SMN1 exon 7 copy number quantification. Purpose of the test:
The AmplideX® SMA Plus kit is an assay based on the amplification of exon 7 of the SMN1 and SMN2 genes together with an endogenous control (EC) starting from purified genomic DNA: the fluorescent amplicons specific for SMN1 and SMN2 are separated by capillary electrophoresis and compared to the co-amplified endogenous control for determine the respective number of copies.
The kit also allows the detection of gene conversion events (SMN1-SMN2 and SMN2-SMN1), the presence of the c.*3+80T>G and c.*211_*212del variants of SMN1 associated with gene duplication on single chromosome and the c.859G>C variant of SMN2 correlated with a less severe disease phenotype due to a more efficient splicing mechanism of SMN2.
The number of copies of exon 7 of SMN2 and the possible presence of "hybrid" genes (with sequences belonging to both SMN1 and a SMN2) and variants of SMN1 (c.*3+80T>G and c.*211_*212del) and SMN2 (c.859G>C) will only be reported if clinically useful to determine the phenotype of the disease.
Specificity: >99%; Sensitivity: 71-95% (variable in relation to ethnicity) Limitations: The analysis is designed to perform quantitative measurement of exon 7 of the SMN1 and SMN2 genes, while it is not capable of highlighting nonsense, missense or frameshift variants of the gene sequence.
Approximately 3-8% of individuals who are healthy carriers of Spinal Muscular Atrophy have two copies of the SMN1 gene on a single chromosome and zero copies on the other chromosome; these subjects are defined as "silent carriers" or "2+0 carriers". The analysis quantitative is not able to identify the silent carrier state. The silent carrier state can, however, be discriminated by the subject having one copy of the gene on each chromosome thanks to the presence of single nucleotide variants that occur in linkage disequilibrium with gene duplication on a single chromosome.
The presence of the SMN1 variants c.*3+80 T>G (g.27134 T>G) and c.*211_*212del (g.27706_27707 delAT) suggest the presence of a gene duplication on a single chromosome and therefore of a silent carrier. The probability of being a silent carrier varies based on the presence/absence of the aforementioned variants in different ethnicities (Luo et al. 2014; Alías et al. 2018).
The two variants are not specific to the SMN1 gene or the SMN2 gene, but are detected independently their presence on one or the other gene. The annealing sites of the primers contained in the AmplideX® SMA Plus kit have no known nucleotide variants with minor allele frequency (MAF) greater than 0.005, ho-

wever the presence of rare variants could alter the dosage quantity of SMN1 and SMN2 genes (Prior et al. 2011)







Cystic Fibrosis is a common disease as it affects 1:2500 both males and females, it is generally serious, present from birth.

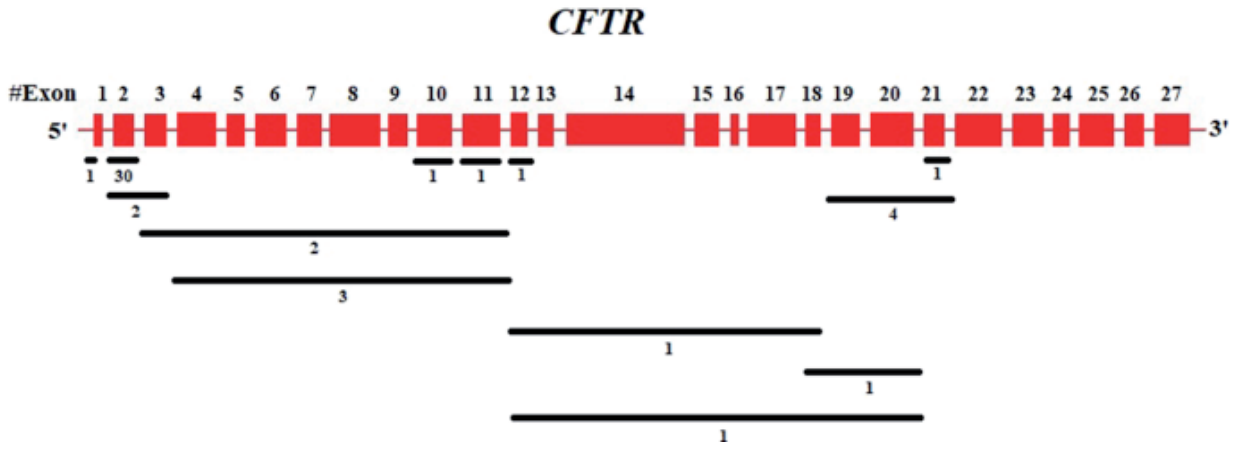
It is inherited from parents who are, almost always without knowing it, healthy carriers. The bronchi and lungs suffer the most damage where mucus tends to stagnate, generating infection and inflammation which, over time, leads to respiratory failure. The damage also involves the pancreas, intestine and liver.
The severity and type of symptoms may vary from person to person. The median survival at 40 years is 50%. In Italy there is one healthy carrier for every 25 people.
Two healthy carriers, in each pregnancy, have a 25% chance of transmitting the disease to each of their children. Fetal analysis is performed with chorionic villus sampling or amniotic fluid.

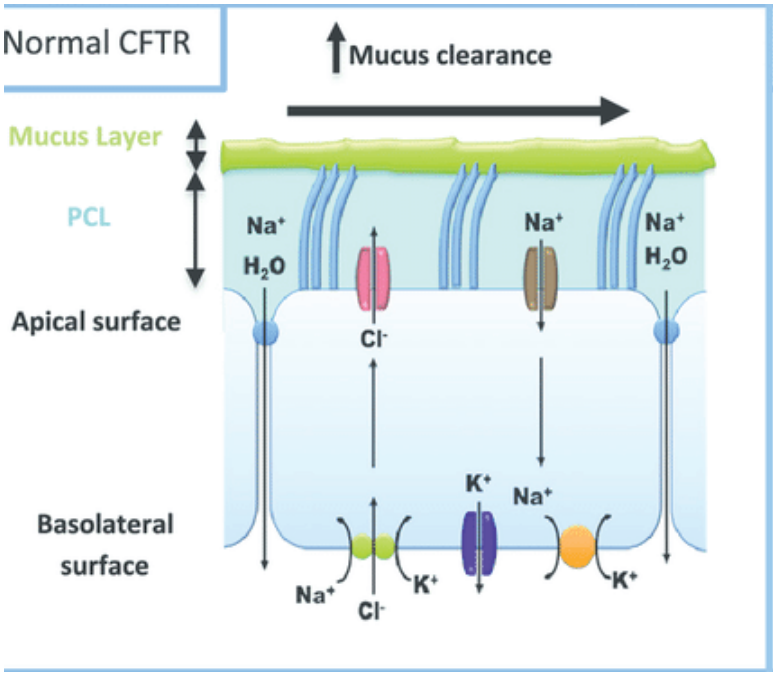
The CFTR gene variants investigated are specifically chosen because they represent the complete group of clinically validated variants classified as causing cystic fibrosis in the CFTR2 database at John s Hopkins University, a product of the CFTR2 (Clinical and Functional Translation of CFTR) initiative.

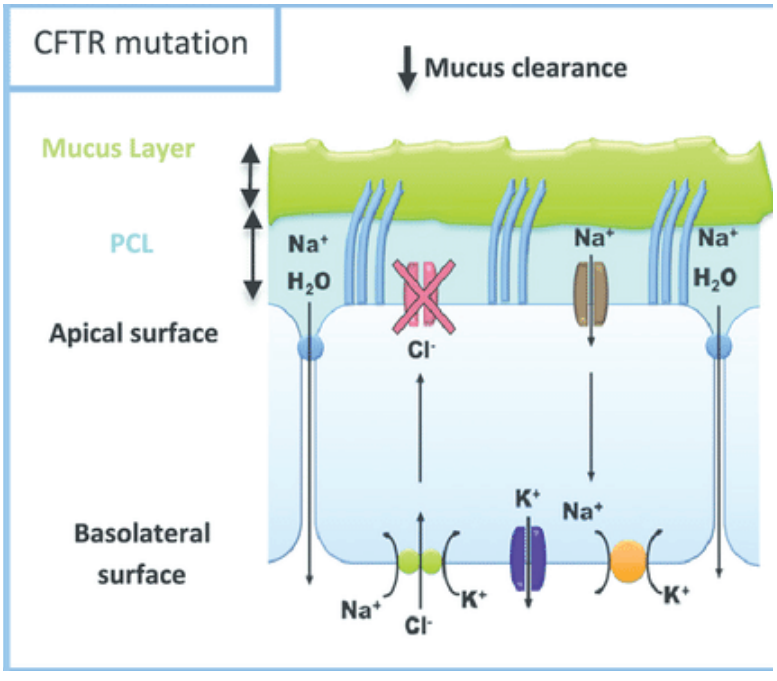
The variations include those recommended in 2004 by the American College of Medical Genetics (ACMG)1 and in 2011 by the Amer ican College of Obstetricians and Gynecologists (ACOG)2.
The essay tests: 134 variants that cause cystic fibrosis; a variant of the panel recommended by the ACMG (R 117H, classified as a mutation of various clinical consequences, MVCC, from CFTR2); a modifying variant rip ortated conditionally (PolyT); and three conditionally reported benign variants (I506V, I507V, F508C)14

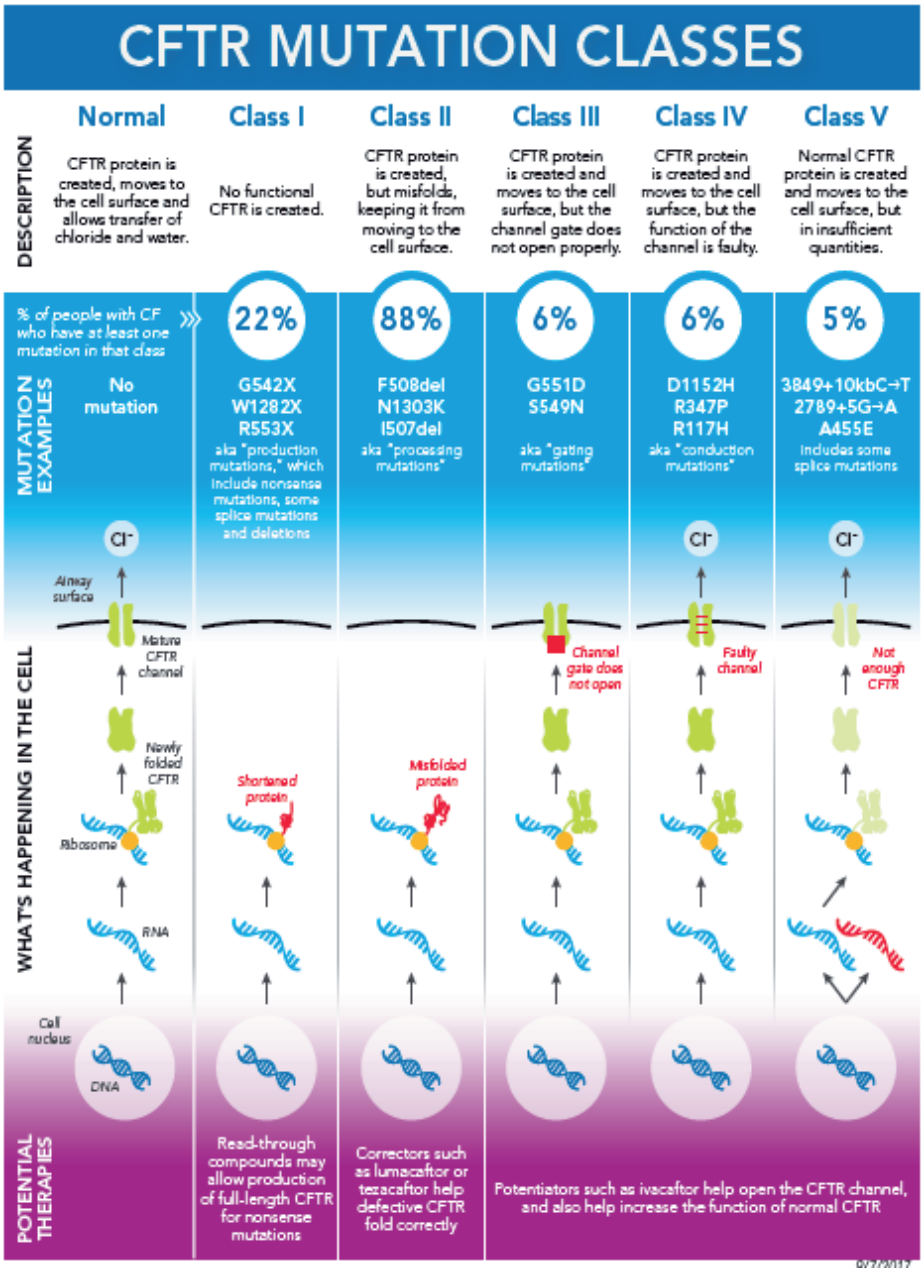
For a total of 139 variants reported. The 134 cystic fibrosis-causing variants correspond to 129 cystic fibrosis-causing variants contained n the CFTR2 database.
The CFTR2 database includes five cystic fibrosis-causing variants for which the same c changes in protein level can occur from two distinct nucleotide changes [e.g., S466X(C>A) and S466X(C>G)].
These five variants are listed by amino acid codon in the CFTR2 database (e.g e.g., S466X) while the assay reports each individual variant [e.g., S466X(C>A) and S466X(C>G)].

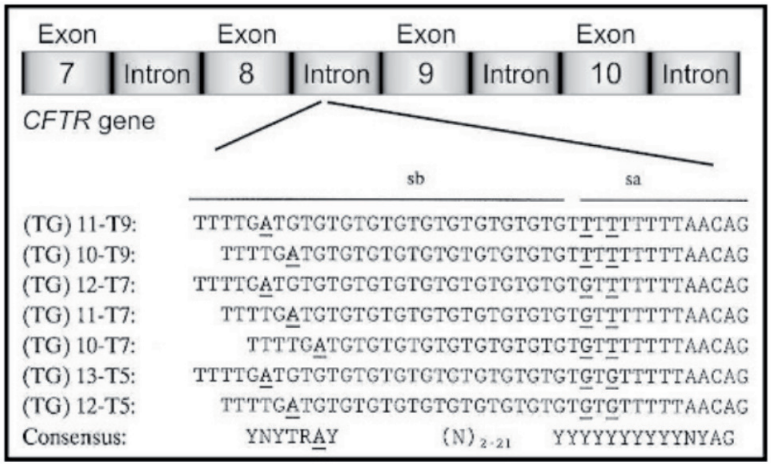
5T/TG12-13 CFTR genotype
In recent years, patients with cystic fibrosis (CF) conductance regulator (CFTR) variant poly(T) sequences have been increasingly reported with a wide spectrum of clinical severity.
The long-term clinical outcomes and progression to a CF diagnosis over time in a large Italian cohort of patients carrying the CFTR F508del/5T;TG12 genotype.
After a median follow-up of 6.7 years (range 0.2-25 years), 15 patients progressed to CF, bringing the total number of CF diagnoses to 45/129 (34.9%).
Most of these patients had mild lung diseases with pancreatic sufficiency and a low prevalence of CF-related complications.


CFTR
#Exon 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
5'
3'
1
30
2
2
3
1
1
1
4
1
1
1


Normal CFTR
Mucus clearance
Mucus Layer
PCL
Apical surface
Basolateral surface
Na+
H2O
Cl-
K+


CFTR mutation
Mucus clearance
Mucus Layer
PCL
Apical surface
Basolateral surface
Na+
H2O
Cl-
K+

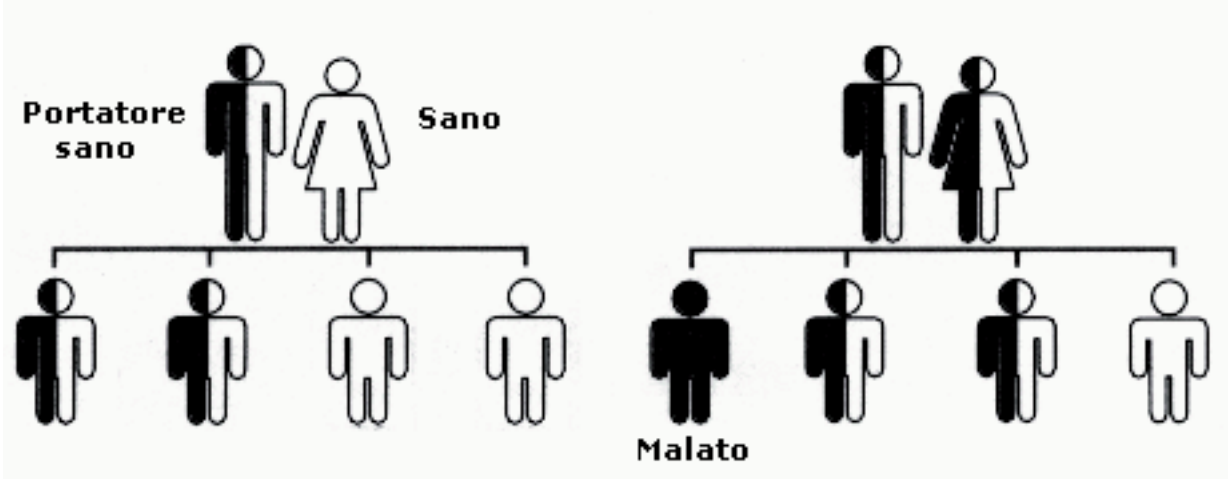

CFTR MUTATION CLASSES
Normal
Class I
Class II
Class III
Class IV
Class V
DESCRIPTION
CFTR protein is created, moves to the cell surface and allows transfer of chloride and water.
No functional CFTR is created.
CFTR protein is created, but misfolds, keeping it from moving to the cell surface.
CFTR protein is created and moves to the cell surface, but the channel gate does not open properly.
CFTR protein is created and moves to the cell surface, but the function of the channel is faulty.
Normal CFTR protein is created and moves to the cell surface, but in insufficient quantities.
% of people with CF who have at least one mutation in that class
22%
88%
6%
6%
5%
MUTATION EXAMPLES
No mutation
G542X
W1282X
R553X
aka "production mutations," which include nonsense mutations, some splice mutations and deletions
F508del
N1303K
I507del
aka "processing mutations"
G551D
S549N
aka "gating mutations"
D1152H
R347P
R117H
aka "conduction mutations"
3849+10kbC→T
2789+5G→A
A455E
Includes some splice mutations
Cl-
Airway surface
Mature CFTR channel
Channel gate does not open
Faulty channel
Not enough CFTR
WHAT'S HAPPENING IN THE CELL
Newly folded CFTR
Shortened protein
Misfolded protein
Ribosome
RNA
Cell nucleus
DNA
POTENTIAL THERAPIES
Read-through compounds may allow production of full-length CFTR for nonsense mutations
Correctors such as lumacaftor or tezacaftor help defective CFTR fold correctly
Potentiators such as ivacaftor help open the CFTR channel, and also help increase the function of normal CFTR


Exon
7
Intron
Exon
8
Intron
Exon
9
Intron
Exon
10
Intron
CFTR gene
sb
sa
(TG) 11-T9: TTTTGATGTGTGTGTGTGTGTGTGTGTTTTTTTTTAACAG
(TG) 10-T9: TTTTGATGTGTGTGTGTGTGTGTGTTTTTTTTTAACAG
(TG) 12-T7: TTTTGATGTGTGTGTGTGTGTGTGTGTTTTTTTTAACAG
(TG) 11-T7: TTTTGATGTGTGTGTGTGTGTGTGTTTTTTTTAACAG
(TG) 10-T7: TTTTGATGTGTGTGTGTGTGTGTGTTTTTTTTAACAG
(TG) 13-T5: TTTTGATGTGTGTGTGTGTGTGTGTGTTTTTTAACAG
(TG) 12-T5: TTTTGATGTGTGTGTGTGTGTGTGTTTTTTAACAG
Consensus: YNYTRAY (N)2-21 YYYYYYYYYYNYAG

CARRIER STATUS SENSITIVITY AND REPRODUCTIVE RISK (AUTOSOMIC RECESSIVE)

	CYSTIC FIBROSIS	SMA
???? / ????	(1/25 x 1/25 x ¼) 1/2500	(1/50 x 1/50 x ¼) 1/10000
Neg 1 / ????	(1/25 x ¼ x 1/25 x ¼) 1/10000	(1/50 x 1/20 x 1/50 x ¼) 1/200 000
Neg 1 / Neg 1	(1/25 x ¼ x 1/25 x ¼ x ¼) 1/40000	(1/50 x 1/20 x 1/50 x 1/20 x ¼) 1/4 000 000
Etero / ???	(1 x 1/25 x ¼) 1/100	(1 x 1/50 x ¼) 1/200
Etero / Neg 1(75)	(1 x 1/25 x ¼x ¼) 1/400	Etero /Neg 1(95) (1 x 1/50 x 1/20 x ¼) 1/4000
Etero / Neg 2(90)	(1 x 1/25 x 1/10 x ¼) 1/1000	
Etero / Neg 3(95)	(1 x 1/25 x 1/20 x ¼) 1/2000	
Etero / Neg 4(99)	(1 x 1/25 x 1/100 x ¼) 1/10000	
Etero / Etero	1/4	Etero / Etero 1/4

Mutazioni: Neg 1(37), Neg3 (139-152), Neg4 (intero gene)



Fragile X Syndrome (Martin Bell Syndrome). Fragile X syndrome is the most frequent form of hereditary mental retardation. It is caused by a mutation of the FMR1 gene located on the X chromosome (FRAXA mutation).

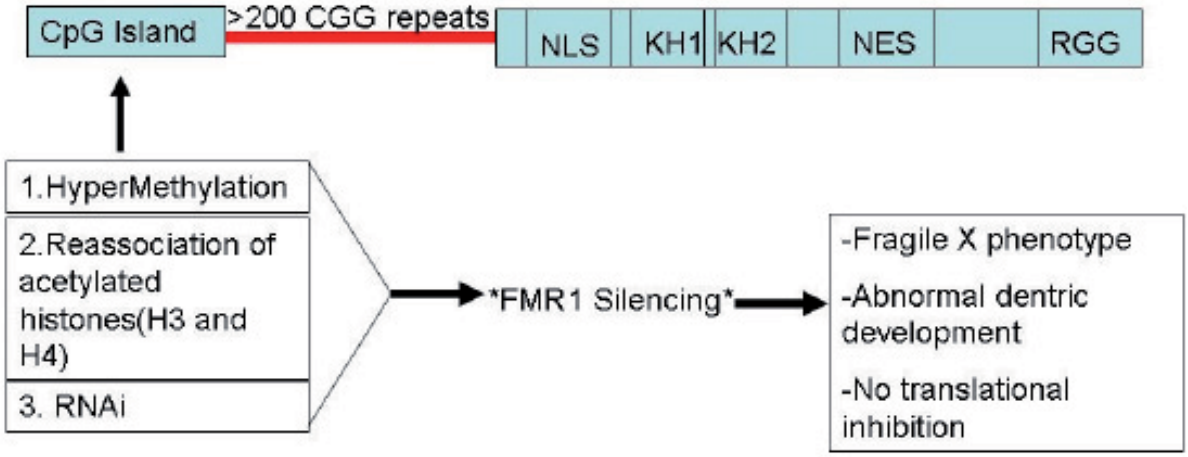
This mutation consists of an amplification and subsequent methylation of a CGG triplet sequence localized in the transcribed and non-translated portion of the first exon of the gene and is responsible for blocking its transcription.
Since the gene codes for a protein necessary for the normal development of the central nevous system, the lack of the protein causes mental retardation.
Normal alleles have a number of triplets between 5 and 45; in mutated alleles, solely responsible for the syndrome, this number is greater than 200 (complete mutation). Alleles with a number of triplets between 56 and 200 (premutation) are normally expressed but are unstable, with a tendency to transition towards complete mutation during female meiosis. Therefore, healthy women who have a premutation have a high risk of passing on a full mutation to a son or daughter.

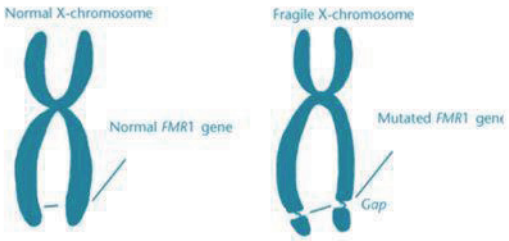
Methodology: Amplification conducted using fluorescent primers of the 5' UTR region of the FMR1 gene (NM_002024) containing a variable number of CGG trinucleotide repeats. Genotyping conducted by electrophoretic run on a ThermoFisher SeqStudio capillary sequencer Flex.
Limits: The technique does not allow the highlighting of single nucleotide variations (aka point mutations), deletions and/or gene duplications as well as the Methylation status of the FMR1 gene Amplification and subsequent genotyping are capable of measuring alleles of lengths up to 80 CGG triplets.
The methodology may not highlight the presence of large triplet expansions (>80 repeats). If a single allele is identified, in subjects of female sex or with two X chromosomes, it is not possible to discriminate between a homozygous genotype and a compound heterozygous genotype having a allele larger than 80 repeats.
In this case it is advisable to proceed with genotyping using second level analysis. Sensitivity of the method >99%.

Long Range FMR1 PCR technology can resolve many of the technological challenges that limit routine fragile X testing.
This method reproducibly amplified alleles with greater than 1,000 CGG repeats, and demonstrated excellent concordance with Southern blot in an assessment of clinical specimens whose FMR1 alleles spanned the entire range of CGG repeats.
The consistency and sensitivity of the reagents to detect premutation and full mutation alleles, including mosaic species that may only be present in a few percent of cells, also resolved ambiguities in identifying female homozygous samples that can confound conventional FMR1 PCR assays.
Reproducible detection of full mutation alleles by PCR has implications for the broader adoption of FMR1 analysis.Reference ranges:
Normal alleles: 5-45 CGG repeats Alleles in the Gray Zone range: 46-54 CGG repeats Alleles in the premutation range: 55-200 CGG repeats: Alleles in the full mutation range: >200 CGG repeats

Normal FMR1 Gene

Fragile X





Screening carrier for AR and X linked mendelian variants (300 genes)

Medical Reproduction, Infertility. Screening for AR and X linked mendelian disorders in phisiological Reproduction, normal phenotype.

Tabella 1: GeneScreen® Easy - Elenco dei geni analizzati e delle malattie genetiche investigate

Gene	OMIM Gene	Disease	OMIM Disease	Inheritance
ABCD1	300371	Adrenoleukodystrophy	300100	XLR
ABCC8	600509	Diabetes mellitus, noninsulin-dependent	125853	AD
		Diabetes mellitus, permanent neonatal	606176	AD, AR
		Diabetes mellitus, transient neonatal 2	610374	
		Hyperinsulinemic hypoglycemia, familial, 1	256450	AD, AR
		Hypoglycemia of infancy, leucine-sensitive	240800	AD
ABCB11	603201	Cholestasis, benign recurrent intrahepatic, 2	605479	AR
		Cholestasis, progressive familial intrahepatic 2	601847	AR
ADAMTS2	604539	Ehlers-Danlos syndrome, dermatosparaxis type	225410	AR
EVC	604831	?Weyers acrofacial dysostosis	193530	AD
ACADS	606885	Acyl-CoA dehydrogenase, short-chain, deficiency of	201470	AR
ACADM	607008	Acyl-CoA dehydrogenase, medium chain, deficiency of	201450	AR
EVC2	607261	Ellis-van Creveld syndrome	225500	AR
ACAT1	607809	Alpha-methylacetoacetic aciduria	203750	AR
ADA	608958	Adenosine deaminase deficiency, partial	102700	SMo, AR
ACADVL	609575	VLCAD deficiency	201475	AR
ACOX1	609751	Peroxisomal acyl-CoA oxidase deficiency	264470	AR
AGL	610860	Glycogen storage disease IIIa	232400	AR
ACAD9	611103	Mitochondrial complex I deficiency, nuclear type 20	611126	AR
AGA	613228	Aspartylglucosaminuria	208400	AR
		Severe combined immunodeficiency due to ADA deficiency	102700	SMo, AR
		Glycogen storage disease IIIb	232400	AR
AGPS	603051	Rhizomelic chondrodysplasia punctata, type 3	600121	AR
AGXT	604285	Hyperoxaluria, primary, type 1	259900	AR
AIRE	607358	Autoimmune polyendocrinopathy syndrome , type I, with or without reversible metaphyseal dysplasia	240300	AD, AR
ALDH3A2	609523	Sjogren-Larsson syndrome	270200	AR
ALDOB	612724	Fructose intolerance, hereditary	229600	AR
ALG6	604566	Congenital disorder of glycosylation, type Ic	603147	AR

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CHIAMATA GRATUITA NUMEROVERDE

GENOMA Molecular Genetics Laboratories Group

AZIENDA CON SISTEMA DI GESTIONE QUALITÀ CERTIFICATO DA DNV GL = ISO 9001 =

ALMS1	606844	Alstrom syndrome	203800	AR
ALPL	171760	Hypophosphatasia, adult	146300	AD, AR
		Hypophosphatasia, childhood	241510	AR
		Hypophosphatasia, infantile	241500	AR
		Odontohypophosphatasia	146300	AD, AR
AMT	238310	Glycine encephalopathy	605899	AR
AR	313700	Androgen insensitivity	300068	XLR
		Androgen insensitivity, partial, with or without breast cancer	312300	XLR
		Hypospadias 1, X-linked	300633	XLR
		Spinal and bulbar muscular atrophy of Kennedy	313200	XLR
		Prostate cancer, susceptibility to	176807	AD, SMu
ARG1	608313	Argininemia	207800	AR
ARSA	607574	Metachromatic leukodystrophy	250100	AR
ARSB	611542	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200	AR
ARSE	300180	Chondrodysplasia punctata, X-linked recessive	302950	XLR
ASL	608310	Argininosuccinic aciduria	207900	AR
ASNS	108370	Asparagine synthetase deficiency	615574	AR
ASPA	608034	Canavan disease	271900	AR
ASS1	603470	Citrullinemia	215700	AR
ATM	607585	Ataxia-telangiectasia	208900	AR
		Lymphoma, B-cell non-Hodgkin, somatic		
		Lymphoma, mantle cell, somatic		
		T-cell prolymphocytic leukemia, somatic		
		Breast cancer, susceptibility to	114480	AD, SMu
ATP6V1B1	192132	Renal tubular acidosis with deafness	267300	AR
ATP7A	300011	Menkes disease	309400	XLR
		Occipital horn syndrome	304150	XLR
		Spinal muscular atrophy, distal, X-linked 3	300489	XLR
ATP7B	606882	Wilson disease	277900	AR
ATRX	300032	Alpha-thalassemia myelodysplasia syndrome, somatic	300448	
		Alpha-thalassemia/mental retardation syndrome	301040	XLD

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CHIAMATA GRATUITA NUMEROVERDE

		Mental retardation-hypotonic facies syndrome, X-linked	309580	XLR
BBS1	209901	Bardet-Biedl syndrome 1	209900	AR, DR
BBS10	610148	Bardet-Biedl syndrome 10	615987	AR
BBS12	610683	Bardet-Biedl syndrome 12	615989	AR
BBS2	606151	Bardet-Biedl syndrome 2	615981	AR
BBS2	606151	Retinitis pigmentosa 74	616562	AR
BCHE	177400	Butyrylcholinesterase deficiency	617936	
BCHE	177400	Apnea, postanesthetic, susceptibility to, due to BCHE deficiency	617936	
BCKDHA	608348	Maple syrup urine disease, type Ia	248600	AR
BCKDHB	248611	Maple syrup urine disease, type Ib	248600	AR
BCS1L	603647	Bjornstad syndrome	262000	AR
BCS1L	603647	GRACILE syndrome	603358	
BCS1L	603647	Leigh syndrome	256000	Mi, AR
BCS1L	603647	Mitochondrial complex III deficiency, nuclear type 1	124000	AR
BLM	604610	Bloom syndrome	210900	AR
BSND	606412	Bartter syndrome, type 4a	602522	AR
BSND	606412	Sensorineural deafness with mild renal dysfunction	602522	AR
BTD	609019	Biotinidase deficiency	253260	AR
BTK	300300	Agammaglobulinemia, X-linked 1	300755	XLR
BTK	300300	Isolated growth hormone deficiency, type III, with agammaglobulinemia	307200	XLR
CAPN3	114240	Muscular dystrophy, limb-girdle, autosomal dominant 4	618129	AD
CAPN3	114240	Muscular dystrophy, limb-girdle, autosomal recessive 1	253600	AR
CBS	613381	Homocystinuria, B6-responsive and nonresponsive types	236200	AR
CBS	613381	Thrombosis, hyperhomocysteinemic	236200	AR
CC2D2A	612013	COACH syndrome	216360	AR
CC2D2A	612013	Joubert syndrome 9	612285	AR
CC2D2A	612013	Meckel syndrome 6	612284	AR
CD40LG	300386	Immunodeficiency, X-linked, with hyper-IgM	308230	XLR
CDH23	605516	Deafness, autosomal recessive 12	601386	AR
CDH23	605516	Usher syndrome, type 1D	601067	AR, DR
CDH23	605516	Usher syndrome, type 1D/F digenic	601067	AR, DR

		Pituitary adenoma 5, multiple types	617540	AD
CEP290	610142	Bardet-Biedl syndrome 14	615991	AR
CEP290	610142	Joubert syndrome 5	610188	AR
CEP290	610142	Leber congenital amaurosis 10	611755	
CEP290	610142	Meckel syndrome 4	611134	AR
CEP290	610142	Senior-Loken syndrome 6	610189	AR
CERKL	608381	Retinitis pigmentosa 26	608380	
CFTR	602421	Congenital bilateral absence of vas deferens	277180	AR
CFTR	602421	Cystic fibrosis	219700	AR
CFTR	602421	Sweat chloride elevation without CF		
CFTR	602421	Bronchiectasis with or without elevated sweat chloride 1, modifier of	211400	AD
CFTR	602421	Hypertrypsinemia, neonatal		
CFTR	602421	Pancreatitis, hereditary	167800	AD
CHM	300390	Choroideremia	303100	XLD
CHRNE	100725	Myasthenic syndrome, congenital, 4A, slow-channel	605809	AD, AR
CHRNE	100725	Myasthenic syndrome, congenital, 4B, fast-channel	616324	AR
CHRNE	100725	Myasthenic syndrome, congenital, 4C, associated with acetylcholine receptor deficiency	608931	AR
CLN3	607042	Ceroid lipofuscinosis, neuronal, 3	204200	AR
CLN5	608102	Ceroid lipofuscinosis, neuronal, 5	256731	AR
CLN6	606725	Ceroid lipofuscinosis, neuronal, 6	601780	AR
CLN6	606725	Ceroid lipofuscinosis, neuronal, Kufs type, adult onset	204300	AR
CLN8	607837	Ceroid lipofuscinosis, neuronal, 8	600143	AR
CLN8	607837	Ceroid lipofuscinosis, neuronal, 8, Northern epilepsy variant	610003	AR
CLRN1	606397	Retinitis pigmentosa 61	614180	
CLRN1	606397	Usher syndrome, type 3A	276902	AR
CNGB3	605080	Achromatopsia 3	262300	AR
CNGB3	605080	Macular degeneration, juvenile	248200	AR
COL17A1	113811	Epidermolysis bullosa, junctional, localisata variant	226650	AR
COL17A1	113811	Epidermolysis bullosa, junctional, non-Herlitz type	226650	AR
COL17A1	113811	Epithelial recurrent erosion dystrophy	122400	AD
COL4A3	120070	Alport syndrome 2, autosomal recessive	203780	AR

DNAI1	604366	Ciliary dyskinesia, primary, 1, with or without situs inversus	244400	AR
DNAI2	605483	Ciliary dyskinesia, primary, 9, with or without situs inversus	612444	
DOK7	610285	Fetal akinesia deformation sequence	208150	AR
		Myasthenic syndrome, congenital, 10	254300	AR
DPYD	612779	5-fluorouracil toxicity	274270	AR
		Dihydropyrimidine dehydrogenase deficiency	274270	AR
DYSF	603009	Miyoshi muscular dystrophy 1	254130	AR
		Muscular dystrophy, limb-girdle, autosomal recessive 2	253601	AR
		Myopathy, distal, with anterior tibial onset	606768	AR
EDA	300451	Ectodermal dysplasia 1, hypohidrotic, X-linked	305100	XLR
		Tooth agenesis, selective, X-linked 1	313500	XLD
EDAR	604095	Ectodermal dysplasia 10A, hypohidrotic/hair/nail type, autosomal dominant	129490	AD
		Ectodermal dysplasia 10B, hypohidrotic/hair/tooth type, autosomal recessive	224900	AR
		Hair morphology 1, hair thickness	612630	
EIF2AK3	604032	Wolcott-Rallison syndrome	226980	AR
EIF2B1	606686	Leukoencephalopathy with vanishing white matter	603896	AR
EIF2B2	606454	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EIF2B3	606273	Leukoencephalopathy with vanishing white matter	603896	AR
EIF2B4	606687	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EIF2B5	603945	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EMD	300384	Emery-Dreifuss muscular dystrophy 1, X-linked	310300	XLR
ERCC6	609413	Cerebrooculofacioskeletal syndrome 1	214150	AR
		Cockayne syndrome, type B	133540	AR
		De Sanctis-Cacchione syndrome	278800	AR
		Premature ovarian failure 11	616946	AD
		UV-sensitive syndrome 1	600630	AR
		Lung cancer, susceptibility to	211980	AD, SMu
		Macular degeneration, age-related, susceptibility to, 5	613761	

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ERCC8	609412	Cockayne syndrome, type A	216400	AR
		UV-sensitive syndrome 2	614621	AR
ESCO2	609353	Roberts syndrome	268300	AR
		SC phocomelia syndrome	269000	AR
ETFA	608053	Glutaric acidemia IIA	231680	AR
ETFB	130410	Glutaric acidemia IIB	231680	AR
ETFDH	231675	Glutaric acidemia IIC	231680	AR
ETHE1	608451	Ethylmalonic encephalopathy	602473	AR
EVC2	607261	Ellis-van Creveld syndrome	225500	AR
		Weyers acrofacial dysostosis	193530	AD
EYS	612424	Retinitis pigmentosa 25	602772	AR
F11	264900	Factor XI deficiency, autosomal dominant	612416	
		Factor XI deficiency, autosomal recessive	612416	
F8	300841	Hemophilia A	306700	XLR
F9	300746	Hemophilia B	306900	XLR
		Thrombophilia, X-linked, due to factor IX defect	300807	
		Deep venous thrombosis, protection against	300807	
		Warfarin sensitivity	122700	AD
FAH	613871	Tyrosinemia, type I	276700	AR
FANCA	607139	Fanconi anemia, complementation group A	227650	AR
FANCC	613899	Fanconi anemia, complementation group C	227645	AR
FANCG	602956	Fanconi anemia, complementation group G	614082	
FH	136850	Fumarase deficiency	606812	AR
		Leiomyomatosis and renal cell cancer	150800	AD
FKRP	606596	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 5	613153	AR
		Muscular dystrophy-dystroglycanopathy (congenital with or without mental retardation), type B, 5	606612	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 5	607155	AR
FKTN	607440	Cardiomyopathy, dilated, 1X	611615	AR
		Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4	253800	AR
		Muscular dystrophy-dystroglycanopathy (congenital without mental retardation), type B, 4	613152	AR

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Tabella 1: GeneScreen® Easy - Elenco dei geni analizzati e delle malattie genetiche investigate

Gene	OMIM Gene	Disease	OMIM Disease	Inheritance
ABCD1	300371	Adrenoleukodystrophy	300100	XLR
ABCC8	600509	Diabetes mellitus, noninsulin-dependent	125853	AD
		Diabetes mellitus, permanent neonatal	606176	AD, AR
		Diabetes mellitus, transient neonatal 2	610374	
		Hyperinsulinemic hypoglycemia, familial, 1	256450	AD, AR
		Hypoglycemia of infancy, leucine-sensitive	240800	AD
ABCB11	603201	Cholestasis, benign recurrent intrahepatic, 2	605479	AR
		Cholestasis, progressive familial intrahepatic 2	601847	AR
ADAMTS2	604539	Ehlers-Danlos syndrome, dermatosparaxis type	225410	AR
EVC	604831	?Weyers acrofacial dysostosis	193530	AD
ACADS	606885	Acyl-CoA dehydrogenase, short-chain, deficiency of	201470	AR
ACADM	607008	Acyl-CoA dehydrogenase, medium chain, deficiency of	201450	AR
EVC2	607261	Ellis-van Creveld syndrome	225500	AR
ACAT1	607809	Alpha-methylacetoacetic aciduria	203750	AR
ADA	608958	Adenosine deaminase deficiency, partial	102700	SMo, AR
ACADVL	609575	VLCAD deficiency	201475	AR
ACOX1	609751	Peroxisomal acyl-CoA oxidase deficiency	264470	AR
AGL	610860	Glycogen storage disease IIIa	232400	AR
ACAD9	611103	Mitochondrial complex I deficiency, nuclear type 20	611126	AR
AGA	613228	Aspartylglucosaminuria	208400	AR
		Severe combined immunodeficiency due to ADA deficiency	102700	SMo, AR
		Glycogen storage disease IIIb	232400	AR
AGPS	603051	Rhizomelic chondrodysplasia punctata, type 3	600121	AR
AGXT	604285	Hyperoxaluria, primary, type 1	259900	AR
AIRE	607358	Autoimmune polyendocrinopathy syndrome , type I, with or without reversible metaphyseal dysplasia	240300	AD, AR
ALDH3A2	609523	Sjogren-Larsson syndrome	270200	AR
ALDOB	612724	Fructose intolerance, hereditary	229600	AR
ALG6	604566	Congenital disorder of glycosylation, type Ic	603147	AR

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ALMS1	606844	Alstrom syndrome	203800	AR
ALPL	171760	Hypophosphatasia, adult	146300	AD, AR
		Hypophosphatasia, childhood	241510	AR
		Hypophosphatasia, infantile	241500	AR
		Odontohypophosphatasia	146300	AD, AR
AMT	238310	Glycine encephalopathy	605899	AR
AR	313700	Androgen insensitivity	300068	XLR
		Androgen insensitivity, partial, with or without breast cancer	312300	XLR
		Hypospadias 1, X-linked	300633	XLR
		Spinal and bulbar muscular atrophy of Kennedy	313200	XLR
		Prostate cancer, susceptibility to	176807	AD, SMu
ARG1	608313	Argininemia	207800	AR
ARSA	607574	Metachromatic leukodystrophy	250100	AR
ARSB	611542	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200	AR
ARSE	300180	Chondrodysplasia punctata, X-linked recessive	302950	XLR
ASL	608310	Argininosuccinic aciduria	207900	AR
ASNS	108370	Asparagine synthetase deficiency	615574	AR
ASPA	608034	Canavan disease	271900	AR
ASS1	603470	Citrullinemia	215700	AR
ATM	607585	Ataxia-telangiectasia	208900	AR
		Lymphoma, B-cell non-Hodgkin, somatic		
		Lymphoma, mantle cell, somatic		
		T-cell prolymphocytic leukemia, somatic		
		Breast cancer, susceptibility to	114480	AD, SMu
ATP6V1B1	192132	Renal tubular acidosis with deafness	267300	AR
ATP7A	300011	Menkes disease	309400	XLR
		Occipital horn syndrome	304150	XLR
		Spinal muscular atrophy, distal, X-linked 3	300489	XLR
ATP7B	606882	Wilson disease	277900	AR
ATRX	300032	Alpha-thalassemia myelodysplasia syndrome, somatic	300448	
		Alpha-thalassemia/mental retardation syndrome	301040	XLD

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		Hemoglobin H disease, nondeletional	613978	
		Methemoglobinemia, alpha type	617973	
		Thalassemias, alpha-	604131	
HBA2	141850	Erythrocytosis 7	617981	
		Heinz body anemia	140700	AD
		Hemoglobin H disease, deletional and nondeletional	613978	
		Thalassemia, alpha-	604131	
HBB	141900	Delta-beta thalassemia	141749	AD
		Erythrocytosis 6	617980	
		Heinz body anemia	140700	AD
		Hereditary persistence of fetal hemoglobin	141749	AD
		Methmoglobinemia, beta type	617971	
		Sickle cell anemia	603903	AR
		Thalassemia, beta	613985	
		Thalassemia-beta, dominant inclusion-body	603902	
		Malaria, resistance to	611162	
HEXA	606869	GM2-gangliosidosis, several forms	272800	AR
		Tay-Sachs disease	272800	AR
		Hex A pseudodeficiency	272800	AR
HEXB	606873	Sandhoff disease, infantile, juvenile, and adult forms	268800	AR
HFE	613609	Hemochromatosis	235200	AR
		Transferrin serum level QTL2	614193	
		Alzheimer disease, susceptibility to	104300	AD
		Microvascular complications of diabetes 7	612635	
		Porphyria cutanea tarda, susceptibility to	176100	AD, AR
		Porphyria variegata, susceptibility to	176200	AD
HFE2	608374	Hemochromatosis, type 2A	602390	AR
HGD	607474	Alkaptonuria	203500	AR
HGSNAT	610453	Mucopolysaccharidosis type IIIC (Sanfilippo C)	252930	AR
		Retinitis pigmentosa 73	616544	AR
HLCS	609018	Holocarboxylase synthetase deficiency	253270	AR

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HMGCL	613898	HMG-CoA lyase deficiency	246450	AR
HOGA1	613597	Hyperoxaluria, primary, type III	613616	
HPS1	604982	Hermansky-Pudlak syndrome 1	203300	AR
HPS3	606118	Hermansky-Pudlak syndrome 3	614072	AR
HSD17B4	601860	D-bifunctional protein deficiency	261515	AR
		Perrault syndrome 1	233400	AR
HSD3B2	613890	Adrenal hyperplasia, congenital, due to 3-beta-hydroxysteroid dehydrogenase 2 deficiency	201810	AR
HYLS1	610693	Hydrolethalus syndrome	236680	AR
IDS	300823	Mucopolysaccharidosis II	309900	XLR
IDUA	252800	Mucopolysaccharidosis Ih	607014	AR
		Mucopolysaccharidosis Ih/s	607015	AR
		Mucopolysaccharidosis Is	607016	AR
IKBKAP	603722	Dysautonomia, familial	223900	AR
IL2RG	308380	Combined immunodeficiency, X-linked, moderate	312863	XLR
		Severe combined immunodeficiency, X-linked	300400	XLR
ISPD	614631	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 7	614643	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 7	616052	AR
IVD	607036	Isovaleric acidemia	243500	AR
KCNJ11	600937	Diabetes mellitus, transient neonatal, 3	610582	AD
		Diabetes, permanent neonatal, with or without neurologic features	606176	AD, AR
		Hyperinsulinemic hypoglycemia, familial, 2	601820	AR
		Maturity-onset diabetes of the young, type 13	616329	AD
		Diabetes mellitus, type 2, susceptibility to	125853	AD
L1CAM	308840	Corpus callosum, partial agenesis of	304100	XLR
		CRASH syndrome	303350	XLR
		Hydrocephalus due to aqueductal stenosis	307000	XLR
		Hydrocephalus with congenital idiopathic intestinal pseudoobstruction	307000	XLR
		Hydrocephalus with Hirschsprung disease	307000	XLR

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		Alport syndrome 3, autosomal dominant	104200	AD
		Hematuria, benign familial	141200	AD
COL4A4	120131	Alport syndrome 2, autosomal recessive	203780	AR
		Hematuria, familial benign	141200	AD
COL4A5	303630	Alport syndrome 1, X-linked	301050	XLD
COL7A1	120120	EBD inversa	226600	AR
		EBD, Bart type	132000	AD
		EBD, localisata variant		
		Epidermolysis bullosa dystrophica, AD	131750	AD
		Epidermolysis bullosa dystrophica, AR	226600	AR
		Epidermolysis bullosa pruriginosa	604129	AD, AR
		Epidermolysis bullosa, pretibial	131850	AD, AR
		Toenail dystrophy, isolated	607523	AD
		Transient bullous of the newborn	131705	AD, AR
CPS1	608307	Carbamoylphosphate synthetase I deficiency	237300	AR
		Pulmonary hypertension, neonatal, susceptibility to	615371	
		Venoocclusive disease after bone marrow transplantation		
CPT1A	600528	CPT deficiency, hepatic, type IA	255120	AR
CPT2	600650	CPT II deficiency, infantile	600649	AR
		CPT II deficiency, lethal neonatal	608836	AR
		CPT II deficiency, myopathic, stress-induced	255110	AD, AR
		Encephalopathy, acute, infection-induced, 4, susceptibility to	614212	AD, AR
CRB1	604210	Leber congenital amaurosis 8	613835	
		Pigmented paravenous chorioretinal atrophy	172870	AD
		Retinitis pigmentosa-12	600105	AR
CRTAP	605497	Osteogenesis imperfecta, type VII	610682	AR
CSTB	601145	Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg)	254800	AR
CTNS	606272	Cystinosis, atypical nephropathic	219800	AR
		Cystinosis, late-onset juvenile or adolescent nephropathic	219900	AR
		Cystinosis, nephropathic	219800	AR
		Cystinosis, ocular nonnephropathic	219750	AR

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CTSD	116840	Ceroid lipofuscinosis, neuronal, 10	610127	AR
CTSK	601105	Pycnodysostosis	265800	AR
CYP11B1	610613	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	202010	AR
		Aldosteronism, glucocorticoid-remediable	103900	AD
CYP11B2	124080	Aldosterone to renin ratio raised		
		Hypoaldosteronism, congenital, due to CMO I deficiency	203400	AR
		Hypoaldosteronism, congenital, due to CMO II deficiency	610600	AR
		Low renin hypertension, susceptibility to		
CYP17A1	609300	17,20-lyase deficiency, isolated	202110	AR
		17-alpha-hydroxylase/17,20-lyase deficiency	202110	AR
CYP19A1	107910	Aromatase deficiency	613546	
		Aromatase excess syndrome	139300	AD
CYP1B1	601771	Anterior segment dysgenesis 6, multiple subtypes	617315	
		Glaucoma 3A, primary open angle, congenital, juvenile, or adult onset	231300	AR
CYP21A2	613815	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency	201910	AR
		Hyperandrogenism, nonclassic type, due to 21-hydroxylase deficiency	201910	AR
CYP27A1	606530	Cerebrotendinous xanthomatosis	213700	AR
DBT	248610	Maple syrup urine disease, type II	248600	AR
DCLRE1C	605988	Omenn syndrome	603554	AR
		Severe combined immunodeficiency, Athabascan type	602450	AR
DHCR7	602858	Smith-Lemli-Opitz syndrome	270400	AR
DHDDS	608172	Congenital disorder of glycosylation, type 1bb	613861	AR
		Developmental delay and seizures with or without movement abnormalities	617836	AD
		Retinitis pigmentosa 59	613861	AR
DKC1	300126	Dyskeratosis congenita, X-linked	305000	XLR
DLD	238331	Dihydrolipoamide dehydrogenase deficiency	246900	AR
DMD	300377	Becker muscular dystrophy	300376	XLR
		Cardiomyopathy, dilated, 3B	302045	XL
		Duchenne muscular dystrophy	310200	XLR
DNAH5	603335	Ciliary dyskinesia, primary, 3, with or without situs inversus	608644	

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DNAI1	604366	Ciliary dyskinesia, primary, 1, with or without situs inversus	244400	AR
DNAI2	605483	Ciliary dyskinesia, primary, 9, with or without situs inversus	612444	
DOK7	610285	Fetal akinesia deformation sequence	208150	AR
		Myasthenic syndrome, congenital, 10	254300	AR
DPYD	612779	5-fluorouracil toxicity	274270	AR
		Dihydropyrimidine dehydrogenase deficiency	274270	AR
DYSF	603009	Miyoshi muscular dystrophy 1	254130	AR
		Muscular dystrophy, limb-girdle, autosomal recessive 2	253601	AR
		Myopathy, distal, with anterior tibial onset	606768	AR
EDA	300451	Ectodermal dysplasia 1, hypohidrotic, X-linked	305100	XLR
		Tooth agenesis, selective, X-linked 1	313500	XLD
EDAR	604095	Ectodermal dysplasia 10A, hypohidrotic/hair/nail type, autosomal dominant	129490	AD
		Ectodermal dysplasia 10B, hypohidrotic/hair/tooth type, autosomal recessive	224900	AR
		Hair morphology 1, hair thickness	612630	
EIF2AK3	604032	Wolcott-Rallison syndrome	226980	AR
EIF2B1	606686	Leukoencephalopathy with vanishing white matter	603896	AR
EIF2B2	606454	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EIF2B3	606273	Leukoencephalopathy with vanishing white matter	603896	AR
EIF2B4	606687	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EIF2B5	603945	Leukoencephalopathy with vanishing white matter	603896	AR
		Ovarioleukodystrophy	603896	AR
EMD	300384	Emery-Dreifuss muscular dystrophy 1, X-linked	310300	XLR
ERCC6	609413	Cerebrooculofacioskeletal syndrome 1	214150	AR
		Cockayne syndrome, type B	133540	AR
		De Sanctis-Cacchione syndrome	278800	AR
		Premature ovarian failure 11	616946	AD
		UV-sensitive syndrome 1	600630	AR
		Lung cancer, susceptibility to	211980	AD, SMu
		Macular degeneration, age-related, susceptibility to, 5	613761	

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ERCC8	609412	Cockayne syndrome, type A	216400	AR
		UV-sensitive syndrome 2	614621	AR
ESCO2	609353	Roberts syndrome	268300	AR
		SC phocomelia syndrome	269000	AR
ETFA	608053	Glutaric acidemia IIA	231680	AR
ETFB	130410	Glutaric acidemia IIB	231680	AR
ETFDH	231675	Glutaric acidemia IIC	231680	AR
ETHE1	608451	Ethylmalonic encephalopathy	602473	AR
EVC2	607261	Ellis-van Creveld syndrome	225500	AR
		Weyers acrofacial dysostosis	193530	AD
EYS	612424	Retinitis pigmentosa 25	602772	AR
F11	264900	Factor XI deficiency, autosomal dominant	612416	
		Factor XI deficiency, autosomal recessive	612416	
F8	300841	Hemophilia A	306700	XLR
F9	300746	Hemophilia B	306900	XLR
		Thrombophilia, X-linked, due to factor IX defect	300807	
		Deep venous thrombosis, protection against	300807	
		Warfarin sensitivity	122700	AD
FAH	613871	Tyrosinemia, type I	276700	AR
FANCA	607139	Fanconi anemia, complementation group A	227650	AR
FANCC	613899	Fanconi anemia, complementation group C	227645	AR
FANCG	602956	Fanconi anemia, complementation group G	614082	
FH	136850	Fumarase deficiency	606812	AR
		Leiomyomatosis and renal cell cancer	150800	AD
FKRP	606596	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 5	613153	AR
		Muscular dystrophy-dystroglycanopathy (congenital with or without mental retardation), type B, 5	606612	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 5	607155	AR
FKTN	607440	Cardiomyopathy, dilated, 1X	611615	AR
		Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4	253800	AR
		Muscular dystrophy-dystroglycanopathy (congenital without mental retardation), type B, 4	613152	AR

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CHIAMATA GRATUITA NUMEROVERDE 800 501651

PEX26	608666	Peroxisome biogenesis disorder 7A (Zellweger)	614872	AR
		Peroxisome biogenesis disorder 7B	614873	AR
PEX6	601498	Heimler syndrome 2	616617	AR
		Peroxisome biogenesis disorder 4A (Zellweger)	614862	AR
		Peroxisome biogenesis disorder 4B	614863	AD, AR
PEX7	601757	Peroxisome biogenesis disorder 9B	614879	AR
		Rhizomelic chondrodysplasia punctata, type 1	215100	AR
PFKM	610681	Glycogen storage disease VII	232800	AR
PHGDH	606879	Neu-Laxova syndrome 1	256520	AR
		Phosphoglycerate dehydrogenase deficiency	601815	AR
PKHD1	606702	Polycystic kidney disease 4, with or without hepatic disease	263200	AR
PLA2G6	603604	Infantile neuroaxonal dystrophy 1	256600	AR
		Neurodegeneration with brain iron accumulation 2B	610217	AR
		Parkinson disease 14, autosomal recessive	612953	AR
PMM2	601785	Congenital disorder of glycosylation, type Ia	212065	AR
POLG	174763	Mitochondrial DNA depletion syndrome 4A (Alpers type)	203700	AR
		Mitochondrial DNA depletion syndrome 4B (MNGIE type)	613662	AR
		Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE)	607459	AR
		Progressive external ophthalmoplegia, autosomal dominant 1	157640	AD
		Progressive external ophthalmoplegia, autosomal recessive 1	258450	AR
POMGNT1	606822	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 3	253280	AR
		Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 3	613151	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 3	613157	AR
		Retinitis pigmentosa 76	617123	AR
POMT1	607423	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 1	236670	AR

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		Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 1	613155	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 1	609308	AR
POMT2	607439	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 2	613150	AR
		Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 2	613156	AR
		Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 2	613158	AR
PPT1	600722	Ceroid lipofuscinosis, neuronal, 1	256730	AR
PROP1	601538	Pituitary hormone deficiency, combined, 2	262600	AR
PRPS1	311850	Arts syndrome	301835	XLR
		Charcot-Marie-Tooth disease, X-linked recessive, 5	311070	XLR
		Deafness, X-linked 1	304500	XL
		Gout, PRPS-related	300661	XLR
		Phosphoribosylpyrophosphate synthetase superactivity	300661	XLR
PSAP	176801	Combined SAP deficiency	611721	AR
		Gaucher disease, atypical	610539	
		Krabbe disease, atypical	611722	AR
		Metachromatic leukodystrophy due to SAP-b deficiency	249900	AR
PTS	612719	Hyperphenylalaninemia, BH4-deficient, A	261640	AR
PUS1	608109	Myopathy, lactic acidosis, and sideroblastic anemia 1	600462	AR
PYGM	608455	McArdle disease	232600	AR
RAB23	606144	Carpenter syndrome	201000	AR
RAG1	179615	Alpha/beta T-cell lymphopenia with gamma/delta T-cell expansion, severe cytomegalovirus infection, and autoimmunity	609889	
		Combined cellular and humoral immune defects with granulomas	233650	AR
		Omenn syndrome	603554	AR
		Severe combined immunodeficiency, B cell-negative	601457	AR

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RAG2	179616	Combined cellular and humoral immune defects with granulomas	233650	AR
		Omenn syndrome	603554	AR
		Severe combined immunodeficiency, B cell-negative	601457	AR
RAPSN	601592	Fetal akinesia deformation sequence	208150	AR
		Myasthenic syndrome, congenital, 11, associated with acetylcholine receptor deficiency	616326	AR
RARS2	611524	Pontocerebellar hypoplasia, type 6	611523	AR
RDH12	608830	Leber congenital amaurosis 13	612712	AD, AR
RMRP	157660	Anauxetic dysplasia 1	607095	AR
		Cartilage-hair hypoplasia	250250	AR
		Metaphyseal dysplasia without hypotrichosis	250460	AR
RPE65	180069	Leber congenital amaurosis 2	204100	AR
		Retinitis pigmentosa 20	613794	AR
RPGRIP1L	610937	COACH syndrome	216360	AR
		Joubert syndrome 7	611560	AR
		Meckel syndrome 5	611561	AR
RS1	300839	Retinoschisis	312700	XLR
SACS	604490	Spastic ataxia, Charlevoix-Saguenay type	270550	AR
SAMHD1	606754	Chilblain lupus 2	614415	AD
		Aicardi-Goutieres syndrome 5	612952	AR
SBDS	607444	Shwachman-Diamond syndrome	260400	AR
		Aplastic anemia, susceptibility to	609135	
SEPSECS	613009	Pontocerebellar hypoplasia type 2D	613811	AR
SERPINA1	107400	Emphysema due to AAT deficiency	613490	AR
		Emphysema-cirrhosis, due to AAT deficiency	613490	AR
		Hemorrhagic diathesis due to antithrombin Pittsburgh	613490	AR
		Pulmonary disease, chronic obstructive, susceptibility to	606963	
SGCA	600119	Muscular dystrophy, limb-girdle, autosomal recessive 3	608099	AR
SGCB	600900	Muscular dystrophy, limb-girdle, autosomal recessive 4	604286	AR
SGCG	608896	Muscular dystrophy, limb-girdle, autosomal recessive 5	253700	AR
SGSH	605270	Mucopolysaccharidosis type IIIA (Sanfilippo A)	252900	AR

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SLC12A3	600968	Gitelman syndrome	263800	AR
SLC12A6	604878	Agenesis of the corpus callosum with peripheral neuropathy	218000	AR
SLC17A5	604322	Salla disease	604369	AR
		Sialic acid storage disorder, infantile	269920	AR
SLC22A5	603377	Carnitine deficiency, systemic primary	212140	AR
SLC25A13	603859	Citrullinemia, adult-onset type II	603471	AR
		Citrullinemia, type II, neonatal-onset	605814	AR
SLC25A15	603861	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	238970	AR
SLC25A20	613698	Carnitine-acylcarnitine translocase deficiency	212138	AR
SLC26A2	606718	Achondrogenesis Ib	600972	AR
		Atelosteogenesis, type II	256050	AR
		De la Chapelle dysplasia	256050	AR
		Diastrophic dysplasia	222600	AR
		Diastrophic dysplasia, broad bone-platyspondylic variant	222600	AR
		Epiphyseal dysplasia, multiple, 4	226900	AR
SLC26A4	605646	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct	600791	AR
		Pendred syndrome	274600	AR
SLC37A4	602671	Glycogen storage disease Ib	232220	AR
		Glycogen storage disease Ic	232240	AR
SLC39A4	607059	Acrodermatitis enteropathica	201100	AR
SLC4A11	610206	Corneal dystrophy, Fuchs endothelial, 4	613268	
		Corneal endothelial dystrophy and perceptive deafness	217400	AR
		Corneal endothelial dystrophy, autosomal recessive	217700	AR
SLC6A8	300036	Cerebral creatine deficiency syndrome 1	300352	XLR
SMN1	600354	Spinal muscular atrophy-1	253300	AR
		Spinal muscular atrophy-2	253550	AR
		Spinal muscular atrophy-3	253400	AR
		Spinal muscular atrophy-4	271150	AR
SMPD1	607608	Niemann-Pick disease, type A	257200	AR
STAR	600617	Lipoid adrenal hyperplasia	201710	AR

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SUMF1	607939	Multiple sulfatase deficiency	272200	AR
TAT	613018	Tyrosinemia, type II	276600	AR
TCIRG1	604592	Osteopetrosis, autosomal recessive 1	259700	AR
TFR2	604720	Hemochromatosis, type 3	604250	AR
TGM1	190195	Ichthyosis, congenital, autosomal recessive 1	242300	AR
TH	191290	Segawa syndrome, recessive	605407	AR
TMEM216	613277	Joubert syndrome 2	608091	AR
		Meckel syndrome 2	603194	AR
TPP1	607998	Ceroid lipofuscinosis, neuronal, 2	204500	AR
		Spinocerebellar ataxia, autosomal recessive 7	609270	AR
TREX1	606609	Aicardi-Goutieres syndrome 1, dominant and recessive	225750	AD, AR
		Chilblain lupus	610448	AD
		Vasculopathy, retinal, with cerebral leukodystrophy	192315	AD
		Systemic lupus erythematosus, susceptibility to	152700	AD
TRIM37	605073	Mulibrey nanism	253250	AR
TSEN2	608753	Pontocerebellar hypoplasia type 2B	612389	AR
TSEN34	608754	Pontocerebellar hypoplasia type 2C	612390	
TSEN54	608755	Pontocerebellar hypoplasia type 5	610204	AR
		Pontocerebellar hypoplasia type 2A	277470	AR
		Pontocerebellar hypoplasia type 4	225753	AR
TSFM	604723	Combined oxidative phosphorylation deficiency 3	610505	AR
TSHB	188540	Hypothyroidism, congenital, nongoitrous 4	275100	AR
TTC37	614589	Trichohepatoenteric syndrome 1	222470	AR
TTPA	600415	Ataxia with isolated vitamin E deficiency	277460	AR
TYMP	131222	Mitochondrial DNA depletion syndrome 1 (MNGIE type)	603041	AR
TYR	606933	Albinism, oculocutaneous, type IA	203100	AR
		Albinism, oculocutaneous, type IB	606952	
		Waardenburg syndrome/albinism, digenic	103470	AD
		Skin/hair/eye pigmentation 3, blue/green eyes	601800	
		Skin/hair/eye pigmentation 3, light/dark/freckling skin	601800	
		Melanoma, cutaneous malignant, susceptibility to, 8	601800	

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UGT1A1	191740	Crigler-Najjar syndrome, type I	218800	AR
		Crigler-Najjar syndrome, type II	606785	AR
		Hyperbilirubinemia, familial transient neonatal	237900	AR
		Bilirubin, serum level of, QTL1	601816	
		Gilbert syndrome	143500	AR
USH1C	605242	Deafness, autosomal recessive 18A	602092	AR
		Usher syndrome, type 1C	276904	AR
USH2A	608400	Retinitis pigmentosa 39	613809	
		Usher syndrome, type 2A	276901	AR
VPS13A	605978	Choreoacanthocytosis	200150	AR
VPS13B	607817	Cohen syndrome	216550	AR
VRK1	602168	Pontocerebellar hypoplasia type 1A	607596	AR
WAS	300392	Neutropenia, severe congenital, X-linked	300299	XLR
		Thrombocytopenia, X-linked	313900	XLR
		Thrombocytopenia, X-linked, intermittent	313900	XLR
		Wiskott-Aldrich syndrome	301000	XLR
WNT10A	606268	Odontoonychodermal dysplasia	257980	AR
		Schopf-Schulz-Passarge syndrome	224750	AR
		Tooth agenesis, selective, 4	150400	AD, AR
XPA	611153	Xeroderma pigmentosum, group A	278700	AR
XPC	613208	Xeroderma pigmentosum, group C	278720	AR
ZFYVE26	612012	Spastic paraplegia 15, autosomal recessive	270700	AR

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Conventional Karyotype, and **array-CGH**
Syndromic definition, genetic definition of unsolved diseases, one or more congenital anomalies

DUEinUNO is a magnifying glass on the karyotype: chromosomal analysis under the microscope and an oligo array together for new cytogenetics.

1) Easychip Agilent (Custom ChIP-on-chip/DNA Methylation, 8x15k)
Next generation genomic microarray for molecular karyotyping.

Increase the sensitivity of conventional karyotyping.
It analyzes the copy number imbalance of genomic sequences at a resolution much higher than that possible with traditional metaphase cytogenetic techniques.
The investigation therefore provides rapid and accurate information relating to a series of rearrangements not identifiable with traditional chromosomal investigations as syndromic regions associated with known microdeletion and microduplication pathologies including those involving the subtelomeric regions (i.e. the ends of chromosomes, often the site of anomalies related to mental retardation).

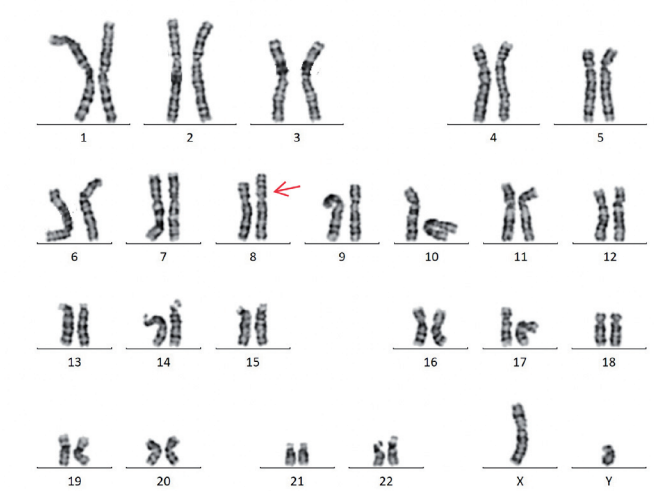
PGT3™ is designed to minimize the occurrence of CNVs, especially small ones, which are present in the general population as benign variants, i.e. without clinical significance, or of unknown significance. In the absence of abnormal ultrasound signs or other conditions of increased genetic risk of the fetus, this fact may reduce the prognostic value of the test.

Easychip Agilent (Custom ChIP-on-chip/DNA Methylation, 8x15k) (15k) is a platform designed to integrate prenatal chromosomal analysis: 1) identifies CNVs along the genome with a resolution of 3-4 Mb (4-5 times greater than chromosomal analysis) reducing CNVs without clinical significance or of unknown significance from 26% to 3%. 2) Analyze the subtelomeric regions at a higher resolution (300-500 kb) to search for any cryptic imbalances 3) Analyze at the maximum resolution of 200-250 kb the microduplications or deletions in the syndromic regions associated with 43 syndromes known from microdup/del .

2) Kit Genetisure Cyto 8x60 K Agilent
Whole genome, Mean Resolution 150 kb. Referring genome: Male DNA (Promega)

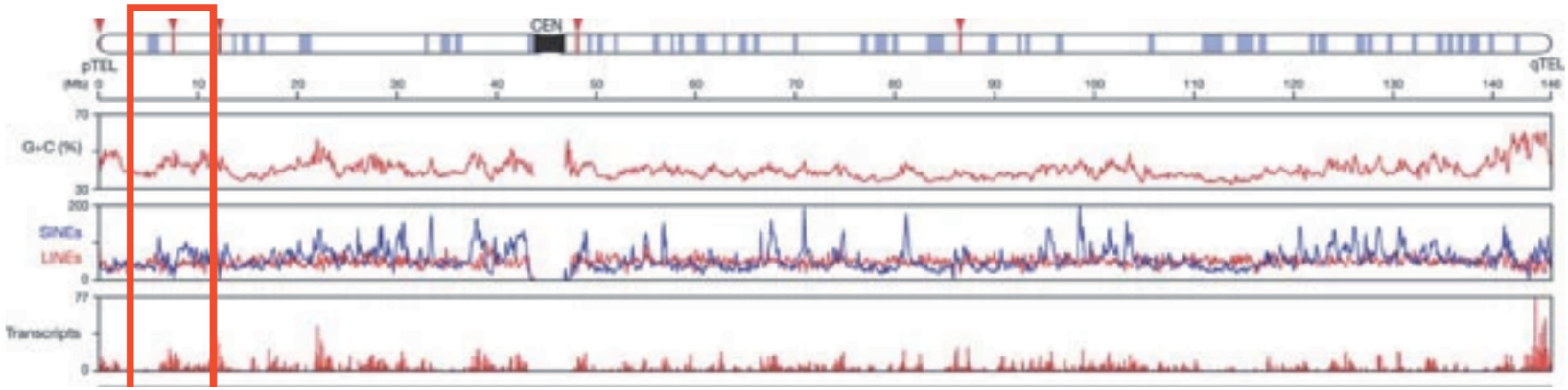
CASE REPORT

Male patient seeking eligibility in IVF trial with oligospermia, congenital heart defect (aorta stenosis, ventricular defects), congenital brain defects (hippocampus and amygdala), sporadic epileptic episodes, mild dyslexia.
Conventional karyotype: 46,XY, dup(8p).



CytoSure Aneuploidy Array (cat. no. 020024)

Component	Contents
Slides	8x15k format oligonucleotide microarray slides (eight arrays with ~15,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file



Array-CGH 15k: arr[GRCh37] 8p23.2p23.1 (2433724_11860230)x3.
Platform: Agilent Custom HD-CGH. Microarray 8x15K.
Choromosomal regions: all genome.
Mean Resolution: 3 Mb nel Backbone; 300 kb in subtelomeric regions; 150 kb in 43 genomic regions linked to 52 microdelezion/microduplication syndromes
Referring DNA: male DNA (Promega).
Bioinformatic Software: Agilent Cytogenomics 4.0.3.12.
Referring Database: Genome build GRCh37(hg19).
The analysis highlighted a duplication of 9.426 Mb at the level of chromosome 8, between 2.433 Mb (8p23.2) and 11.860 Mb (8p23.1), in a male genomic profile.
The duplication on chromosome 8 includes 163 genes including 38 OMIM genes, 8 OMIM genes Morbid ANGPT2 (*601922), BLK (*191305), CTSB (*116810), FDFT1 (*184420), GATA4 (*600576), MCPH1 (*607117), MFHAS1 (*605352), RP1L1 (*608581), and 117 RefSeq genes.
The chromosomal analysis conducted with a-CGH technique demonstrated a duplication of chromosome 8 in the 8p23.1 region of approximately 9MB (see report) with the consequence that the genes included in that stretch are present in three copies instead of two resulting in overexpression of the product or function of the genes.
The “8p23.1 duplication syndrome” is configured which has variable clinical effects depending on the size of the duplication and which includes the cardiac and neurological symptoms of the patient, particularly attributable to overexpression of the GATA4 gene, regulator of cardiac morphogenesis, and CTSB described in neurocerebral association.
This duplication is transmissible to offspring, the probability that the duplicated chromosome 8 is involved in fertilization is 50%. In the PMA process, we recommend carrying out the Pre-implantation Chromosomal Diagnosis (PGT-SR Preimplantation Genetic Testing for Structural chromosomal Rearrangement).
Polymorphic or frequently observed copy number variations (CNVs) were not considered in the interpretation of the results in the general population, although they do not reach the frequency of 1% (http://dgv.tcag.ca/dgv/app/home), the CNVs of the loci described as devoid of genes, CNVs of uncertain significance in the subtelomeric regions (<1Mb) not associated with syndromic conditions and CNVs within the syndromes that are not clearly causative of pathology.
However, it is possible that some of these CNVs may subsequently be found to be pathogenic in the literature. In accordance with the indications of the Italian Society of Human Genetics, some recurrent CNVs for which the role pathogenetic is currently still controversial or with a penetrance of less than 15% (Rosenfeld et al., 2013; Coe et al., 2014; Maya et al., 2018).
The following CNVs are part of this group: del/dup 15q11.2 (NIPA1); del/dup 16p13.11 (MYH11); of the PAR1 region (not including the SHOX gene); dup PAR1 region (although including the SHOX gene); dup Xp22.31 (STS and adjacent region); del/dup region PAR2.

Whole Exome Sequencing (WES) with Next Generation Sequencing Technique, if Karyotype and array-CGH negative
Syndromic definition, genetic definition of unsolved diseases, one or more congenital anomalies

With this approach, based on the enrichment of genomic fragments that refer to gene sequences coding for proteins and for selected subclasses of RNA that have a regulatory function (e.g. microRNA), it is possible to limit the analysis to 1-2% of the genome, thus excluding non-coding regions and, consequently, losing information that can impact gene expression.
Current knowledge on the genetic causes of Mendelian diseases suggests that most of their mutations consist of changes in the coding sequence of a gene or an abnormality in transcript processing. Therefore, the exome is an enriched portion of the genome, in which it is useful to search for mutations with potential clinical impact.
It follows that its sequencing is fundamental in the diagnosis of rare diseases and in understanding the molecular basis of many Mendelian pathologies, as documented by the diagnoses obtained in recent years on large cohorts of undiagnosed patients and the hundreds of disease genes identified with this technique.

CASE REPORT
Male boy, age 8, with Spastic Paraparesis/Paraplegia. Negative for array-CGH and selected gene panels.

The analysis of the exome (WES) highlighted the presence of some variants in genes sensitive to the anamnestic characteristics of the proband; the report contains a detailed description of the variants.
The variants are defined in different ways: in heterozygosity (present in only one chromosome of the two, healthy carrier if recessive, affected if dominant), in homozygosity or compound heterozygosity (present in both chromosomes, affected), pathogenic (predispose to the disease) , of doubtful significance or potentially benign (if at the moment there are no data to define them as pathogenic).
It should be noted that the definition “of doubtful significance” or “probably benign” derives from in silico evaluations and/or sporadic data open to updates. It is also highlighted that the indications “dominant AD” or “recessive RA” in heterozygous cases derive from described family cases, are not necessarily the rule, as is the heterogeneity or variability of the expression.

The TMEM107 gene presents two variants in the proband (pathogenic paternal/ maternal of doubtful significance) in compound heterozygosity.
The report illustrates a complex series of pathologies linked (in homozygosity or compound heterozygosity) to gene variants and overlapping sequences: OMIM (MECKEL SYNDROME 13; MKS13; OROFACIODIGITAL SYNDROME XVI; OFD16; OMIM: #617562; #617563). OMIM (LEUKOENCEPHALOPATHY , BRAIN CALCIFICATIONS,

AND CYSTS; LCC; OMIM: #614561).
The KIF1A gene (previously described as maternally inherited) presents two variants in the proband (paternal of doubtful significance/maternal of doubtful significance) in compound heterozygosity.
The report illustrates a complex series of pathologies linked (in homozygosity or compound heterozygosity) to variants of the gene: OMIM (NESCAV SYNDROME; NESCAVS; NEUROPATHY, HEREDITARY SENSORY, TYPE IIC; HSN2C; SPASTIC PARAPLEGIA 30, AUTOSOMAL DOMINANT, SPG30 and SPASTIC PARAPLEGIA 30 , AUTOSOMAL RECESSIVE, INCLUDED; OMIM: #614255; #614213; #610357).
The FBXO38 gene presents a heterozygous variant (maternal of doubtful significance) in the proband.
The report illustrates a complex series of pathologies linked (heterozygous, AD) to variants of the gene: OMIM (NEURONOPATHY, DISTAL HEREDITARY MOTOR, AUTOSOMAL DOMINANT 6; HMND6; OMIM: #615575).

The CPT2 gene presents a heterozygous (maternal pathogenetic) variant in the proband.
The gene variants are described AD/AR also heterosymptomatic.
The report illustrates a complex series of pathologies linked to gene variants (see report).
It is advisable to submit the genetic report to a neurologist specialist for appropriate checks with respect to the proband's medical history.
From the hereditary point of view relating to the parents, in the case of pregnancy the risk of fetal recurrence of the variants in compound heterozygosity (TMEM107 and KIF1A) is 25%, while the risk of recurrence of the variants in heterozygosity (FBXO38 and CPT2) is 50 %.

* 601255

KINESIN FAMILY MEMBER 1A; KIF1A

Alternative titles; symbols

AXONAL TRANSPORTER OF SYNAPTIC VESICLES; ATSV
UNC104, C. ELEGANS, HOMOLOG OF; UNC104
KINESIN, HEAVY CHAIN, MEMBER 1A, MOUSE, HOMOLOG OF

HGNC Approved Gene Symbol: *KIF1A*

Cytogenetic location: *2q37.3* ***Genomic coordinates (GRCh38):*** *2:240,713,767-240,821,403* (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype View Clinical Synopses	Phenotype MIM number	Inheritance	Phenotype mapping key
2q37.3	NESCAV syndrome	614255	AD	3
	Neuropathy, hereditary sensory, type IIC	614213	AR	3
	Spastic paraplegia 30, autosomal dominant	610357	AD, AR	3
	Spastic paraplegia 30, autosomal recessive	610357	AD, AR	3

PRENATAL DIAGNOSIS TECHNIQUES

Prenatal diagnosis techniques include instrumental and laboratory investigations, developed over the last 50 years, with the aim of monitoring the concept starting from the early stages of embryonic development up to the moments preceding birth. Prenatal ultrasound, i.e. pregnancy monitoring using ultrasound, is the non-technique most important and widespread invasive prenatal diagnosis. It is used to monitor the development of the embryo and fetus, check their well-being, follow the evolution of the pregnancy and as a support for invasive investigations involving the acquisition of fetal tissues. The not invasiveness and harmlessness of the technique, which allows it to be repeated during pregnancy, together with the high degree of resolution obtained with the latest generation equipment, justify the extraordinary diffusion of prenatal ultrasound which occurs in industrialized countries used in almost all pregnancies, offering itself as a real tool prenatal screening. The potential of the technique correlates directly with the gestational age in which it is used, the resolution of the equipment and the experience of the operator.

Amniocentesis is the most widely used invasive prenatal diagnosis technique aimed at acquisition, via transabdominal puncture, below ultrasound check of the amniotic fluid, ideally around the 15th-16th week amenorrhea. The risk of miscarriage, linked to the invasiveness of the technique, is calculated at approximately 1:200, but varies widely depending on the experience of the operator.
Amniotic fluid contains a non-corpuscular part, i.e. cell-free, which is isolated by centrifugation of the sample, and a corpuscular part, formed by amniocytes, i.e. the cells that derive from the skin, from mucous membranes, the genitourinary tract, the gastrointestinal tract of the fetus and the membranes amniotic.
On the non-corpuscular portion it is possible to measure alphafetoprotein (AFP) and, possibly, other biochemical markers, while amniocytes are used, primarily, for cytogenetic investigations, and possibly for molecular and biochemical analyses, both directly than on cultured cells.

Chorionic villus sampling is an invasive technique used for trophoblast sampling by transabdominal puncture, ideally under ultrasound control around the X-XII week of amenorrhea. The risk of abortion, linked to the invasiveness of the technique, it is approximately 2-3%, but varies significantly depending on the experience of the operator. The sample acquired can be used for cytogenetic analysis, directly on the cells of the cytotrophoblast or on cultures (villus mesenchymal cells).
The combined use of the two techniques provides information on cell populations that have a different embryonic origin, allowing, in most cases, to resolve the potential problem of discrepancies between the placental karyotype and the fetal karyotype (found in approximately 2% of samples), which is attributable to a condition of postzygotic mosaicism.

Chorionic villus sampling allows to acquire biological material in relatively abundant quantities and is therefore the technique of choice for molecular diagnosis of disease genes and for biochemical analyses. The advantage of the earliness of technique, compared to amniocentesis, is counterbalanced by its greater invasiveness and by acquisition of placental and non-fetal tissue.

Cordocentesis is the technique of acquiring fetal blood, by transabdominal puncture, around the 18th week of amenorrhea. The risk of abortion, linked to the invasiveness of the technique, it is approximately 2%, but varies significantly based on the experience of the operator. The technique is heavily out of use, being used mainly for monitor some infectious pathologies and possibly to resolve non-cytogenetic results informative with the analysis of amniocytes.

Non-invasive prenatal screening, developed over the last 30 years, is essentially based on the analysis of biochemical markers on maternal blood, combined with ultrasound investigations. The prototype of these analyzes was the alpha fetoprotein (AFP) assay, initially used as a marker of neural tube defects (increased value) and, subsequently, of Down syndrome (SD; reduced value). Over time these screenings, based on the association of different markers, have achieved increasing development in the calculation of the probability of fetal aneuploidies, especially in mothers who fell into the low probability age group chromosomal pathologies in the fetus, and therefore not candidates for invasive monitoring of pregnancy.
The triple-test (or tri-test) based on the dosage, in the second trimester, of AFP, of chorionic gonadotropin and unconjugated estriol, combined with maternal age and gestational rate measured ultrasoundwise, allowed to predict approximately 65% of SDs, with one false positive rate between 5 and 10%.
This protocol has been accompanied by others over time numerous others, based on various markers, in different combinations, and on anticipation of screening from the second to the first trimester. In parallel, biochemical markers were integrated with ultrasound tests, in particular the analysis of the thickness of the nuchal skin (translucency nuchal - TN), which, although not pathognomonic of SD, between the 11th and 14th week of amenorrhea, diagnoses approximately 75% of cases, with a false positive rate of 5%.
In recent years it has affirmed the bi-test, which uses maternal blood acquired around the 11th week, on which the free fraction of beta chorionic gonadotropin and an elevated glycoprotein are measured molecular weight, Pregnancy Associated Plasma Protein A (PAPP-A).
This analysis, integrated with the measurement of TN and maternal age predicts approximately 80% of SDs, with a false percentage positive equal to approximately 6% (see also Annex 1).
The test must also be considered in this context contingent (TN + biochemical markers at 11-13 weeks; ultrasound markers at 12-13 weeks or biochemical tests at 14-16 weeks in the intermediate probability groups) to increase the specificity.

PRIVATE ACCESS TO PRENATAL DIAGNOSIS

7694 CHORIONIC VILLI SAMPLES (first trimester CVS) for chromosome analysis.

The accuracy of prenatal Combined Test for trisomy detection in a private medical care experience.

Sir,
the combined test for the prenatal evaluation of trisomy risk is an indirect and statistic item based on maternal age, nuchal translucency and some biochemical placental values. The method is validated and used in wide population studies with the aim to obtain a more predictable instrument vs. maternal age only. The individual use of the combined test to predict the trisomy risk in a single pregnancy does not have the same accuracy. The present report shows 7694 diagnostic CVS cases, consecutive and from single medical center. The population asking for CVS procedure is divided in two groups:
1. Personal choice without increased risk from combined test (NT value where detected during CVS procedure)
2. Increased trisomy risk from combined test.
In each group the cases are grouped for NT value, age.
Tables 1 and 2 present the cases of the two groups for NT values, all trisomies, trisomies for age over 35, trisomy 21 and related frequencies.
The conclusion is that both groups overlap for all the comparison parameters. The maternal age and NT value play a rule in trisomy detection, while the biochemical (bitest) data do not influence the real capture of trisomies.

Table1. NO COMBINED SCREENING TEST.
CASES SELECTED BY NT MEASUREMENTS BEFORE CVS PROCEDURE
NESSUN TEST. DATI SEPARATI PER MISURE NT ESEGUITE PRIMA DEL CVS

NT mm	CASES	ALL TRISOMIES	ALL TRIS > AGE 35	TRIS 21	TRIS/CASES	TRIS>35/ALL TRIS
0-1,9	3749	9	7	5	**0,002**	0,8
2-3,9	992	29	25	21	**0,03**	0,9
4-5,9	53	15	11	11	**0,28**	0,7
6-9,9	16	8	6	4	**0,5**	0,7

Table2. NT, free β-hCG, PAPP-A, 10-12 weeks gestation. COMBINED TEST.
CASES RESULTED AT RISK FOR TRISOMY. CASES SELECTED BY NT MEASUREMENTS
CASI RISULTATI A RISCHIO PER TRISOMIE. DATI SEPARATI PER MISURE NT

NT mm	CASES	ALL TRISOMIES	ALL TRIS > AGE 35	TRIS 21	TRIS/CASES	TRIS>35/ALL TRIS
0-1,9	2248	6	5	3	**0,002**	0,8
2-3,9	595	17	14	9	**0,03**	0,8
4-5,9	31	10	7	5	**0,32**	0,7
6-9,9	10	6	4	2	**0,6**	0,7

Free DNA in maternal blood was first described by Lo (1997) shawing the presence of the Y chromosome in the plasma of some women with fetuses male, using the analysis of free DNA present in the maternal circulation (cfDNA).
It has been shown that, starting from the first trimester of pregnancy, it is present in the blood circulation maternal free DNA of fetal origin (cell free fetal DNA, cffDNA), which can be recovered in non-invasive manner and used for the study of some fetal pathologies. cfDNA originates from the lysis of maternal and placental cells.
Starting from the fifth week of amenorrhea, the placental cytotrophoblast anchors itself to the uterine parietal decidua, the spiral arteries deciduals supply the gaps between the decidua and the placenta, the cytotrophoblast invades and it covers the walls of the spiral uterine arteries and reshapes them.
Cell turnover trophoblast, which covers the walls of the spiral arteries, mediated by cytokines, releases the DNA. The fragments of degraded fetal DNA contain approximately 180 base pairs (bp) and are suspended in the arterial plasma. The cffDNA can be isolated early starting from the 10th week, when it reaches quantities su
fficient for potential clinical use. Its percentage can vary between <4%, a quantity not useful for diagnosis, and around 40%, with an average of 10%, at the 12th week, when 90% approximately fragments of free DNA circulating in the plasma originate from apoptosis of the epithelia maternal, creating a mix of maternal cfDNA and cffDNA.
The percentage of cffDNA comes defined as “fetal fraction” (FF). The cffDNA is no longer found in the maternal circulation a few hours after childbirth and is probably eliminated through renal excretion.

General information on NIPT and fetal fraction.
The principle of “cff DNA Non Invasive Prenatal Test (NIPT)” protocols, regardless of the technique used, is based on comparisons. Taking chromosome 21 (CR21) as an example, the technique compares the number of fragments belonging to CR21 in the pregnancy under examination, with the number of fragments of another chromosome of the same sample (internal comparison), expected in a disomy condition (two copies of a certain chromosome, for example chromosome 1 or 10 or a combination thereof), or with those from a pool of disomic pregnancies (two CR21) of reference.
If the sample obtained from the pregnancy under examination contains two pairs of CR21 (two of the mother and two of the fetus), the ratio between the counts (number of CR21 fragments in the test/number of fragments in disomic reference samples) is approximately equal to 1.
If a fetus with trisomy 21 (T21) is present in the pregnancy under consideration, the FF increases for presence of additional circulating fragments released by the fetus’s supernumerary CR21.
The entity of the increase depends on the percentage of total FF and the number of bp of CR21, in relation to the bp of the overall genome of the fetus. Maternal plasma contains variable percentages of FF, which differ in different samples.
Around the 12th week, on average, the FF corresponds to approximately 10% of the cfDNA, with a range between <4% and 40%. Depending on the percentage of total FF

present in the sample, the accuracy of the chromosome analysis may vary, similarly to the increase in percentage of the total FF, in the presence of a trisomy.
Taking as a reference a percentage of 10% of the circulating FF, the increase in the FF in presence of a T21 is equal to approximately 5% of the total and the ratio (R) between the number of fragments of the CR21 in the sample under examination and the number of disomic reference fragments increases from 1 to approximately 1.05.
For a percentage of the FF equal to 20%, the increase in the total FF correlated to the presence of a T21 in the fetus is approximately 10%, with the consequent increase in the R value from 1 to approximately 1.10. In the presence of a FF of 4%, the increase in FF related to a fetal T21 is approximately 2% and the R value increases from 1 to approximately 1.02.
Finally, if the FF is less than the threshold value of 4%, R is <1.02, a value that cannot be statistically differentiated from 1, which predicts the disomy of CR21, i.e. the normality of the fetus.
This explains why the threshold ≥4% is critical to avoid having false results negative (FNR), based on the absence/insufficient quantity of FF.
It is therefore appropriate to verify the percentage of FF in the sample under examination, using protocols which involve, before or during the NIPT, another test which is usually based on the analysis of sites single nucleotide polymorphisms (so-called SNPs – Single Nucleotide Polymorphisms).
Some tests NIPT insert the percentage of FF into the algorithm for formulating the probability of presence of the trisomy investigated, while others use predetermined normalization factors, which can still achieve high levels of reliability (Dan et al, 2012; Zhang et al, 2015).

CffDNA Analysis Techniques.
The techniques in use analyze total cfDNA, without differentiating fetal from maternal. Since these are, in fact, investigations based on a mixture of maternal and placental DNA, the NIPT it is not a diagnostic test, but a screening one.
In fact, as in traditional tests, the use of dedicated algorithms allow to define the post-test probability that the fetus is affected by one of the major autosomal trisomies (trisomy 21 [T21], trisomy 18 [T18], trisomy 13 [T13]) or by an aneuploidy of the sex chromosomes (X, XXX, XXY, XYY), or structural chromosome anomalies (deletions – duplications) >7-10 Mb, selectively analyzing the number of the cffDNA fragments contributed by each of the chromosomes being tested.
Three main techniques based on NIPT are used for the analysis of aneuploidies second generation sequencing techniques (Next Generation Sequencing - NGS): NGS of the entire genome; NGS of specific regions; SNPs, i.e. polymorphisms of single nucleotides.
The whole genome NGS technique is based on the sequencing of the cffDNA present in the maternal plasma, to generate millions of short sequences of the entire genome, which are then mapped to a reference sequence of the human genome, to establish their

origin and count the number of fragments originating from the chromosome of interest, compared with the number of fragments obtained from other chromosomes (Fan et al, 2008). So for example, if a fetus has T21, they will be in the maternal plasma present more CR21 fragments than expected in controls without T21.
An alternative NGS technique selectively amplifies specific genomic loci on the chromosome of interest, which are subsequently sequenced. This technique is less expensive, as it reduces the regions to be sequenced, but has the limitation of studying only some regions of interest preselected.
PRENATAL DIAGNOSIS

Invasive sampling techniques, chorionic villi sampling, amniocenthesis

Chromosomal aberrations, aneuplody, deletions and duplications.

DUEinUNO is a magnifying glass on the karyotype: chromosomal analysis (10 Mb resolution) under the microscope and an oligo array (0.5 - 3 Mb resolution, together for new cytogenetics.

PGT3. Easychip Agilent (Custom ChIP-on-chip/DNA Methylation, 8x15k)
Next generation genomic microarray for molecular karyotyping.

Increase the sensitivity of conventional karyotyping.
It analyzes the copy number imbalance of genomic sequences at a resolution much higher than that possible with traditional metaphase cytogenetic techniques.
The investigation therefore provides rapid and accurate information relating to a series of rearrangements not identifiable with traditional chromosomal investigations as syndromic regions associated with known microdeletion and microduplication pathologies including those involving the subtelomeric regions (i.e. the ends of chromosomes, often the site of anomalies related to mental retardation).

PGT3™ is designed to minimize the occurrence of CNVs, especially small ones, which are present in the general population as benign variants, i.e. without clinical significance, or of unknown significance. In the absence of abnormal ultrasound signs or other conditions of increased genetic risk of the fetus, this fact may reduce the prognostic value of the test.

Easychip Agilent (Custom ChIP-on-chip/DNA Methylation, 8x15k) (15k) is a platform designed to integrate prenatal chromosomal analysis: 1) identifies CNVs along the genome with a resolution of 3-4 Mb (4-5 times greater than chromosomal analysis) reducing CNVs without clinical significance or of unknown significance from 26% to 3%. 2) Analyze the subtelomeric regions at a higher resolution (300-500 kb) to search for any cryptic imbalances 3) Analyze at the maximum resolution of 200-250 kb the microduplications or deletions in the syndromic regions associated with 43 syndromes

known from microdup/del.

INFORMED CONSENT
CYTOGENETIC ANALYSIS OF CHORIONIC VILLI (VILLOCENTHESIS)
CYTOGENETIC ANALYSIS OF AMNIOTIC FLUID (AMNIOCENTHESIS)
PLURIGENTEST3 (CONVENTIONAL KARYOTYPE + aCGH-EASYCHIP™)

1) The cytogenetic analysis of the chorionic villi of the placenta or the amniotic fluid cells have the aim of ascertaining the presence of anomalies in the number and shape of the chromosomes in the fetal chromosome set (or karyotype).
2) There are congenital defects which, not being associated with chromosomal anomalies, cannot be diagnosed with prenatal cytogenetic analysis. Chorionic villi can also be used to study genetic diseases with molecular analyzes of DNA,
3) In rare cases, especially due to the inadequacy of the material taken, it may be necessary to repeat the sampling.
4) Conventional cytogenetic diagnosis (optical analysis under the microscope) is based on cultured amniotic fluid cells or on direct examination (result 7 days) and on culture examination of the villi (result approximately 21 days). The two data must be integrated. It is able to identify chromosomal anomalies larger than 10-15 Megabases of DNA.
5) The molecular cytogenetic diagnosis (with CGH oligo15 Easychip™ array) is based on the molecular analysis of the preparation and is able to identify chromosomal anomalies up to 3 Megabases of DNA in size and 45 microdeletion or microduplication syndromes (result in 10 days). It combines with conventional cytogenetic diagnosis. This technique is associated with the rapid molecular investigation of the five most common chromosomal anomalies (trisomies 21, 18, 13, X/Y) with the QFPCR technique.
6) The prenatal cytogenetic diagnosis may not correspond to the real fetal condition in rare cases of: feto-placental discordance, presence of a second reabsorbed fetus, low percentage mosaicisms, massive maternal contamination.
7) To carry out the test it is necessary to take a blood sample from the patient and her partner in EDTA (complete blood count tube).

INFORMED CONSENT TO PERFORM GENETIC TESTS

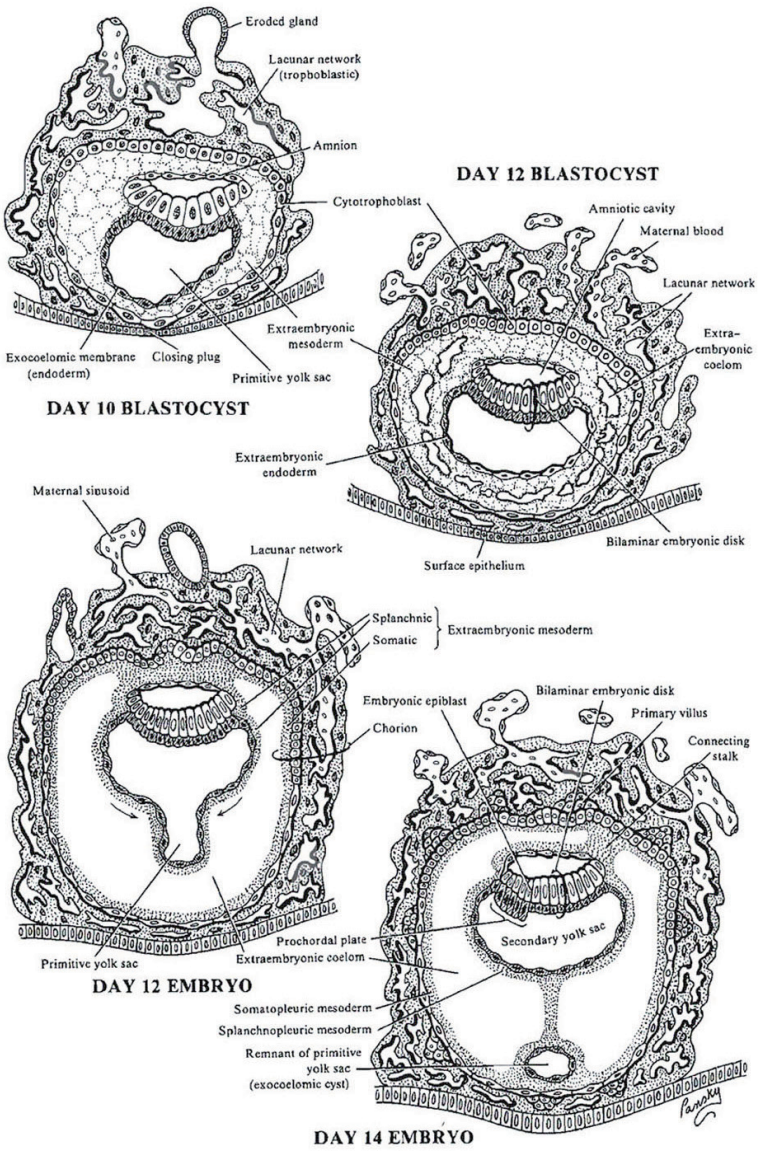
I declare that I have been informed:
1) the purpose of the sampling/consultancy; 2) the limits of the methods used; 3) the characteristics of the possible results: (a) real possibility of identifying the alteration; (b) false positives/false negatives; (c) need for interpretation of any alterations identified during genetic counseling; 4) that the aCGH-EASYCHIP™ tests are performed by the MEDICAL GENETICS LABORATORY, 5) that I have read the specific information relating to the test(s) performed which are attached to this consent . 6) the methods of application of the privacy law for the confidentiality of the data collected; 6) the right to interrupt investigation procedures at any time; 8) that, as a consequence of the results obtained and for completion of the analysis, further tests may be necessary on other

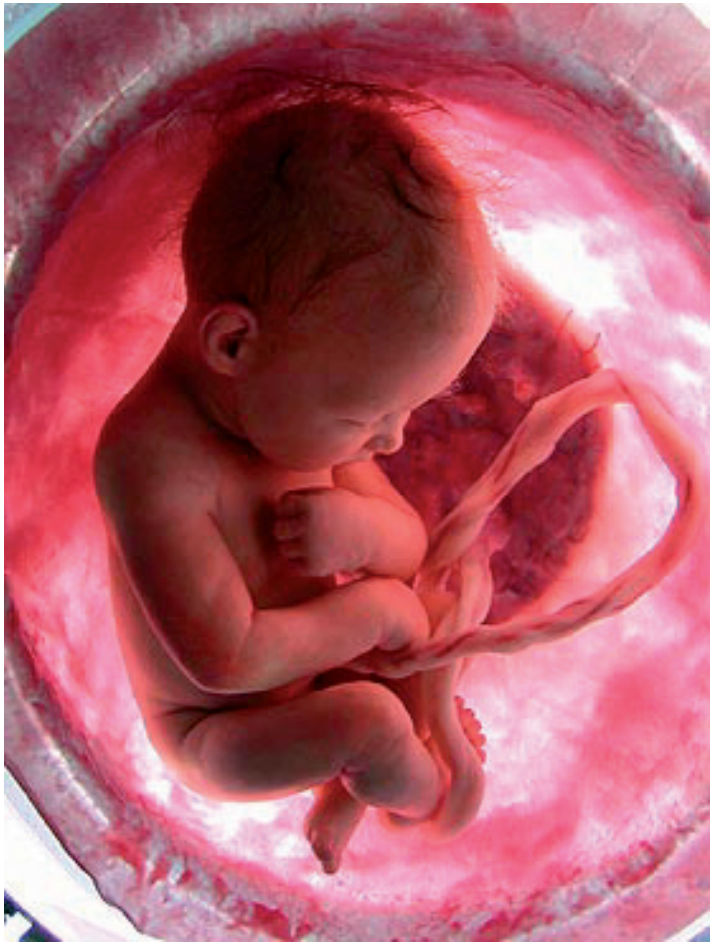
family members; 9) that if the results obtained produce data relevant to the health of the user or family members, the user can choose whether or not to know the results of the research; 10) that, unless otherwise specified, it is not possible to provide an absolute guarantee on the times and certainty of reaching results that allow a definitive diagnosis to be obtained.

Agreement Based on the information obtained for the analysis
 PLURIGENTEST3 (CONVENTIONAL KARYOTYPE+aCGH-EASYCHIP™)
 CONVENTIONAL KARYOTYPE

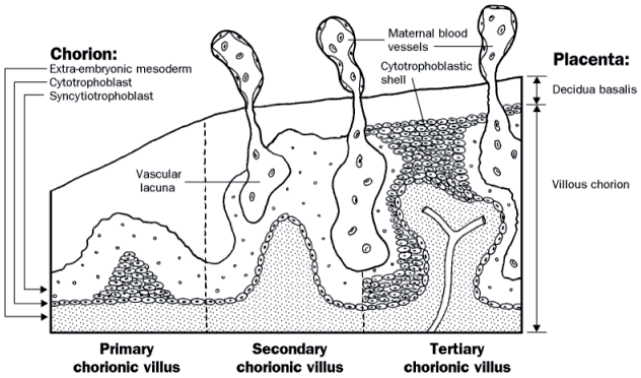
INFORMATION FOR PRENATAL CYTOGENETIC DIAGNOSIS

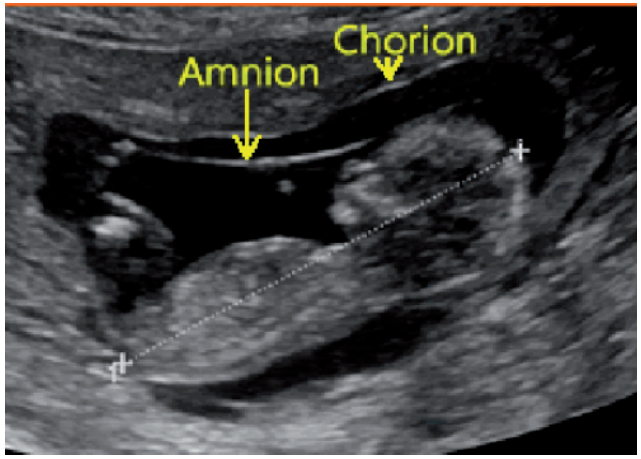
Conventional Karyotype Analysis on Amniotic Fluid, Chorionic Villus.
The prenatal cytogenetic investigation aims to ascertain the presence of numerical and/or structural chromosomal anomalies. The quality of the chromosomal preparations obtained does not allow the identification of loss or acquisition of genetic material (due to deletions, duplications, insertions, translocations, etc.) smaller than 10-15 Megabases
The diagnosis of duplications and/or deletions of small dimensions (submicroscopic) is obtained with the Array-CGH technique, especially useful in high-risk pregnancies (ultrasound indications, chromosomopathies, maternal age, etc.).
There are congenital defects which, not being associated with chromosomal anomalies, cannot be diagnosed by prenatal cytogenetic analysis. In rare cases, the clinical consequences associated with a chromosomal anomaly cannot be established with certainty ; the relevant clarifications will be provided during the consultation.
Sample treatment:
Chorionic villi:
after evaluation of the sample taken, it is divided into two aliquots in order to obtain a direct preparation and a culture preparation. There is a minimum quantity of chorionic villi necessary for the preparation of the two preparations.
Amniotic fluid: The cellular component of amniotic fluid is collected and divided into multiple independent cultures. The minimum quantity of sample for preparing cultures is 10 ml, the optimal one is 16/18 ml. The success of cell cultures is related to the number of viable cells present in the sample.
Diagnosis:
1-The criteria used for the cytogenetic investigation are those recommended by the Guidelines of the Italian Society of Human Genetics and the European Study Group on Prenatal Diagnosis.
Chorionic Villi
The analysis of both the direct preparation and culture optimizes the reliability of the diagnosis. The use of only one of the two analyzes leads to a reliability of 99%, a figure obtained from published international experience. Cases of difference in results between the two preparations cannot be excluded. In this circumstance it may be necessary

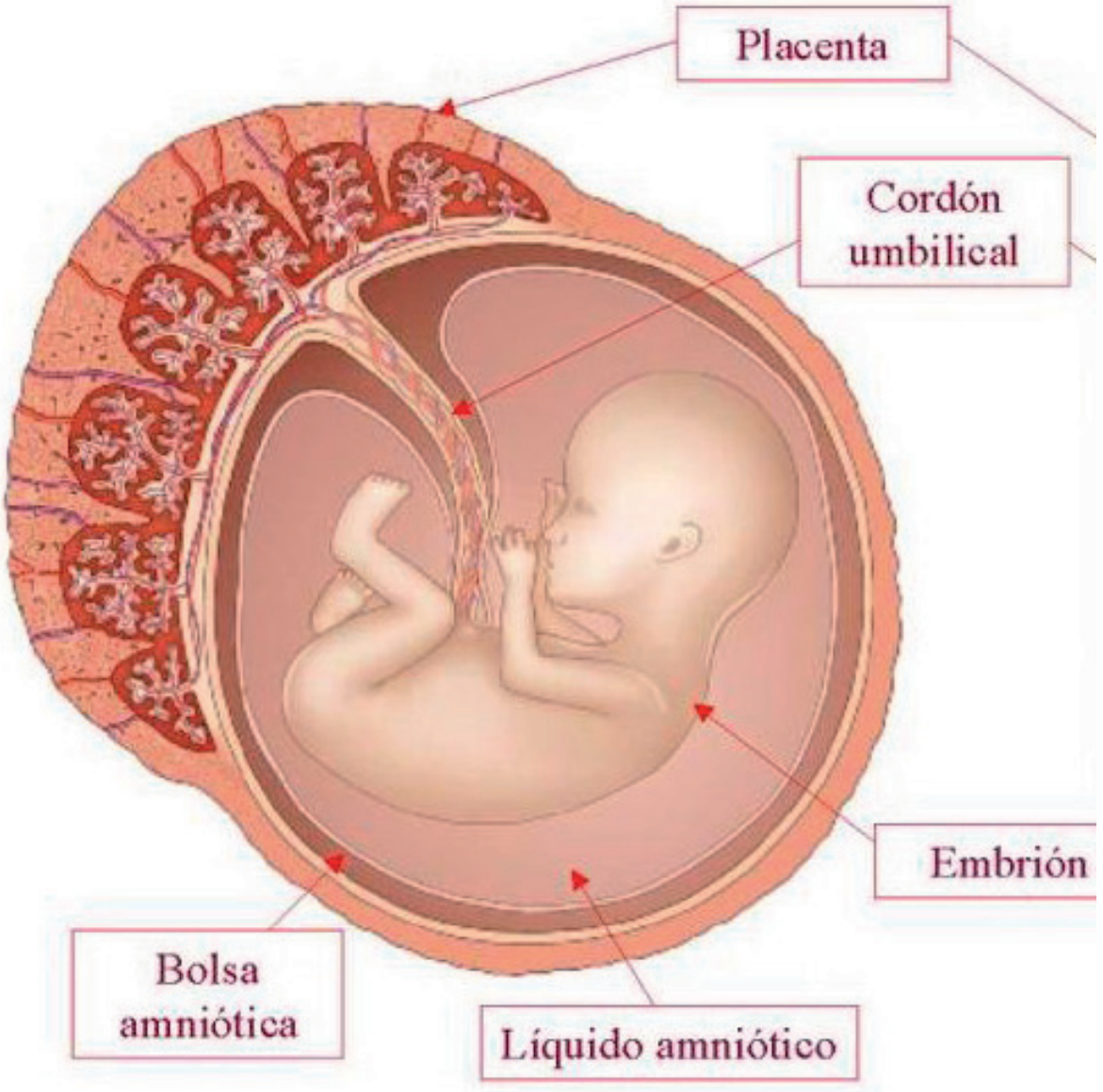


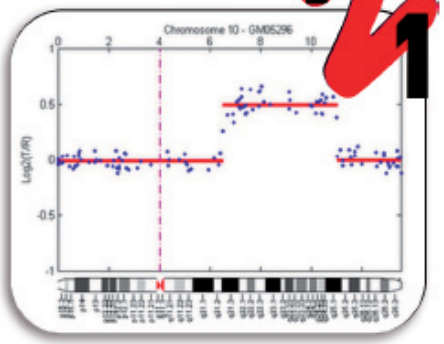


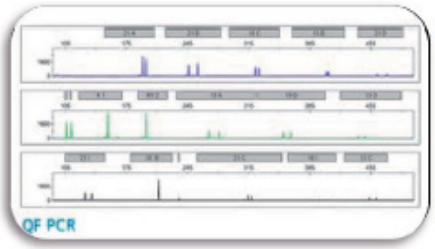
EMBRIO FIRST TWO WEEKS

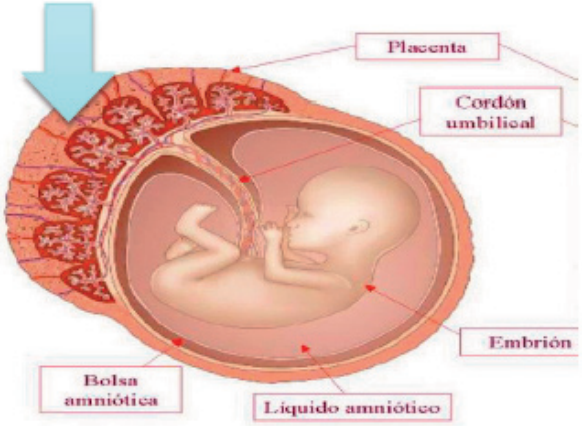

Chorion:
Extra-embryonic mesoderm
Cytotrophoblast
Syncytiotrophoblast
Maternal blood vessels
Cytotrophoblastic shell
Placenta:
Decidua basalis
Vascular lacuna
Villous chorion
Primary chorionic villus
Secondary chorionic villus
Tertiary chorionic villus

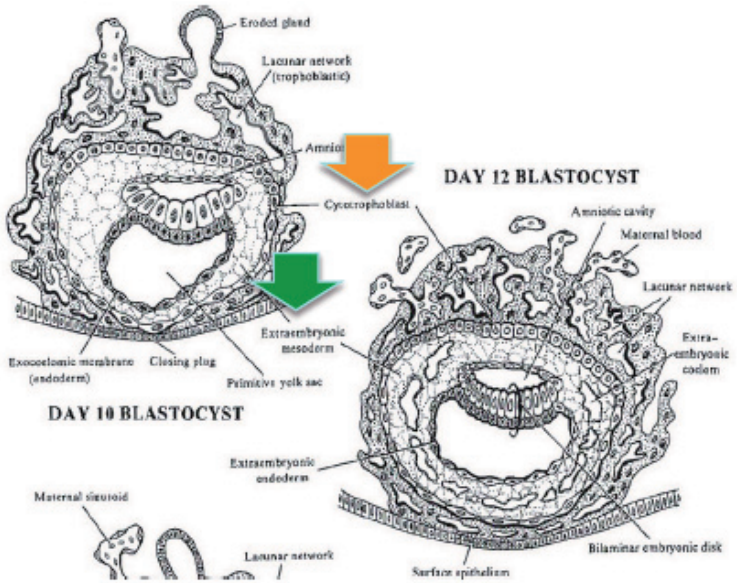

Amnion
Chorion

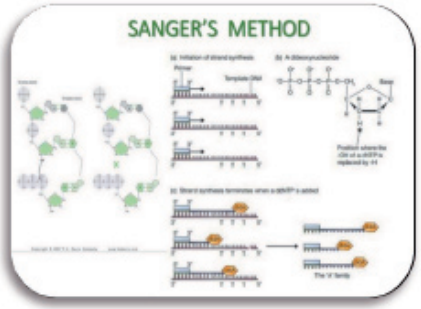

Placenta
Cordón umbilical
Embrión
Bolsa amniótica
Líquido amniótico

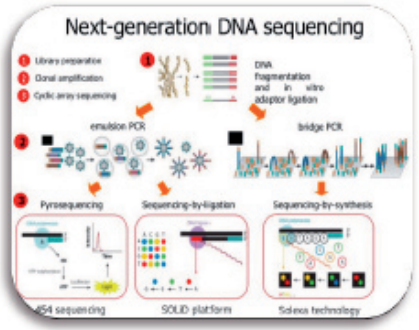












Casi di Anomalie Cromosomiche	‰ prevalence EU	% Anomalie Cromosomiche	CVS Cytotrophoblast (direct method)	CVS Cytotrophoblast (direct method)
Totale	4,4		Specificità%	Sensibilità%
T21 T18 T13	3,1	70 (48<77)	99,9 99,9 99,8	99,5 98,4 98,4
X/Y Trisomies	0,2	5	99,9	99,0
45,X	0,33	8	99,7	99,1

Totale	4,4		PPV%	NPV%
T21 T18 T13	3,1	70 (48<77)	96 92 62	99,98 99,99 99,99
X/Y Trisomies	0,2	5	85	99,00
45,X	0,33	8	43	99,10

FETO PLACENTAL DISCORDANCE IN CVS

to proceed with further investigations, of which the patient will be informed during genetic counseling. The inability to reach a diagnosis can occur in very rare cases, for reasons generally related to reduced growth of the villi in culture and an absence of dividing cells in the direct preparation.

Amniotic fluid

In the case of two or more cell lines with different karyotypes (mosaic), a further cytogenetic investigation on another sample may be necessary. In this circumstance the patient is informed, during genetic counseling, about the possibility of further diagnostics. The impossibility of reaching a diagnosis can occur in very rare cases, for reasons related to the growth of cells in culture or the massive presence of blood or meconium.

2-It is possible that the result requires, for a more correct interpretation, the extension of the cytogenetic examination to the parents or the application of additional molecular investigations

3-The analysis does not highlight very small structural anomalies (mutations, microdeletions) or low percentage mosaics.

4-There is the possibility, limited to very rare cases, of discordance between the outcome of the prenatal cytogenetic diagnosis and the karyotype found at birth. This discordance can be attributed to different causes: contamination of the sample with cells of maternal origin, low percentage mosaics or presence of chromosomal structural anomalies not detectable with the applied investigation techniques.

QF PCR (fluorescent quantitative PCR)

The analysis reveals numerical anomalies of chromosomes 13, 18, 21, X and Y with standardized molecular biology methods. The analysis can also be extended to other chromosomes. (fluorescence quantification of DNA-STR segments)

QF-PCR does not detect structural or sequence alterations other than those used, therefore other chromosomal anomalies are not highlighted. QF-PCR reveals but does not distinguish free and translocation trisomies and is not suitable for highlighting mosaic aneuploidies. QF-PCR has a false negative risk of 1%.

The presence of cells of maternal origin may result in non-informative analyzes or may not allow the analysis to be performed.

TEST SCREENING INFORMATION ARRAY-CGH 15k EASYCHIP™ IN PRENATAL DIAGNOSIS

Molecular analysis of the karyotype on Amniotic Fluid, Chorionic Villi.

In the European population, approximately 2.4% of newborns are affected by a congenital birth defect (so-called species risk), which depends on genetic (chromosomal, genetic, etc.) or non-genetic causes (drugs, infections, etc.) .

Among genetic diseases, the so-called “microdeletion/microduplication syndromes” are made up of a group of pathologies caused by imbalances in the DNA structure called “copy number variations” (CNV: Copy Number Variations), which are associated with clinical pictures distinct. These imbalances are not detectable with conventional microscopic karyotype analysis, but are detectable with high-resolution genomic analysis with a

resolving power of up to one hundred times higher than that possible with conventional karyotyping . The test complements but does not replace conventional karyotyping on amniocytes or chorionic villi. The test is carried out with aCGH (array-based Comparative Genomic Hybridization) technique.

The aCGH EASYCHIP™ platform can identify DNA "copy number variation" (CNV) up to a minimum size of approximately 3Mb across the entire genome, avoiding identifying CNVs that are too small, often present in the general population as benign variants, i.e. without clinical significance, or of unknown significance. EASYCHIP™ (15k) is a platform designed to integrate prenatal chromosome analysis. In particular:

1) it identifies CNVs along the genome with a resolution of 3-4 Mb (4-5 times greater than chromosomal analysis), reducing CNVs without clinical significance or of unknown significance from 26% to 3%.

2) it analyzes subtelomeric regions at higher resolution (300-500 kb) to search for cryptic imbalances

3) it analyzes at a maximum resolution of 200-250 kb the syndromic regions associated with 45 known microduplication or deletion syndromes. List (in brackets the gene involved, when known)

1p36 deletion syndrome 15q24 deletion/duplication syndrome
1q41q42 microdeletion syndrome (DISP1) 16p deletion syndrome (ATR-16) (HBA1, HBA2)
2p15-16.1 microdeletion syndrome (BCL11A) 16q24.1 microdeletion syndrome (FOXF1, FOXC2)
2q33.1 deletion (Glass syndrome) (STAB2) 17p13.3 deletion syndrome (Miller Dieker) (PAFAH1B1, YWHAE)
2q37 deletion syndrome (HDAC4) 17p11.2 deletion syndrome (Smith-Magenis) (RAI1)
3pter-p25 deletion syndrome (CNTN4, ITPR1, SRGAP3, VHL) 17p11.2 duplication syndrome (Potocki Lupski) (RAI1)
3q29 deletion/duplication syndrome (FBXO45, PAK2, DLG1) 17q11.2deletion/ duplication syndrome (NF1, SUZ12)
4p16.3 deletion syndrome (Wolf-Hirschhorn) (LETM1, WHSC1) 17q21.31 deletion syndrome (Koolen-De Vries) (KANSL1)
4q21 deletion syndrome (PRKG2, RASGEF1B) 17q23.1-q23.2 deletion syndrome (TBX2, TBX4)
5p deletion syndrome (Cri du chat) (CTNND2, TERT) 19q13.11 deletion syndrome (LSM14A, UBA2)
5q14.3 deletion syndrome (MEF2C) Down Syndrome critical region (21q22.12q22.2)
6q13-q14 deletion syndrome (COL12A1) 22 partial tetrasomy (Cat Eye)
7q11.23 deletion syndrome (Williams-Beuren) (ELN) 22q11.2 deletion syndrome (DiGeorge) (HIRA, TBX1)
8p23.1 deletion syndrome (GATA4) 22q11.2 distal deletion syndrome (MAPK1)
8q21.11 Microdeletion Syndrome (ZFHX4, PEX2) Xp11.3 deletion syndrome (RP2)
8q24.1 deletion syndrome (Langer-Giedion) (TRPS1, EXT1) Xp11.22

microduplication syndrome (HUWE1)
9q34.3 deletion syndrome (Kleefstra) (EHMT1) Xq12deletion/duplication (OPHN1
10p14p13 deletion syndrome (DiGeorge type 2) (GATA3) Xq22.3 deletion syndrome (AMME COMPLEX) (COL4A5, ACS4)
11p13 deletion syndrome (WAGR) (PAX6, WT1) Xq28 duplication syndrome (MECP2)
11p11.2 deletion syndrome (Potocki-Shaffer) (ALX4)
11q deletion syndrome (Jacobsen)
14q12 microdeletion syndrome (FOXG1)
15q11q13 deletion syndrome (Prader-Willi) (SNRPN)
15q11q13 deletion syndrome (Angelman) (UBE3A)
A negative result does not exclude the onset of some diseases represented on the array, in cases where these are caused by point mutations in the disease gene, rather than by deletions/duplications.

TECHNICAL INDICATIONS FOR CONSTITUTIONAL PRENATAL CYTOGENETIC ANALYSIS

Karyotype on chorionic villi (trophoblast).
The chorionic villus biopsy should be analyzed to determine its suitability for testing. The lab must have a written protocol for verifying suitability. 2.2.1 “Direct” and “culture” methods Cytogenetic analysis of chorionic villi can be performed both with the “direct” and “direct” methods after “culture”. In the “direct” method the cytotrophoblast cells, which divide spontaneously, can be analyzed after a short incubation period.
In the analysis after “culture”, the villus is disintegrated with mechanical techniques and enzymes that allow the cells present in the mesenchyme to be released and made proliferate in culture.
To define the karyotype of the chorionic villi it is necessary to use both methods. Discrepancies between the karyotype obtained with the direct and culture methods are possible. If the trophoblast sample is not sufficient to perform both methods, it is the use of the “direct” method is preferable. If the laboratory replaces the method “direct” with the QF-PCR technique, it is necessary to perform the karyotyping on “culture”.
Combination of “direct” and “culture” methods Karyotype: it is recommended to analyze at least 6 metaphases obtained by direct method and 10 from culture (Hook, 1977) and perform the reconstruction of the karyotype on at least 3 metaphase (1 by direct method) to a resolution indicated (320-400).
In the presence of a mosaic it is necessary to perform at least one karyotype per line cell phone and compare the results obtained with the two methods. Every mosaicism foresees the possibility of further investigation. It is recommended not to process all crops prepared and to retain, if possible, a portion of the sample (culture cells or frustule) of native villus for any further investigations until the analysis is concluded.
Single method. When only one of the two methods can be used: a) the chromosomal investigation is performed by analyzing at least 16 metaphases (Hook, 1977), of which

3 with reconstruction of the karyotype.
In the presence of a mosaic it is recommended to reconstruct at least one karyotype by cell line; b) chromosomal analysis with a single technique increases the probability of a discrepant result from the fetal karyotype, this possibility must be reported in the report (indication of interview with the doctor/geneticist); c) with the application of the post-culture method only, it is recommended to analyze the metaphases from multiple growth areas obtained from two cultures independent; d) with the application of the post-culture method only, in the presence of XX gonosomes, it is recommended to exclude possible maternal contamination with the analysis of DNA polymorphisms.
Karyotype on amniocytes (amniotic fluid).
For each sample of amniotic fluid it is recommended to prepare no less than 3 cultures primaries, using two different incubators. It is good practice to use two types of soil or two plots several of the same land.
Karyotype analysis must be performed on at least 2 primary cultures. “Flask” method: at least 16 metaphases must be analyzed (Hook, 1977) of 2 independent cultures, in which no fewer than 10 colonies have grown in total. “In situ” method: at least 10 metaphases from 10 colonies obtained must be analyzed from at least 2 independent cultures (Claussen 1984).
Regardless of the culture method used, it is recommended to reconstruct the karyotype in at least 3 metaphases, at the resolution indicated in 1.2. In case of mosaicism it is necessary to analyze a greater number of metaphases/colonies, examining other cultures and reconstructing at least one karyotype of each identified cell line.
If the quality of the preparation is poor and the cell growth is not suitable to perform the analysis according to the recommended protocol, the report must include this eventuality.
It is recommended not to process all the cultures established and to retain a portion of cells in culture for any further investigations until the analysis is concluded.

PRENATAL DIAGNOSIS
Non invasive sampling techniques, cell free fetal DNA in maternal blood

CffDNA Analysis Techniques.
The techniques in use analyze total cfDNA, without differentiating fetal from maternal. Since these are, in fact, investigations based on a mixture of maternal and placental DNA, the NIPT it is not a diagnostic test, but a screening one.
In fact, as in traditional tests, the use of dedicated algorithms allow to define the post-test probability that the fetus is affected by one of the major autosomal trisomies (trisomy 21 [T21], trisomy 18 [T18], trisomy 13 [T13]) or by an aneuploidy of the sex chromosomes (X, XXX, XXY, XYY), or structural chromosome anomalies (deletions – duplications) >7-10 Mb, selectively analyzing the number of the cffDNA fragments contributed by each of the chromosomes being tested.
Three main techniques based on NIPT are used for the analysis of aneuploidies second generation sequencing techniques (Next Generation Sequencing - NGS): NGS of the

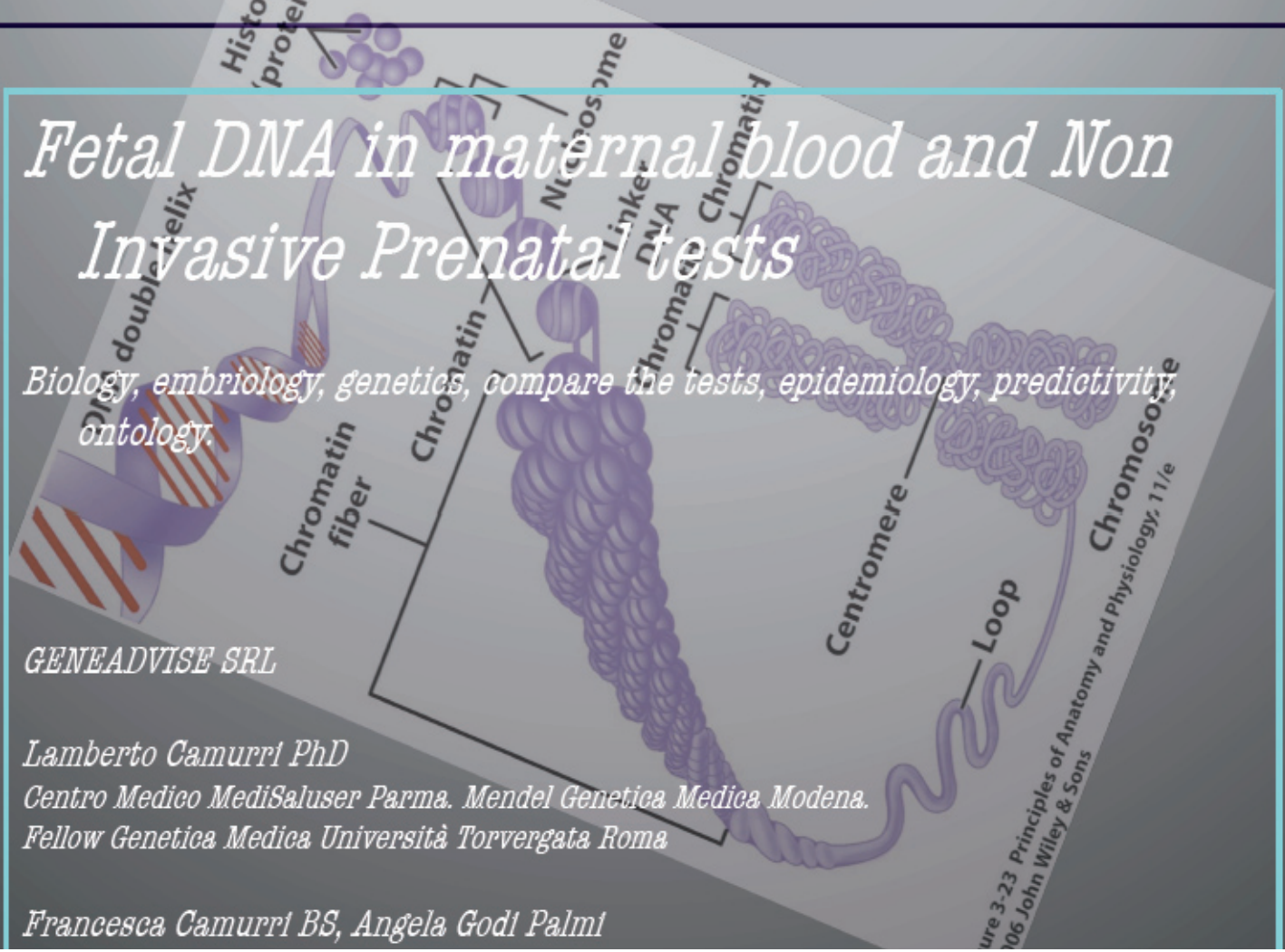
entire genome; NGS of specific regions; SNPs, i.e. polymorphisms of single nucleotides.
The whole genome NGS technique is based on the sequencing of the cffDNA present in the maternal plasma, to generate millions of short sequences of the entire genome, which are then mapped to a reference sequence of the human genome, to establish their origin and count the number of fragments originating from the chromosome of interest, compared with the number of fragments obtained from other chromosomes (Fan et al, 2008). So for example, if a fetus has T21, they will be in the maternal plasma present more CR21 fragments than expected in controls without T21.
An alternative NGS technique selectively amplifies specific genomic loci on the chromosome of interest, which are subsequently sequenced. This technique is less expensive, as it reduces the regions to be sequenced, but has the limitation of studying only some regions of interest preselected.

CFF DNA INSIDE
CffDNA INSIDE shows the story of the non invasive approach to prenatal genetic diagnosis. The experience of Geneadvise team shows the growth of NIPT methodology from the first attempt to the contemporary excellence. Years from 2012 to 2020.
(Geneadvise team: L.Camurri PhD, F.Camurri BS, G.Camurri BS, A.Godi Palmi EA)

cffDNA inside

Fetal DNA in maternal blood and Non Invasive Prenatal tests

Biology, embriology, genetics, compare the tests, epidemiology, predictivity, ontology.



GENEADVISE SRL

Lamberto Camurri PhD
Centro Medico MediSaluser Parma. Mendel Genetica Medica Modena.
Fellow Genetica Medica Università Torvergata Roma

Francesca Camurri BS, Angela Godi Palmi

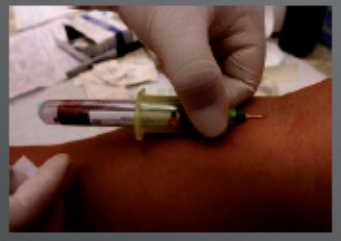
cffDNA inside

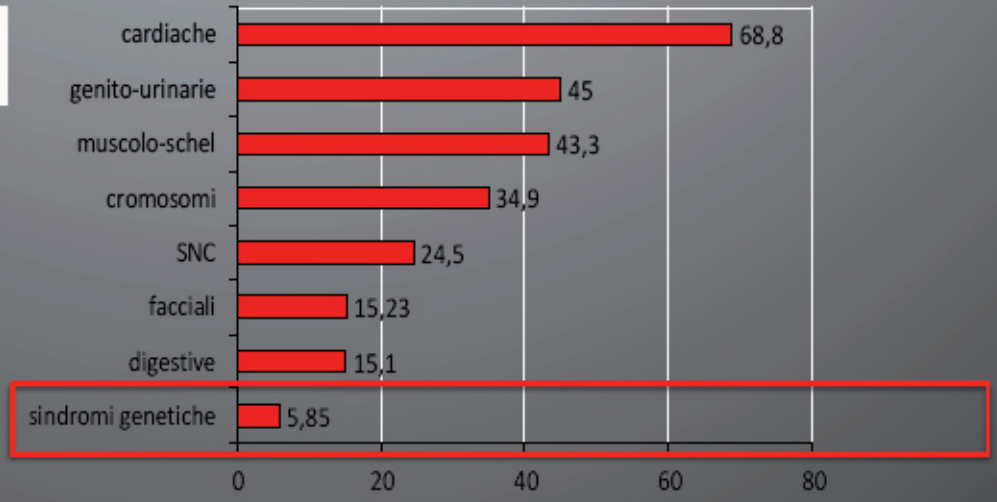
Why?

NIPT: Non Invasive Prenatal Test

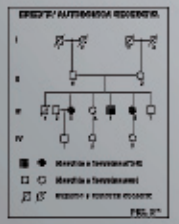
DIAGNOSTIC TEST: MENDELIAN MUTATIONS

PluriGenTEST™

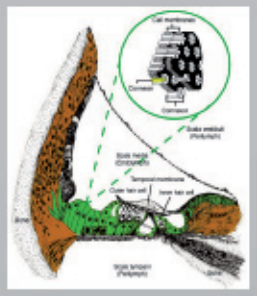




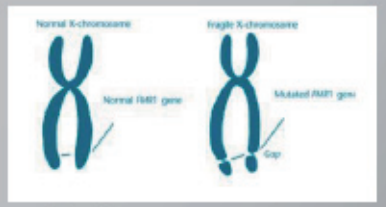
PluriGenTEST™



Fibrosi cistica



Sordità neurosensoriale

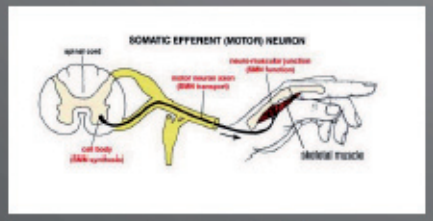


X fragile

Ricerca portatori malattie mendeliane

Fibrosi Cistica	sensibilità tecnica/malattia 75-80%
Atrofia muscolare spinale	sensibilità tecnica/malattia 92%
Sordità NS	sensibilità malattia 90% (rischio residuo 1/350)
Ritardo mentale FRAXA	sensibilità metodo 99%

	SINTOMI FC	ETA' DI COMPARSA	MALATTIA CON CUI VENGONO CONFUSI
RESPIRATORI	○ Tosse frequente ○ Catarro ○ Stanchezza ○ Perdita di peso o appetito	Dalla nascita all'età adulta	○ Asma ○ TBC ○ Bronchite cronica senza cause
INTESTINALI	○ Feci frequenti e abbondanti ○ Pancia gonfia ○ Dolori addominali ○ Arresto di crescita e perdita di peso	Dalla nascita all'età adulta	○ "Colite" ○ Celiachia ○ "Intolleranza alimentare"
	○ Occlusioni intestinali	Più frequenti nell'infanzia ma possibili anche in altre età	○ Appendicite ○ Malformazione intestinale



SMA

Mendelian disease parent-carriers and fetus: I nostri casi

PluriGenTEST™

Ricerca portatori malattie mendeliane

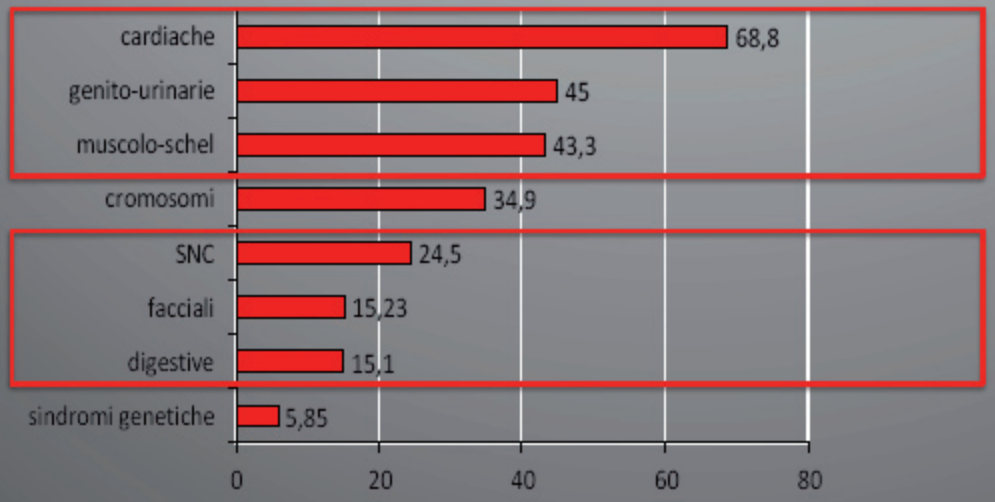
Fibrosi Cistica	90% sensibilità (rischio residuo 1/1000)
Atrofia muscolare spinale	93% sensibilità (rischio residuo 1/2500)
Sordità NS	90% sensibilità (rischio residuo 1/350)
Ritardo mentale FRAXA	99% sensibilità

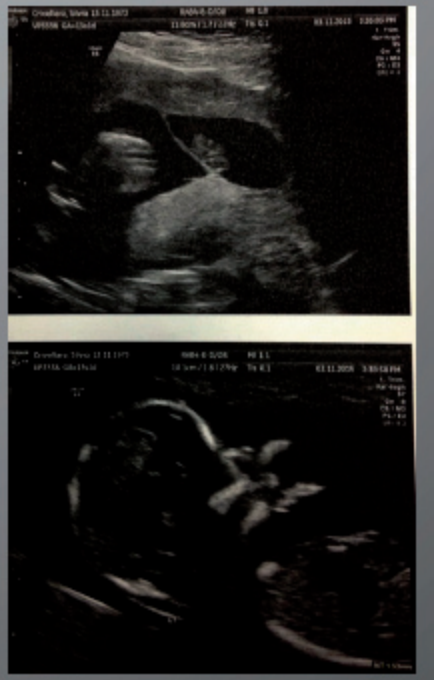
	Singolo eterozigote	Doppio eterozigote	Feto	Prevalenza-coorte /1000	Prevalenza-popolaz /1000	Coorte
CFTR FIBROSI CISTICA	43	3	3 wt/wt/wt	29	40	1570
SMA ATROFIA MUSCOLARE SPINALE	35	2	2 wt/affected	22	20	1570
CX SORDITA' CONGENITA	17	1	0	140	28	1070
FRAXA RITARDO MENTALE	7 57-55-59-56-56-55-55	-	4 wt-wt-56-wt	44	38	1570

cffDNA inside

NIPT: Non Invasive Prenatal Test

DIAGNOSTIC TEST: ULTRASOUND SCAN

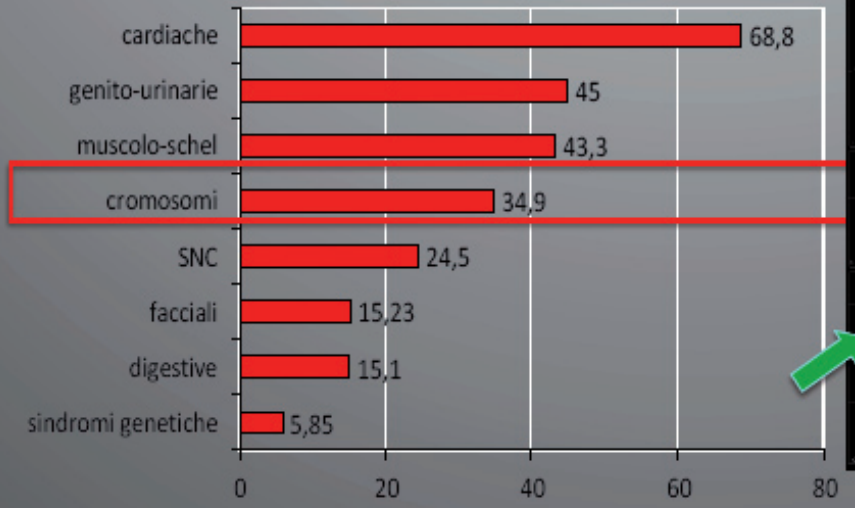


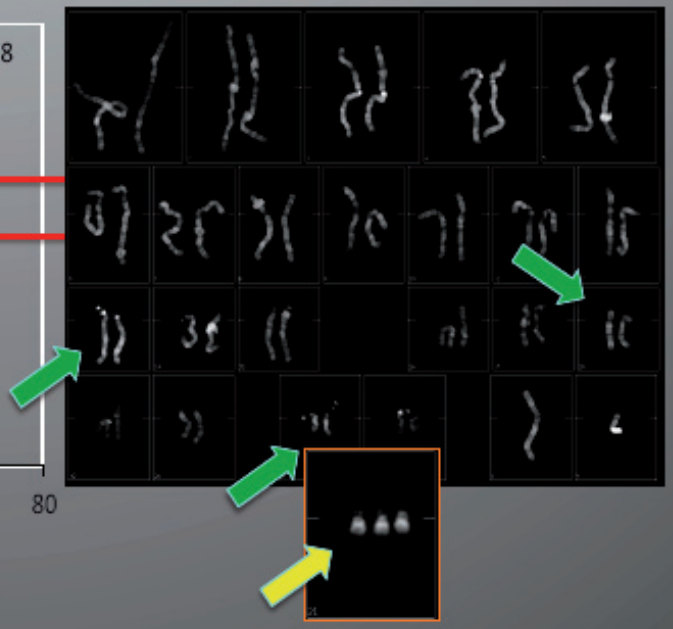


cffDNA inside

NIPT: Non Invasive Prenatal Test

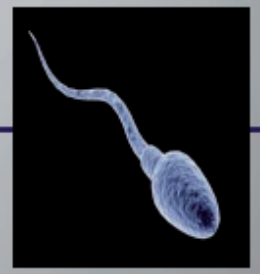
NON DIAGNOSTIC: CHROMOSOMES

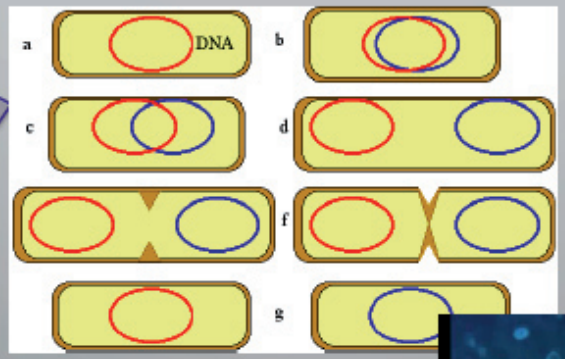


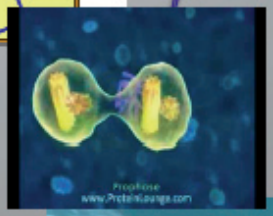


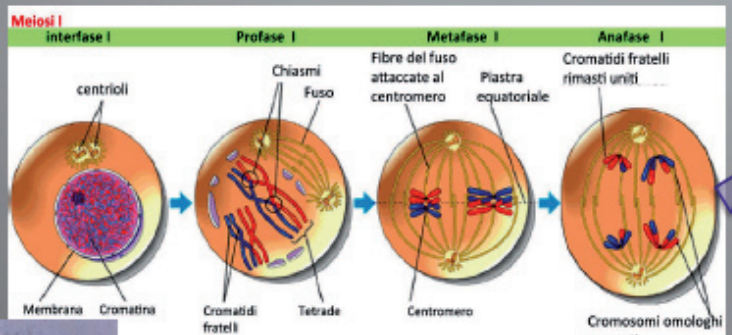
cffDNA inside

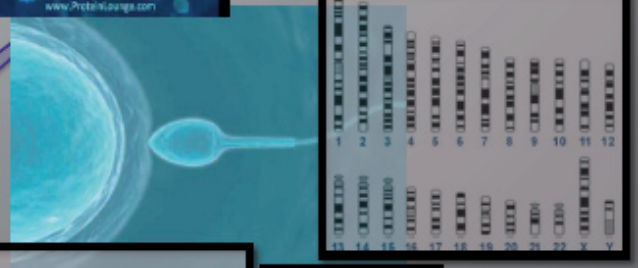
Fecondazione
E sviluppo
Embrionale
Divisione
Cellulare





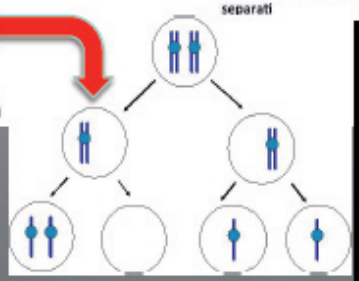


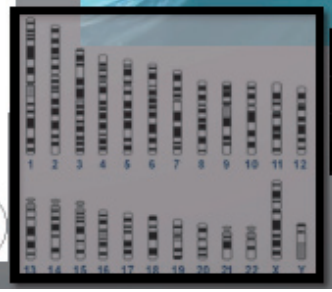


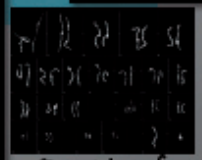


Non disgiunzione

I CROMOSOMI OMOLOGHI
SI APPAIANO E SI SCAMBIANO
DEI SEGMENTI
SINAPSI - APPAIAMENTO
DEI CROMOSOMI OMOLOGHI
E FORMAZIONE DELE TETRADI



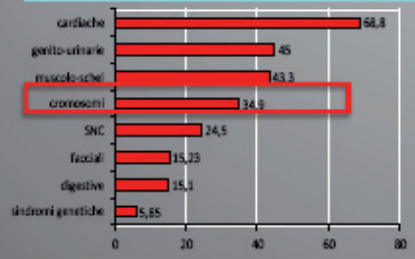


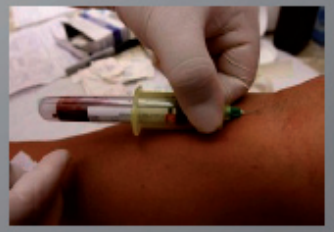


cffDNA inside

NIPT: Non Invasive Prenatal Test

NON DIAGNOSTIC: CHROMOSOMES





TEST COMBINED (NT + PAPPA + βHCG)

INDIRECT

STATISTIC OF NON GENETIC PARAMETERS

TEST SCREENING

HIGHER SENSITIVITY

LOWER SPECIFICITY

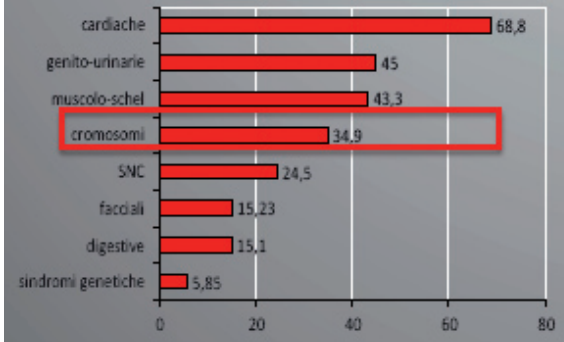
	M+	M-
T+	a	b
T-	c	d

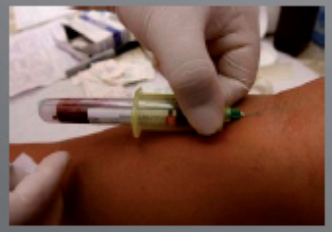
Se = a / (a+c)
Sp = d / (d+b)

cffDNA inside

NIPT: Non Invasive Prenatal Test

NON DIAGNOSTIC: CHROMOSOMES





TEST CROMOSOMAL ANOMALIES cffDNA

TEST DIRECT (non statistic)

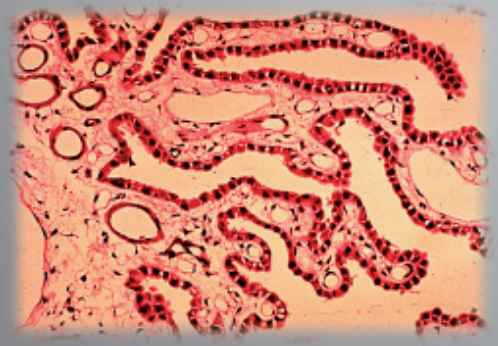
HIGHER SENSITIVITY

HIGHER SPECIFICITY

Confronto fra NIPT DNA fetale e FTS screening primo trimestre

	cffDNA		FTS	
DETECTION RATE	36/36	(100%)	28/36	(77.8%)
FALSE POSITIVE RATE	9/15050	(0.06%)	818/15050	(5.4%)

cffDNA inside

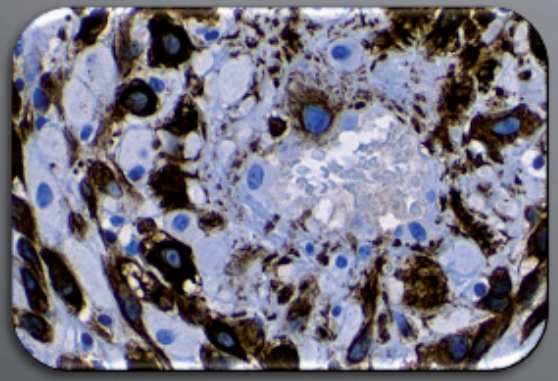


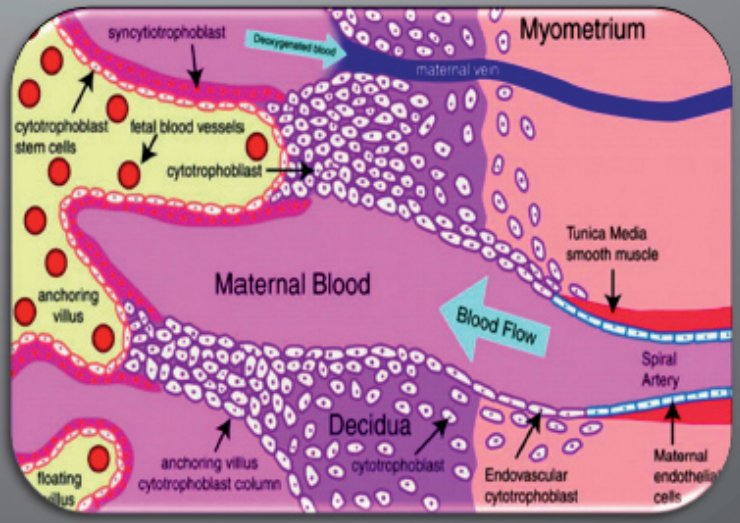
TEST :CROMOSOMAL ANOMALIES cffDNA

TEST DIRECT

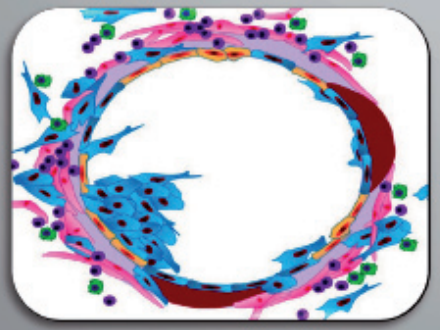
FREE FETAL DNA IN PLASMA COMES FROM TROFOBLAST IN PLACENTA

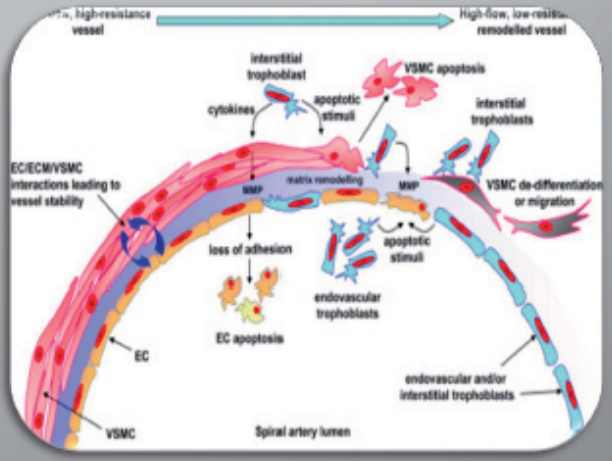
Placenta, miometrio, spiral arteries, cytotrofoblast





cffDNA inside



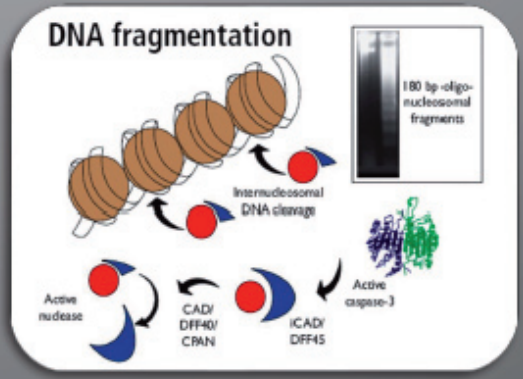


Rimodelling, competitive apoptosis, fragments of DNA

ACCACGAT
GGA
CTGG
CGATTTAACT
...ACCACGATTAACTGGA...

Frammenti di DNA sequenziati

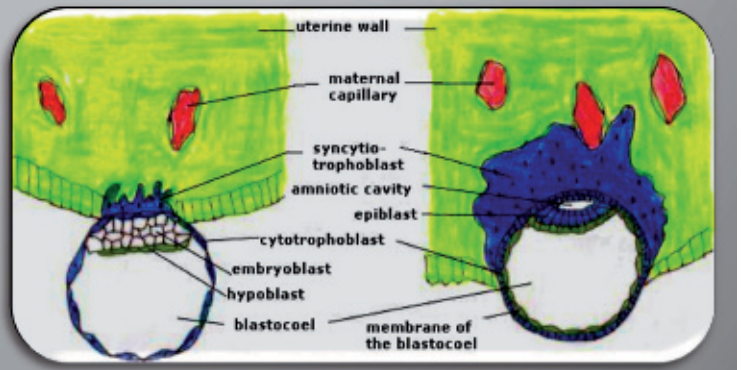
Genoma di riferimento

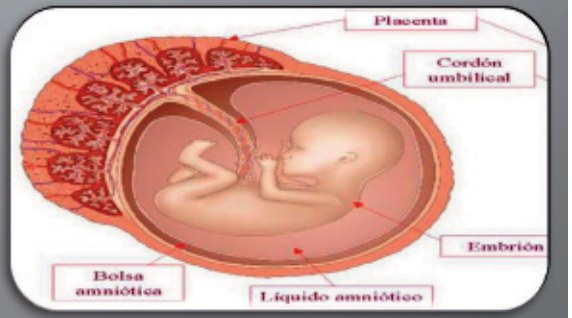


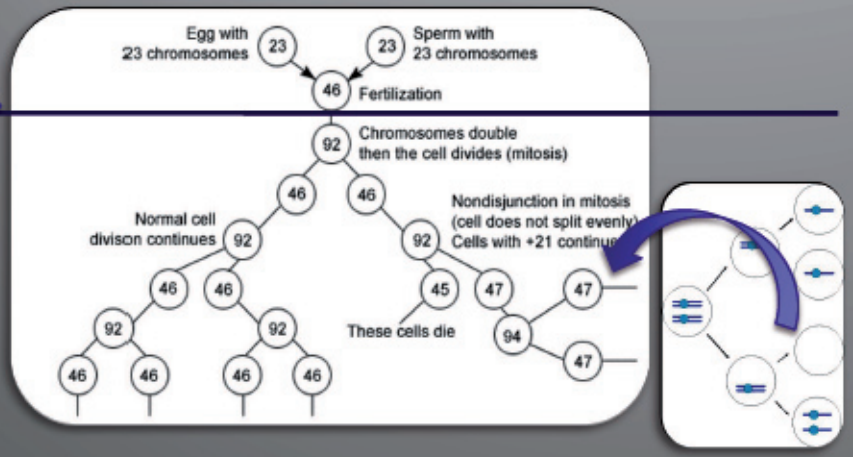
Placenta

Embriology:

On the 10th day the placenta separates from the embryo: it has the genotype of the fetus. Feto-placental discordance.

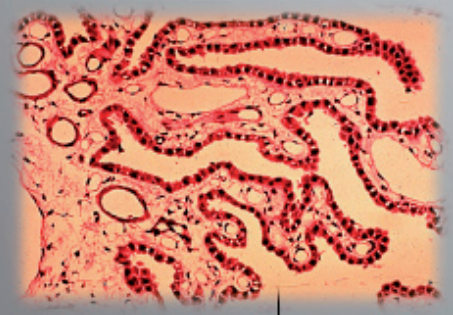






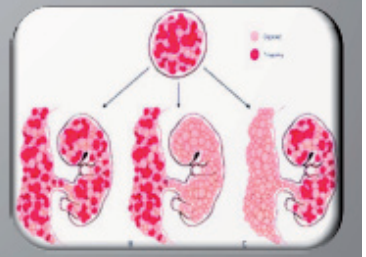
It is the limit of all chromosomal techniques applied to the placenta

cffDNA inside



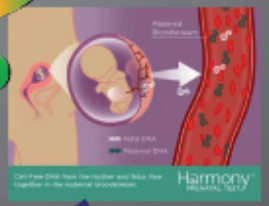
cytotrophoblast

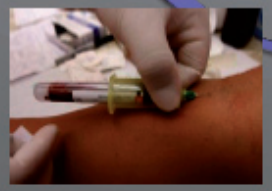
DIAGNOSI



		Trisomy 21	Trisomy 18	Trisomy 13
CVS trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%
62000 cases*	False negative Population rate False negative/sensitivity	0.02% 0.74%	0.01% 1.59%	NS [illegible]
cffDNA total 2013-2015	False positive rate/specificity	0.09%	0.13%	0.[illegible]%
	False negative Population rate False neg. /detection rate	0.08% 0.8%	0.06-0.12% 3.7%	0.18-0.36% 9%

Why?





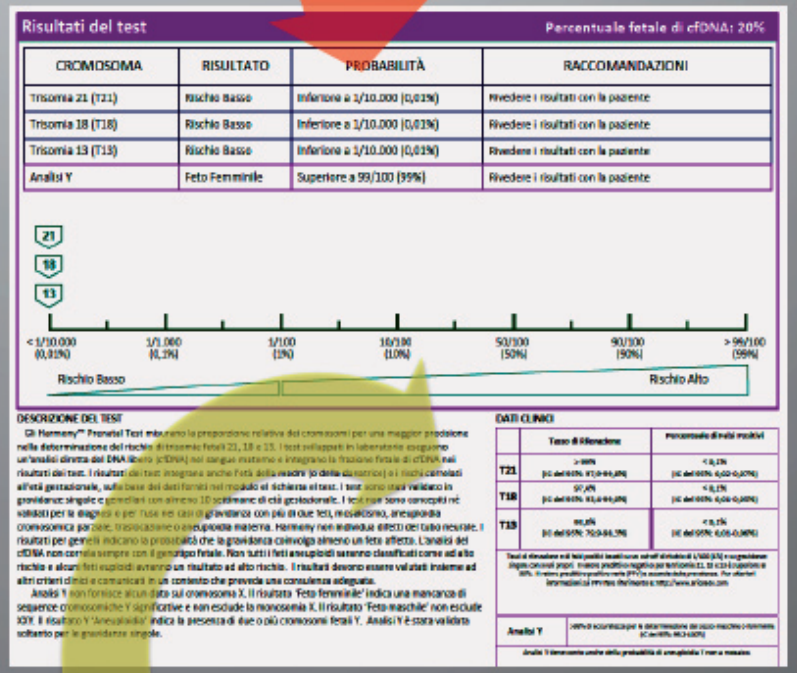
NON DIAGNOSI

cffDNA inside

Incorrect prediction of aneuploidy in cffDNA has two origins:

1. Non-disjunction post fetus-placental divergence: clinical sensitivity and specificity.

2. Sample characteristics influence NGS and processing: sensitivity and specificity of the test



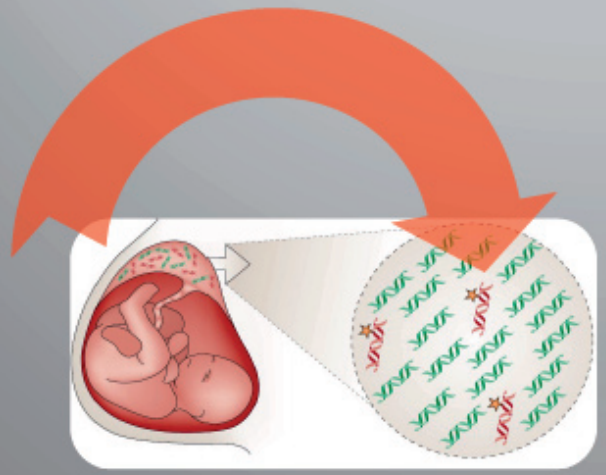
Risultati del test — Percentuale fetale di cfDNA: 20%

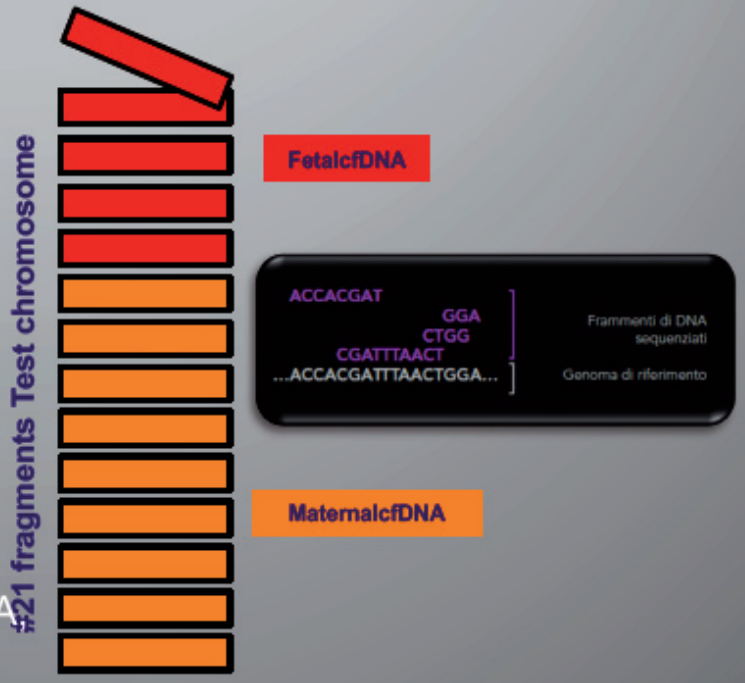
CROMOSOMA	RISULTATO	PROBABILITÀ	RACCOMANDAZIONI
Trisomia 21 (T21)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Trisomia 18 (T18)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Trisomia 13 (T13)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Analisi Y	Feto Femminile	Superiore a 99/100 (99%)	Rivedere i risultati con la paziente

DESCRIZIONE DEL TEST

DATI CLINICI

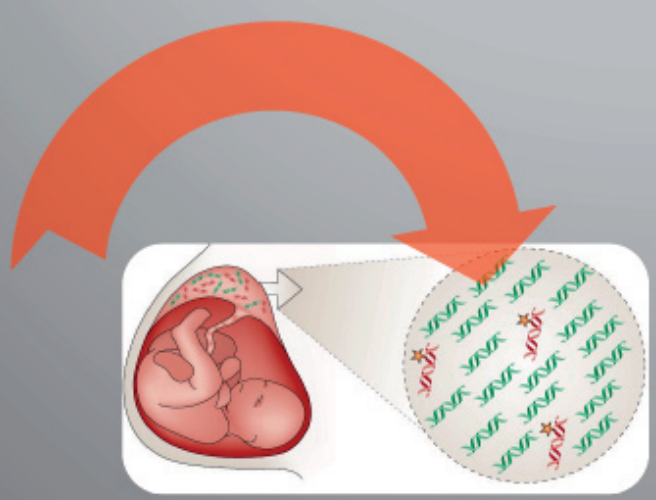
cffDNA inside





The techniques in use analyze total cfDNA without differentiating fetal from maternal. Since these are, in fact, investigations based on a mixture of maternal and placental DNA, the cfDNA test is not a diagnostic test

cffDNA inside



FetalcfDNA

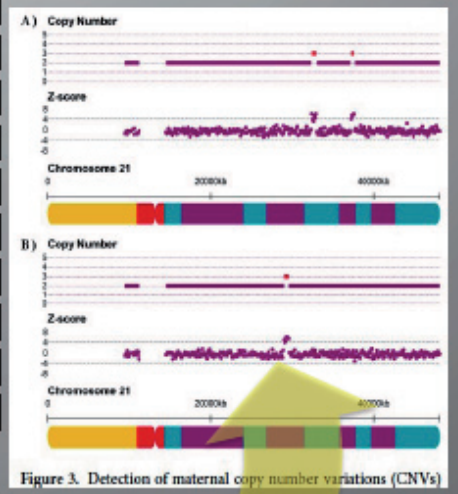


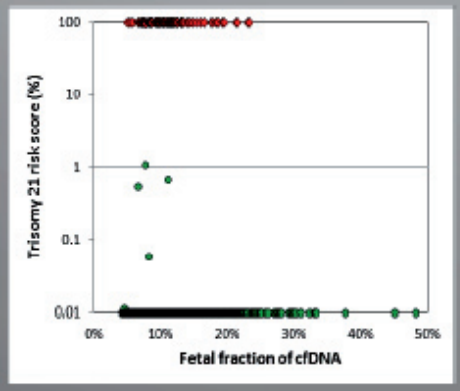
Figure 3. Detection of maternal copy number variations (CNVs)

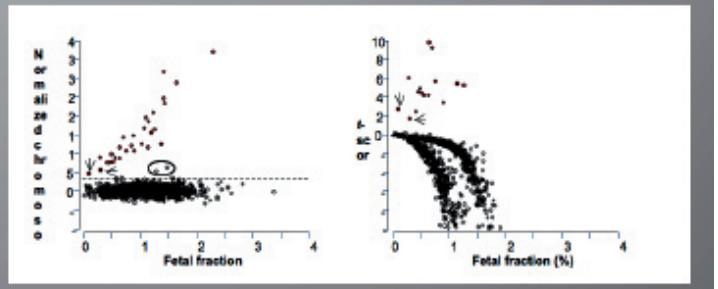
The techniques in use analyze total cfDNA without differentiating fetal from maternal. Since these are, in fact, investigations based on a mixture of maternal and placental DNA, the cfDNA test is not a diagnostic test

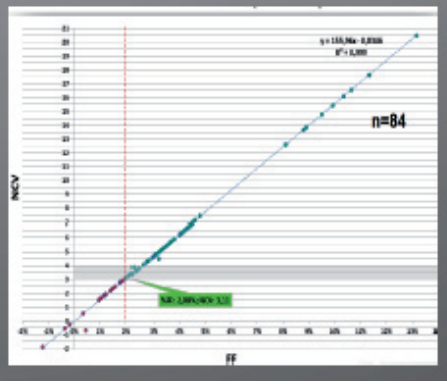
cffDNA inside

Il valore della FF viene.....non viene inserito nell'algoritmo

Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10
40%	1.20



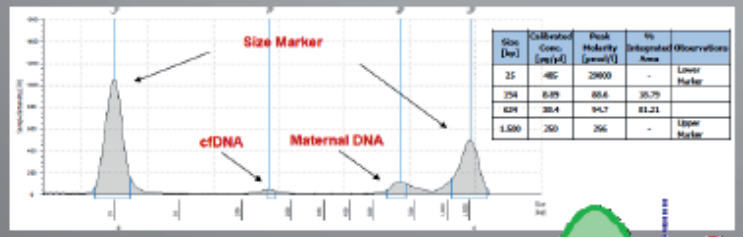


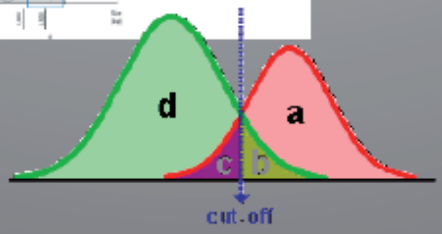


Fetal DNA <> maternal DNA. The techniques in use analyze the total cfDNA, without separating the fetal one from the maternal one. The FF calculation is performed with SNP, X-CNV, #Y, DNA fragment size

cffDNA inside

		Trisomy 21	Trisomy 18	Trisomy 13
CVS trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%
52000 cases*	False negative Population rate False negative/sensitivity	0.02% 0.74%	1.59%	NS 0.74%
NIPT total 2013-2015	**False positive rate/specificity**	**0.09%**	**0.13%**	**0.13%**
	False negative Population rate **False neg. /detection rate**	0.08% **0.8%**	0.06-0.12% **3.7%**	0.18-0.36% **9%**



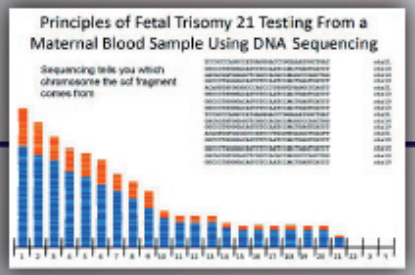


	M+	M-
T+	a	b
T-	c	d

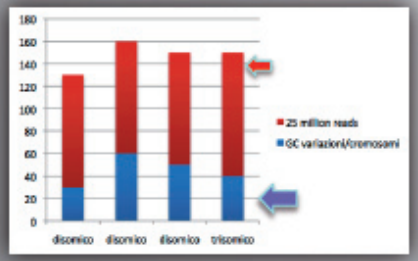
Se = a / (a+c)
Sp = d / (d+b)

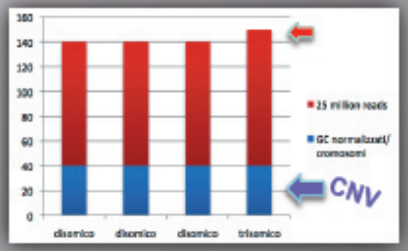
Each cytotrophoblast DNA test has a fixed clinical sensitivity and specificity (in the placenta<>fetus comparison). The cfDNA test adds sensitivity and specificity resulting from the cffDNA/maternalDNA ratio. By increasing the cut off, less FP and more FN, vice versa by decreasing it.

Methodology Capturing fetal DNA: sequencing the genome

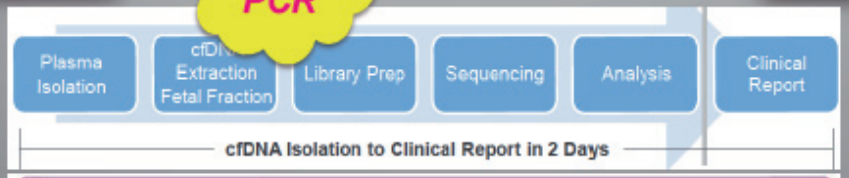


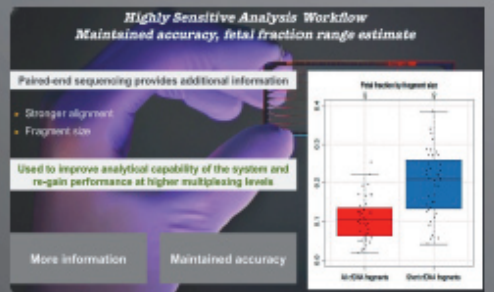
Coverage and depth. Complete massive NGS next-generation sequencing of the human genome can be performed at different read depths. High resolution is 60M (millions) of readings, the average between 15M and 30M, to detect small aneuploidies or base differences. From 1M to 8M the resolution is lower for searching for large numerical anomalies. Paired end sequencing and absence of PCR yield The most stable and reliable sequencing.

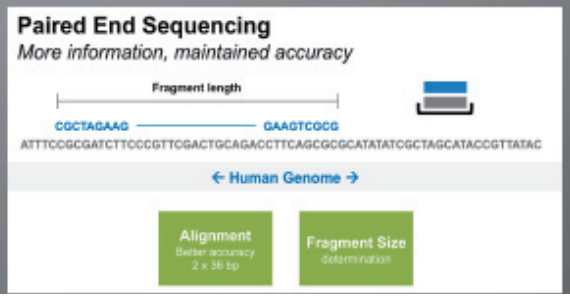


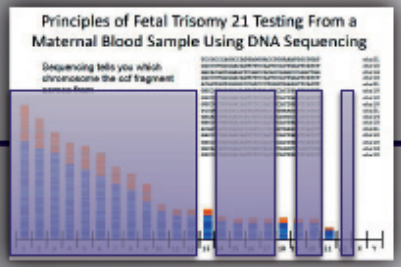


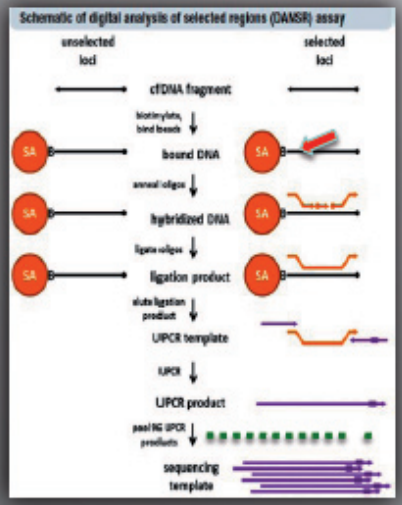
NO PCR

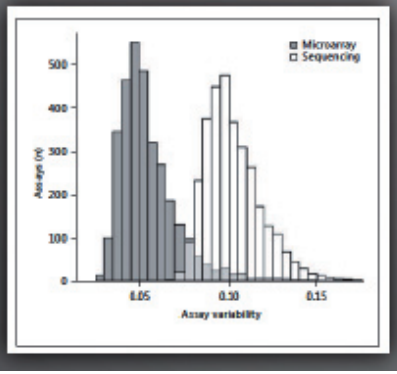






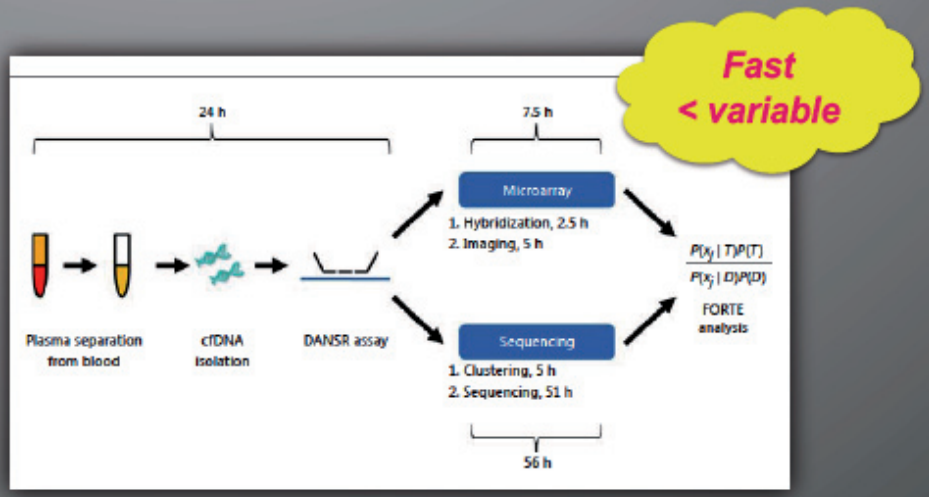




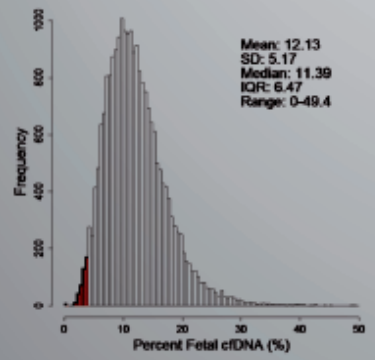


Methodology. The capture of fetal DNA: sequence only chromosomes 21, 18, 13

To identify a trisomy 21 (or 13 or 18) you can select the DNA fragments of the chromosomes, eliminating the rest of the genome. DANSR (Ariosa Harmony) 1. Sequencing: Performs selective (high multiplexed) sequencing of the DNA fragments of chromosomes 21, 18, 13 only (clustering, sequencing). 2. Microarrays: Perform the analysis on an array platform (hybridization, imaging). Reduces variability between samples. The selection of fragments occurs by hybridizing fluorescent probes to: 576 non-polymorphic STR markers of chromosomes (21, 18, 13) for the search for trisomies 192 polymorphic STR markers of chromosomes between 1 and 12 to define the fetal fraction of each sample. Only fragments attached to the fluorescent probes will be sequenced for assay and processing. The sequencing depth is low, 1M reads for three comosomes, which is equivalent to 8M reads A whole genome sequencing



Methodology. The amount of fetal DNA, fetal fraction



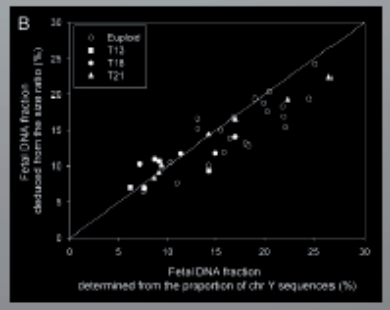
The minimum fetal fraction of each chromosome to identify a trisomy is 4%. With this percentage the ratio of sequence reads between a normal fetus and one trisomic is 1.02, which is the minimum ratio to identify trisomy. The methods are based on SNP polymorphisms, DNA fragment size, CNV, Y chromosome.

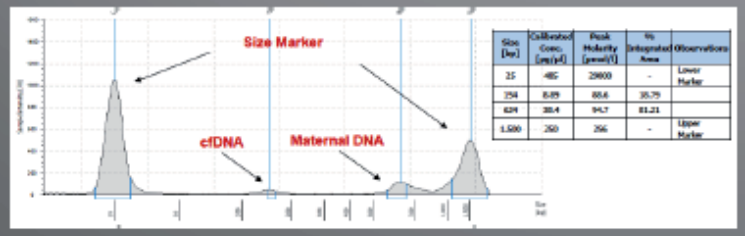
SNP (DANSR)

ABRSJA5517	Maternal (buffy coat)	A/C	G/G	C/T	A/T	A/A	A/G	C/T	C/C	A/C	A/G
	Fetal (cfDNA)					A/G			C/C		

Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10
40%	1.20

Fragment size, #X CNV & #Y (Tscore)





Fragment size distribution

Coverage profile

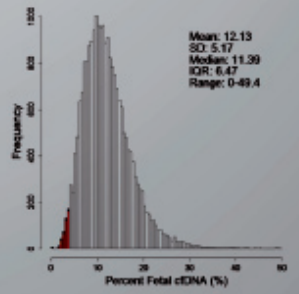
Copy number changes on X

Presence of Y

Methodology. The amount of fetal DNA, fetal fraction

Fetal fraction and Expected ratio for trisomy

Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10
40%	1.20

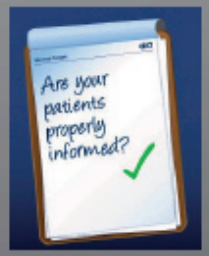


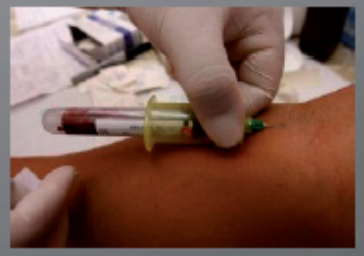
Fetal fraction and test failure

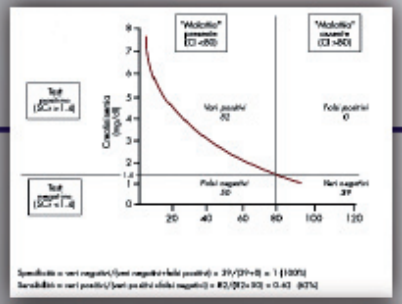
Cases	1300
Successful 1st tier	1286
Successful 2nd tier	8
Low DNA	7
High variance	1
Double fail	4 outcome ok
Low DNA / FIV ovod.	1
High variance / obese	2
No repeat	2
Go to cvs > karyo ok	1
IUD	1

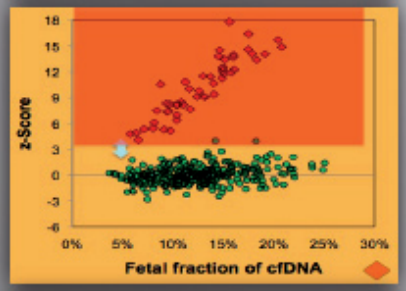
Fetal fraction and gestation weeks

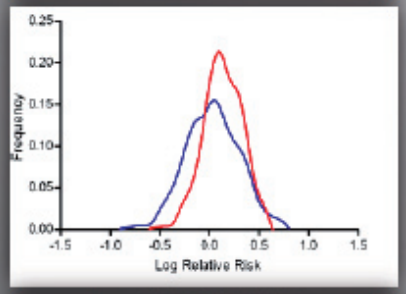
Mean weeks	12,34
Mean fetal fraction	11,5%

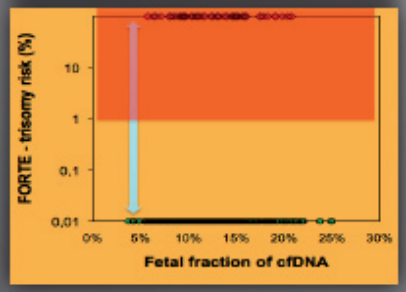






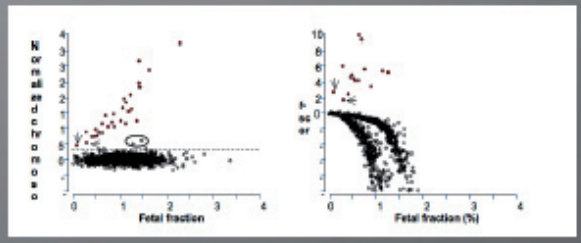






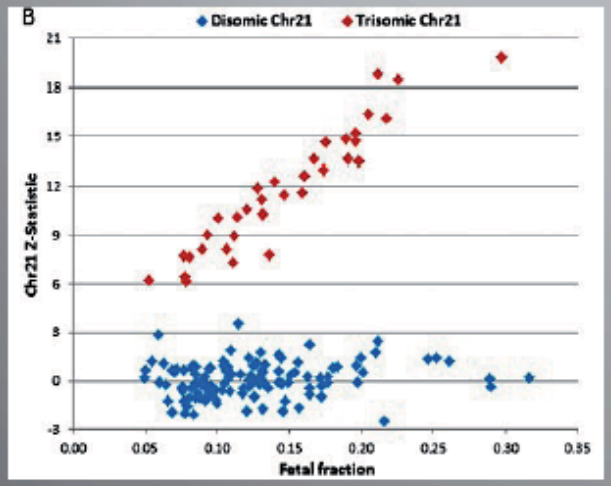
Methodology. Calculation of compatibility with aneuploidies

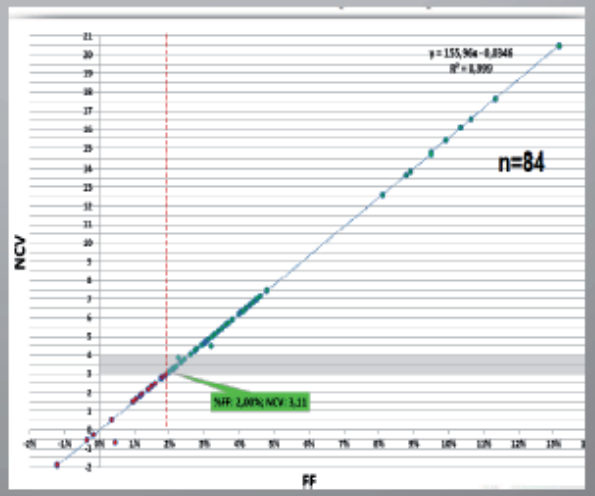
The test calculates the likelihood ratio between the probabilities that the samples contained in a sequence line are disomic or trisomic: examples MPSS SAFeR (Illumuna 2015) Algorithm to define the threshold value of possible trisomy based on: One sample set positive-negative binary hypothesis with t-Student (z-score) and Likelyhood Odds Ratio (likelihood ratio). 2) CNV sequence normalization factor. 3) Run variations between the various sequencing lines corrected with a z-score algorithm. 4) Definition of a threshold value for trisomy (z-score value between 3 and 4) DANSR – FORTE (Ariosa Harmony) - 2012 Algorithm to define the trisomy risk threshold value based on: Multiple sample set The calculation on multiple samples is made possible by the small size of the sequence reading blocks (1 million reads) which allows the analysis in a sequence line of 96 samples which are compared with each other. 1) Percentage hypothesis with Odds ratio (likelihood ratio) between disomic/trisomic models, (normal distribution curves) 2) Calculation of the fetal fraction 3) Montecarlo Simulation which also includes maternal age and gestational age in the calculation of the FORTE algorithm. T-SCORE (Labco Neobona/Illumina) – 2015 Compound z-score like algorithm Fetal fraction calculation Fragment distribution by size Batch-sample/depth comparison sequence



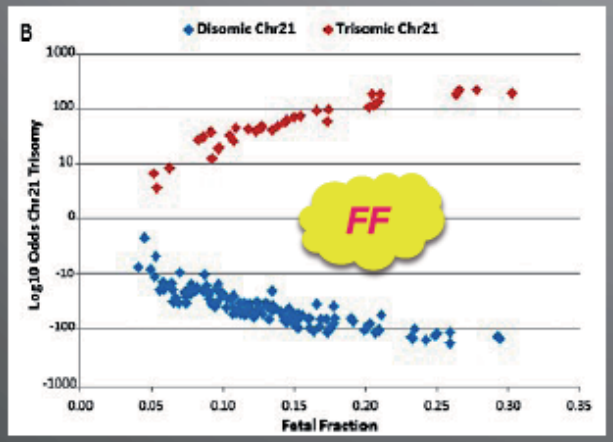
cffDNA inside

Calculation of compatibility with aneuploidies

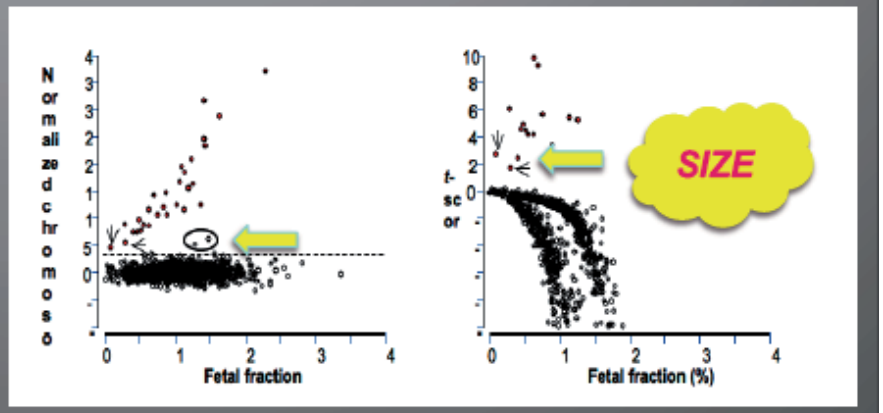




Illumina
SAFeR



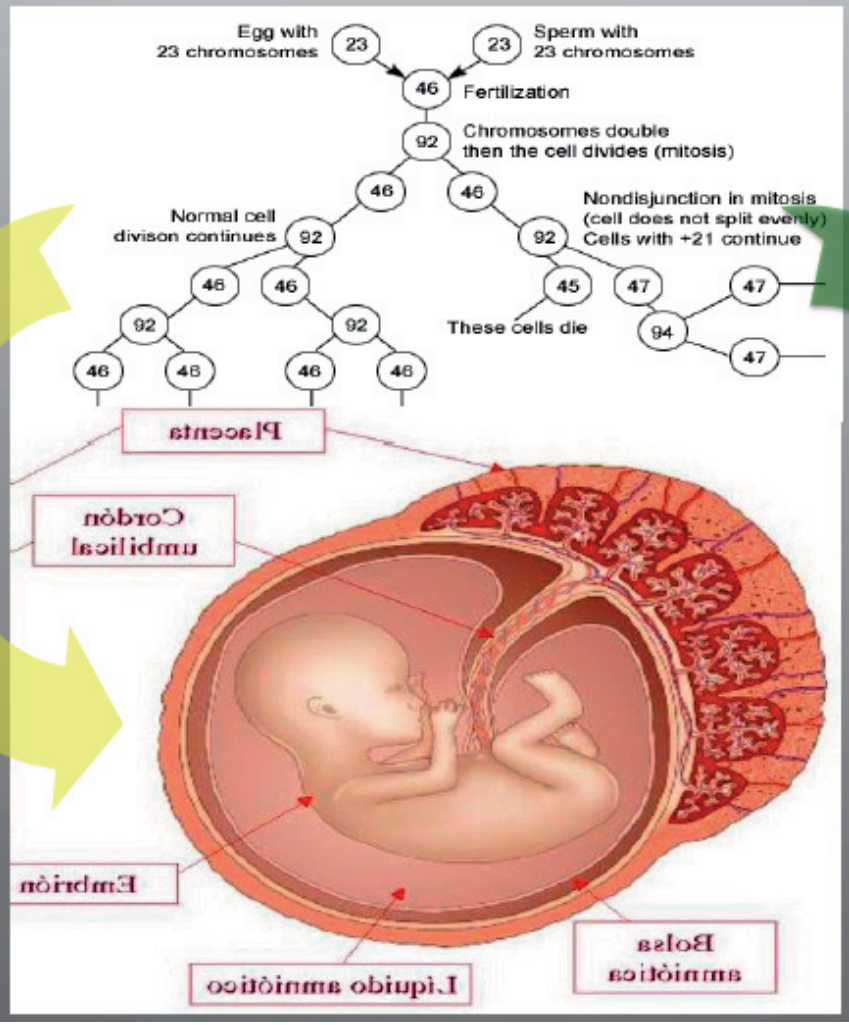
Ariosa FORTE

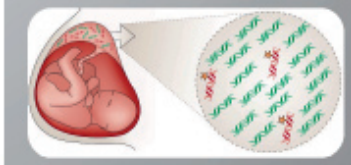


Illumina T-Score (NeoBona)

cffDNA inside

Biological limits, late non-disjunction

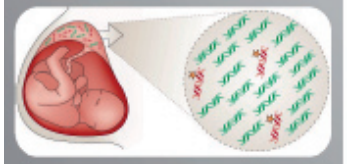




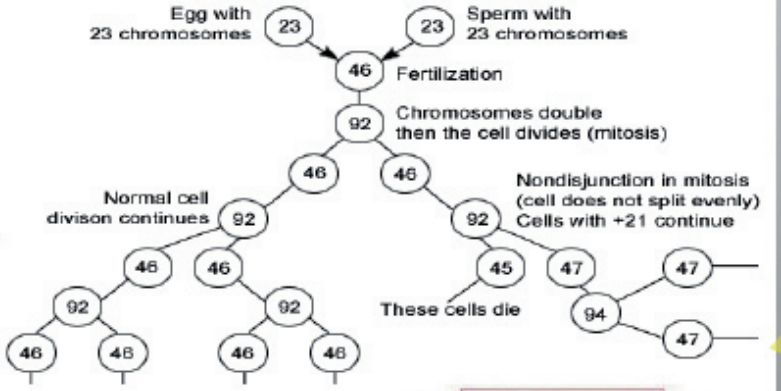
FALSE POSITIVE

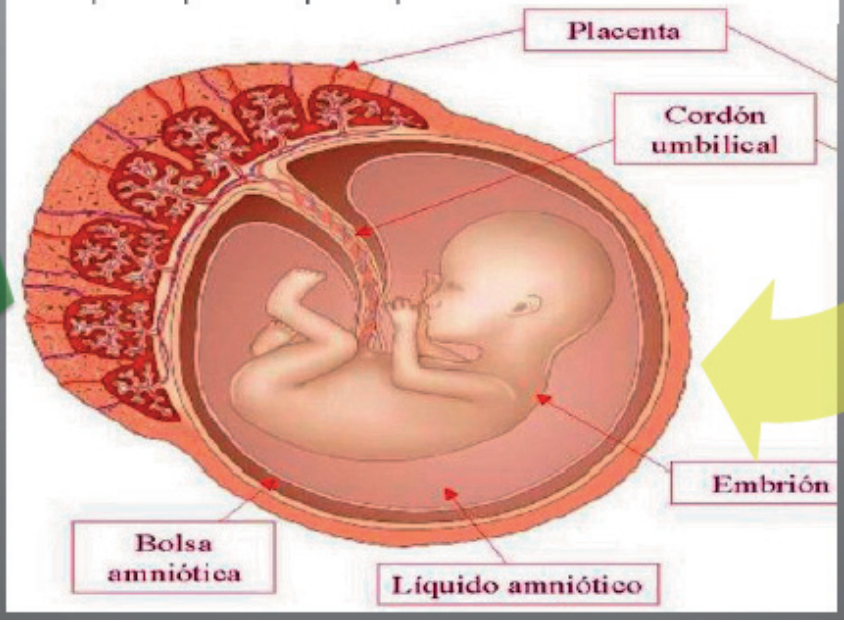
cffDNA inside

Biological limits, late non-disjunction



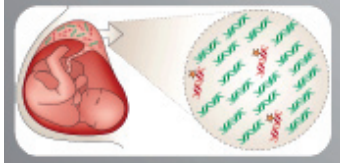
FALSE NEGATIVE

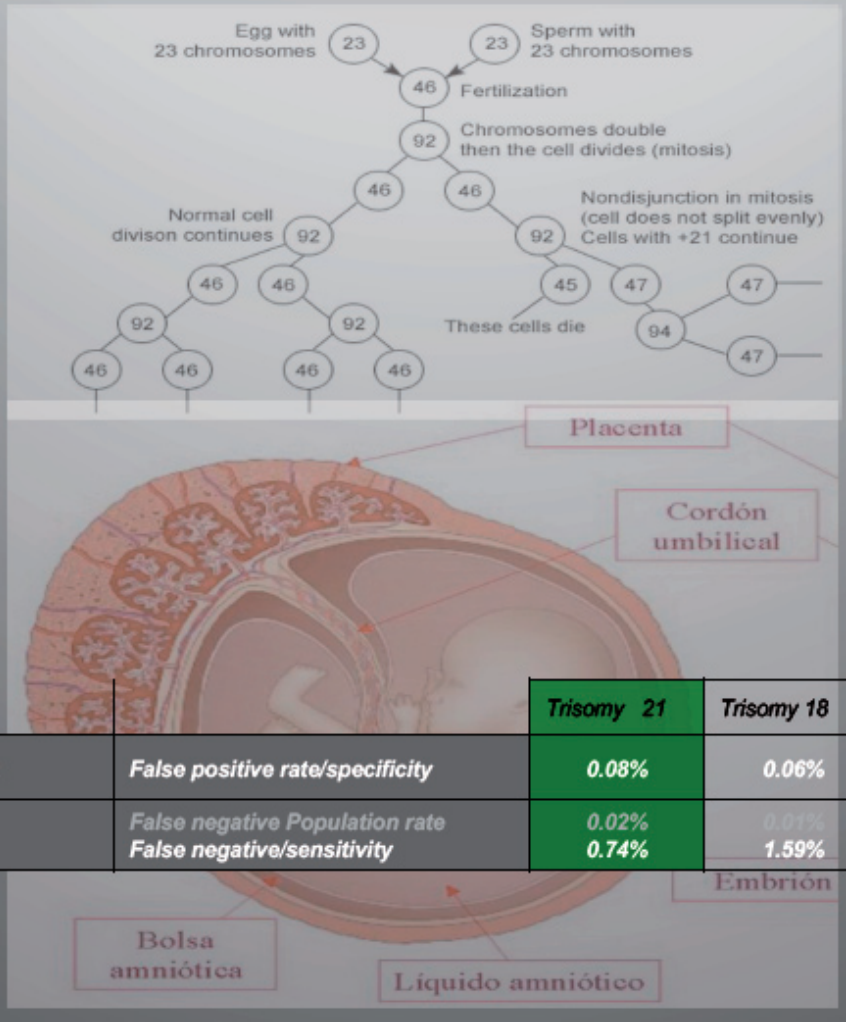




cffDNA inside

Biological limits, late non-disjunction

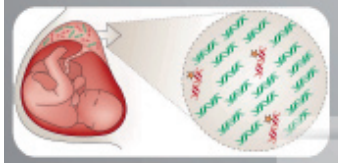




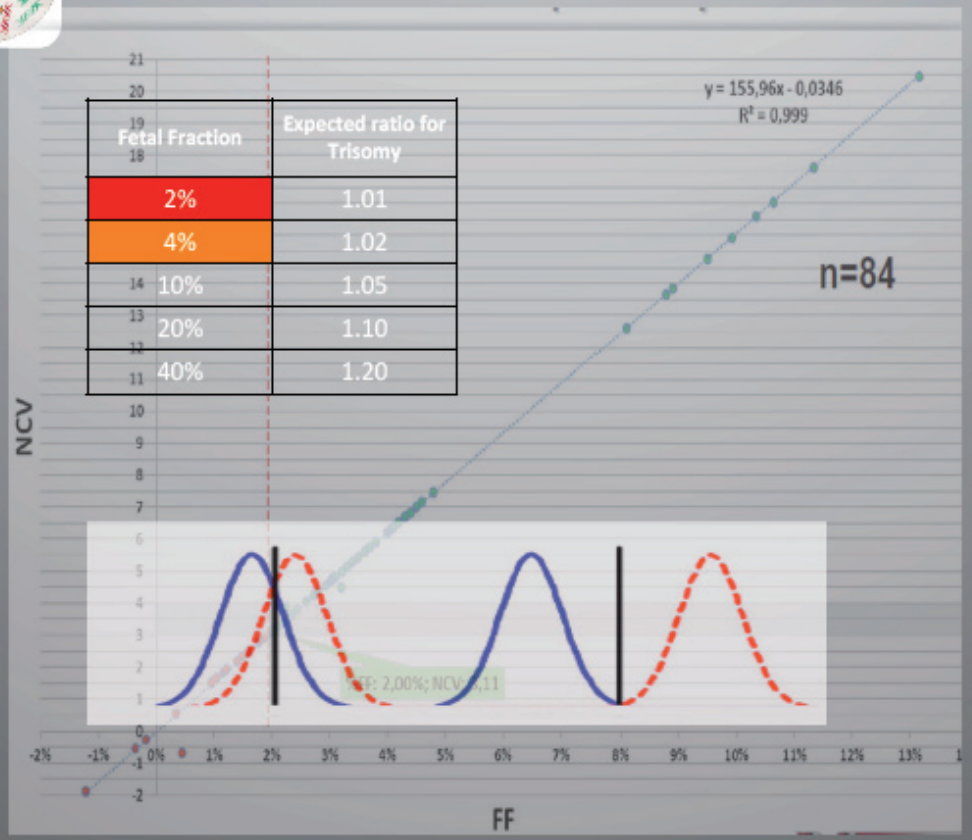
		Trisomy 21	Trisomy 18	Trisomy 13
CVS trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%
62000 cases*	False negative Population rate	0.02%	0.01%	NS
	False negative/sensitivity	0.74%	1.59%	0.74%

cffDNA inside

Technical limitations: fetal and maternal DNA, FF



Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10
40%	1.20



cffDNA inside

General Sensitivity e specificity

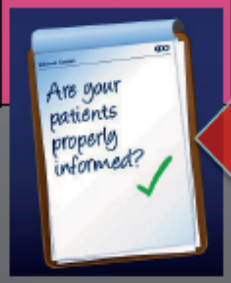
$S = \frac{V_+}{TM_+} = \frac{V_+}{(V_+ + F_-)}$	$S_p = \frac{V_-}{TS_+} = \frac{V_-}{(V_- + F_+)}$	Trisomy 21	Trisomy 18	Trisomy 13	Whole genome* (11932 cases)
VILLI CORIALI trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%	
52000 cases (Grati et al.2014)	False negative Population rate False negative/sensitivity	0.02% 0.74%	0.01% 1.59%	NS 0.74%	
NIPT total 2013-2015	False positive rate/specificity	0.09%	0.13%	0.13%	
(Gill et al.2015)	False negative Population rate False neg. /detection rate	0.08% 0.8%	0.06-0.12% 3.7%	0.18-0.36% 9%	
HARMONY study (Stokowski 2015)	False positive rate/specificity	0.04%	0.02%	0.02%	
23000 cases	False negative False negative Population rate	0.7% 0.03%	2.6% 0.02%	6.2% 0.01	
NEOBONA /Illumina (Cirigliano 2016)	False positive rate/specificity	0.03%	0	0.02%	
6000 cases	False negative/detection rt.	0	0	0	
PRENATALSAFE* (Fiorentino 2016)	False positive rate/specificity	0.02%	0.02%	0.02%	0.01%
31800 cases	False negative/detection rt False negative population rate	0.39% 0.03%	2.08% 0.03%	0	0
BGI Nifty (Zhang et al, 2015)	False positive rate/specificity	0.05%	0.05%	0.04%	
147000 cases	False negative/detection rt.	0.8%	1.76%	0.1%	

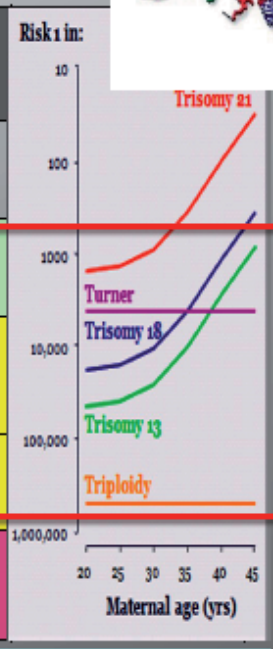
cffDNA inside

Chromosomal Anomalies Prevalence

Sul campo !

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
T21 T18 T13	7335	3,1	70 (48<77)
X-Y trisomies	473	0,2	5
45.X	778	0,33	8
Anomalie cromosomiche rare	1737		17 (40<10)





cffDNA inside

Chromosomal Anomalies Prevalence

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche rare	1737	0,7	17 (40<10)

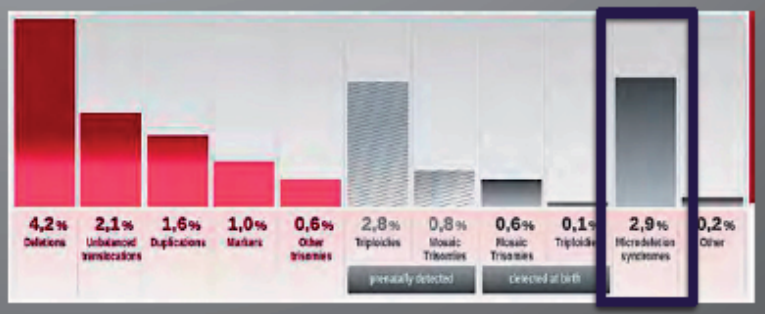
cffDNA inside

Chromosomal Anomalies Prevalence

Microdeletion syndromes

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie strutturali (<10Mb)	1737	0,7	17 (40<10) 3

Sindromi da delezione
Cri du Chat(5p)
1p36
2q33.1
DiGeorge II(10p14)
16p12.2
Jacobsen(11q23)
Van der Woude(1q32.2)
Prader-Willi/Angelman (15q11.2)



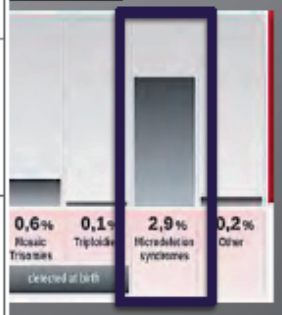
cffDNA inside

Chromosomal Anomalies Prevalence

Microdeletion syndromes

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
crom			<10)
Ano			

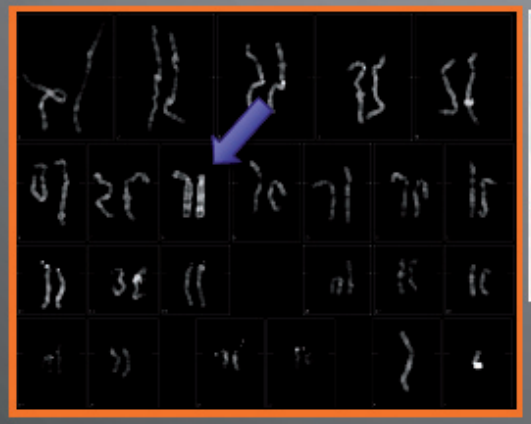
REGIONE INDAGATA	SINDROME ASSOCIATA ALLA MICRODELEZIONE DELLA REGIONE INDAGATA	POPOLAZIONE GENERALE DELLA SINDROME (%AFFETTI CON LA MICRODELEZIONE*)	SINOPSI DELLA SINDROME
22q11.2	Sindrome di Di George-1	1/4.000 (95%)	Anomalie cardiache congenite (tetralogia di Fallot, arco aortico interrotto, difetto del setto ventricolare, tronco arterioso) (74%), anomalie del palato (69%), dimorfismi facciali tipici, difficoltà di apprendimento (70- 90%), disordini autoimmuni, anomalie renali e scheletriche. In epoca prenatale è frequente il riscontro di anomalie cardiache e/o palatoschisi.
10p14	Sindrome di Di George-2		Difetti cardiaci (del setto atriale e ventricolare), ipoparatiroidismo, immunodeficienza da cellule T e caratteristiche peculiari del volto
7q11.2	Sindrome di Williams-Beuren	1/7.500 (95%)	Stenosi aortica sopravalvolare (>80%), ritardo mentale (75%) associato ad un carattere estremamente socievole ed estroverso, ritardo di crescita, invecchiamento precoce, compromissione all'emisfero destro con difficoltà visivo-spaziali e una dissociazione tra gli aspetti pragmatici, fonologici e sintattici del linguaggio.
15q11-q12	Sindrome di Prader-Willi	1/10.000 (70%)	Ip… …talo, scarso … …ma infanzia … …e per una … …o mentale, … …piedi piccoli. … …e una bassa …
	Sindrome di Angelmann	1/15.000 (70%)	… …ti da ritardo … …te, atassia e … …e, lingua … …e); molti … …riso e … …mentazione, ipotonia alla nascita e microcefalia.

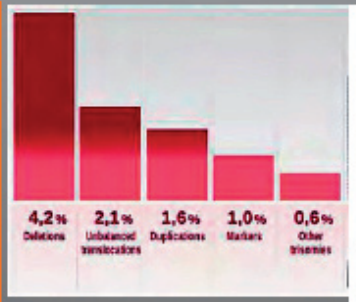


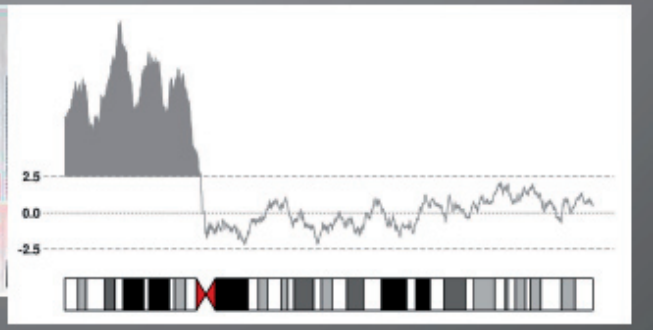
cffDNA inside

Chromosomal Anomalies Prevalence

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie strutturali (>10Mb)	1737	0,7	17 (40<10) 10





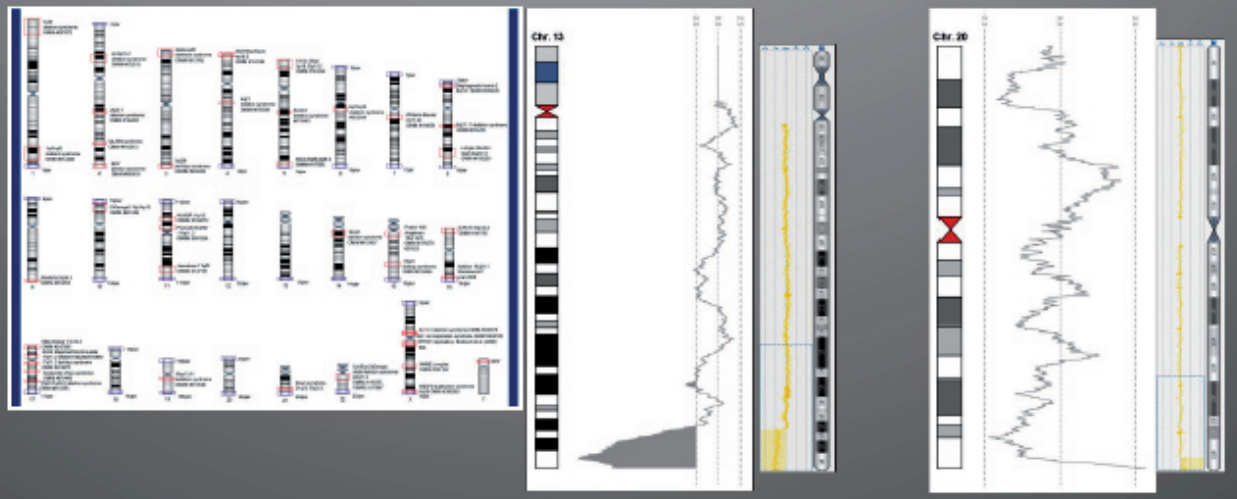


cffDNA inside

Chromosomal Anomalies Prevalence

Whole genome amplification

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie strutturali (>10Mb)	1737	0,7	17 (40<10) 10



cffDNA inside

Chromosomal Anomalies Prevalence

Whole genome amplification

Anomalie Cromosomiche	Popolazione EU ‰ prevalence	Analisi NIPT	Specificità	PPV	Sensibilità	NPV
Anomalie > 10Mb	0,4	tutto genoma	99.3-99.9	66/99	89-100	99,9-100

NIPT DNA WHOLE GENOME ACCURACY

2017 WHOLE GENOME APPROACH 12000 CASES

PrenatalSafe®

Table 3 Performance of the genome-wide cfDNA screening approach

	Trisomy 21 (n = 12 114)	Trisomy 18 (n = 12 114)	Trisomy 13 (n = 12 114)	Sex chromosome aneuploidies (n = 12 114)	Rare aneuploidies (n = 12 114)	Segmental imbalances (n = 12 114)
False positive—no.	1	1	1	12	7	5
False negative—no.	0	0	0	0	0	0
True positive—no.	85	13	12	36	13	8
True negative—no.	12 005	12 008	12 101	12 066	12 097	12 101
Sensitivity (95% CI)—%	100.00% (95.75%–100.00%)	100.00% (75.30%–100.00%)	100.00% (73.54%–100.00%)	100.00% (90.28%–100.00%)	100.00% (74.11%–100.00%)	100.00% (63.06%–100.00%)
Specificity (95% CI)—%	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.94% (99.88%–99.98%)	99.94% (99.88%–99.98%)	99.93% (99.86%–99.97%)
Positive predictive value (95% CI)—%	98.84% (92.54%–99.84%)	92.71% (67.88%–99.07%)	92.31% (63.97%–98.84%)	75.00% (59.67%–86.04%)	65.00% (40.78%–84.61%)	61.54% (31.58%–86.14%)
Negative predictive value (95% CI)—%	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)

2019 WHOLE GENOME APPROACH 20000 CASES

Noninvasive prenatal testing for fetal subchromosomal copy number variations and chromosomal aneuploidy by low-pass whole-genome sequencing

TABLE 4 Evaluation of the NIPSCCD method in detecting CNVs

CNV size	TP	FP	FN	Sensitivity (%)	PPV (%)	FNR (%)
>10 Mb	11	2	1	91.67	84.62	9.35
5 Mb–10 Mb	5	2	0	100.00	71.43	NA
<5 Mb	13	3	6	68.42	81.25	31.58
Total CNVs	29	7	7	80.56	80.56	19.44

Note. TP: true positive NIPSCCD-detected CNVs that were confirmed by amniocyte testing; FP: inconsistent CNVs that were detected by NIPSCCD while not detected by amniocyte testing were classified as false positive; FN: amniocyte testing characterized CNVs that were not detected by the NIPSCCD method; PPV: positive predictive value; FNR, false negative rate; NA, not applicable.

2019 WHOLE GENOME VALIDATION 2000 CASES

illumina®

	Trisomy 21	Trisomy 18	Trisomy 13	RAA[d]	CNVs ≥ 7 Mb	Any anomaly[e]
Sensitivity[a]	> 99.9% (100/100)	> 99.9% (41/41)	> 99.9% (29/29)	99.4% (2768)	74.1% (20/27)	95.5% (318/333)
2-sided 95% CI[b]	97.1%, 100%	91.4%, 100%	87.1%, 100%	82.2%, 99.4%	53.2%, 89.8%	92.7%, 97.2%
Specificity	99.96% (19982/19984)	99.95% (19995/19997)	99.95% (20015/20017)	99.89% (20015/20032)	99.89% (20015/20032)	99.94% (19947/19967)
2-sided 95% CI[b]	99.93%, 99.97%	99.94%, 99.97%	99.94%, 99.97%	99.48%, 99.92%	99.48%, 99.92%	99.87%, 99.91%

a. Seven twin pregnancies reported correctly as T21 not shown in table

b. Basic screen performance is reported for T21, T18, and T13 and excludes 16 samples with known mosaics and an additional 49 samples affected with anomalies for the genome-wide screen only; genome-wide screen performance is reported for RAAs and CNVs

c. CI based on Wilson's score method

d. RAA excludes chromosomes 21, 18, and 13

e. Any anomaly includes samples from SCA/basic and genome-wide screens

PPV	Sen
61	100
71	100
83	74

cffDNA inside

Chromosomal Anomalies Prevalence

Specificità e Sensibilità, pooled

Casi di Anomalie Cromosomiche	‰ prevalence EU	% Anomalie Cromosomiche	CVS Cytotrophoblast (direct method)	CVS Cytotrophoblast (direct method)	cffDNA	cffDNA
Totale	4,4		Specificità%	Sensibilità%	Specificità%	Sensibilità%
T21 T18 T13	3,1	70 (48<77)	99,92 99,94 99,80	99,5 98,4 98,4	99,93 99,97 99,75	99,5 98,4 98,4
X/Y Trisomies	0,2	5	99,99	99,00	99,98	100
45,X	0,33	8	99,73	99,10	99,85	100
Anomalie rare	0,7	17 (40<10)				
Anomalie rare <10Mb		10			99,94	74/100

cffDNA inside

Chromosomal Anomalies Prevalence

Valori Predittivi, pooled

Casi di Anomalie Cromosomiche	‰ prevalence EU	Anomalie Cromosomiche %	CVS Cytotrophoblast (direct method)	CVS Cytotrophoblast (direct method)	cffDNA	cffDNA
Totale	4,4		PPV%	NPV%	PPV%	NPV%
T21 T18 T13	3,1	70 (48<77)	96 92 62	99,98 99,99 99,99	97,7-92,2 88,7-76,6 82,0-32,8	99,99 99,99 100
X/Y Trisomies	0,2	5	85	99,00	73,40	100
45,X	0,33	8	43	99,10	61,60	100
Anomalie rare		17 (40<10)				
Anomalie rare <10Mb		10			61,50	100

cffDNA inside

Chromosomal Anomalies Prevalence

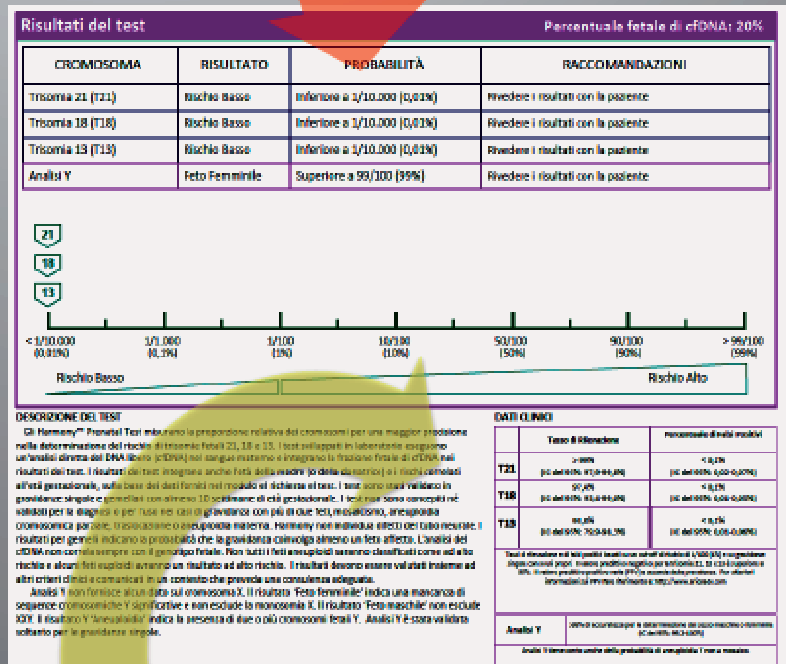
T21	VERI POSITIVI/ TUTTI POSITIVI	VERI NEGATIVI/ TUTTI NEGATIVi	NUMERO CASI	Prevalenza coorte Prev.Italia 15/10000
HARMONY	418/428	22724/22727	23155	*182/10000*
NEOBONA	94/96	5760/5760	5856	*161/10000*
PRENSAFE	257/263	31536/31537	31800	*80/10000*
PS KARYO	87/88	11843/11843	11932	*80/10000*

T18	VERI POSITIVI/ TUTTI POSITIVI	VERI NEGATIVI/ TUTTI NEGATIVi	NUMERO CASI
NEOBONA	17/17	5839/5839	5856
PRENSAFE	47/53	31745/31746	31800
PS KARYO	15/16	11916/11916	11932
HARMONY	147/152	2243/22247	22399
T13	**VERI POSITIVI/ TUTTI POSITIVI**	**VERI NEGATIVI/ TUTTI NEGATIVi**	**NUMERO CASI**
NEOBONA	12/13	5843/5843	5856
PRENSAFE	32/39	31761/31761	31800
PS KARYO	12/13	11919/11919	11932
HARMONY	30/33	14208/14210	14243

cffDNA inside

Incorrect prediction of aneuploidy in cffDNA has two origins:
Non-disjunction post fetus-placental divergence: clinical sensitivity and specificity.

Sample characteristics influence NGS and processing: sensitivity and specificity of the test



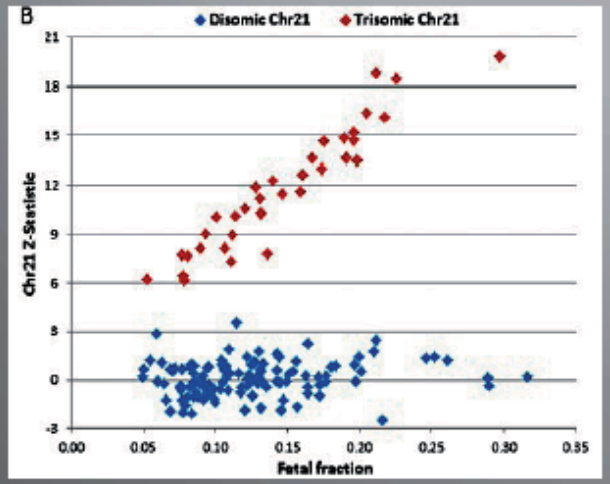
Risultati del test — Percentuale fetale di cfDNA: 20%

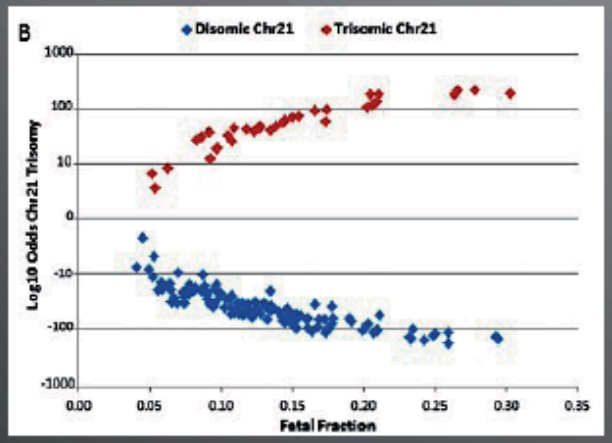
CROMOSOMA	RISULTATO	PROBABILITÀ	RACCOMANDAZIONI
Trisomia 21 (T21)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Trisomia 18 (T18)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Trisomia 13 (T13)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Analisi Y	Feto Femminile	Superiore a 99/100 (99%)	Rivedere i risultati con la paziente

cffDNA inside

Normalizzazione dei sequence counts (osservati/attesi)

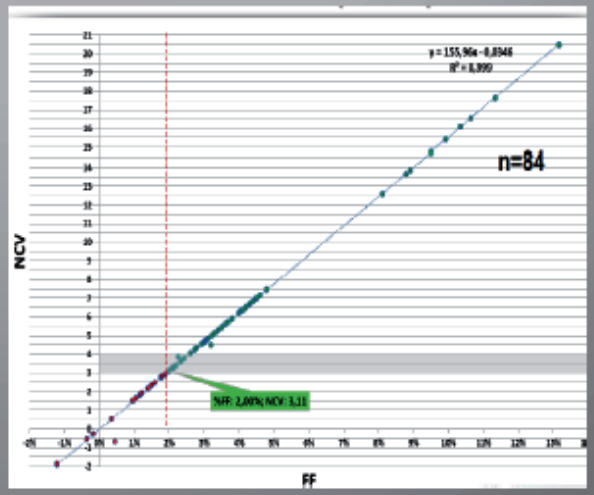
Normalizzazione Cromosomi: Z statistic standadization



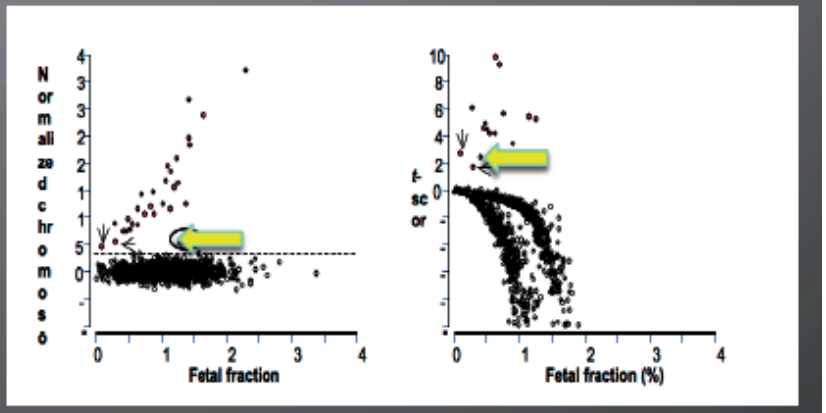


Ariosa FORTE

Normalizzazione Cromosomi: CNV by SAFeR



Illumina SAFeR



Illumina T-Score (NeoBona)

cffDNA inside

Sample characteristics: low FF and aneuploidy

Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10
40%	1.20

3%

NEXT	TOTALE	<4% FF INDETERMINATED DNA	>4% FF	PREV
PAZIENTI	16329	488	15841	
T21	51(3.2%)	13 (2.7%)	38 (0.4%)	31
FOLLOW UP		CONFIRMED	CONFIRMED	

GENOMA	TOTALE	<2% FF	>2<4% FF	>4% FF	PREV
PAZIENTI	3628	79	231	3318	
TRISOMIE	52 (1.4%)	0	16 (6.9%)	36 (1.1%)	143
T21	41	0	12	29	
FOLLOW UP		RECALL 0	CONFIRMED	CONFIRMED	

The frequency of aneuploidy is higher in cohorts of samples with cffDNA < 4% (Harmony) and >2<4% (Illumina, Genome-algorithm)

cffDNA inside

Sample characteristics: CNV interference and algorithm improvement

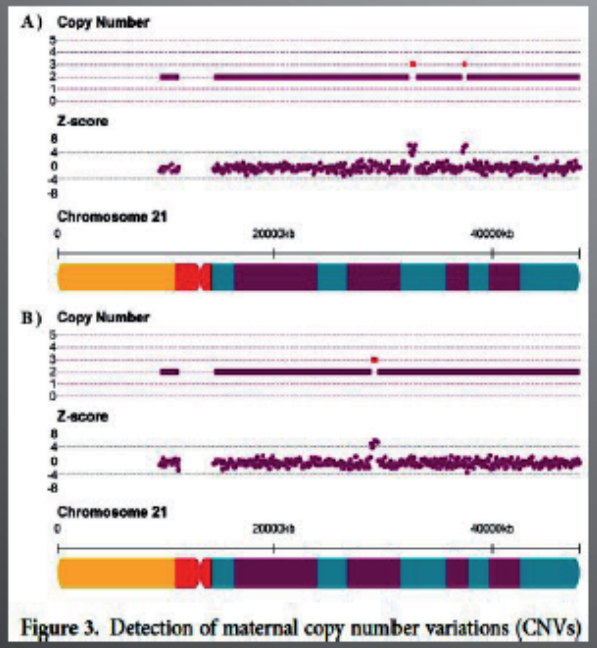


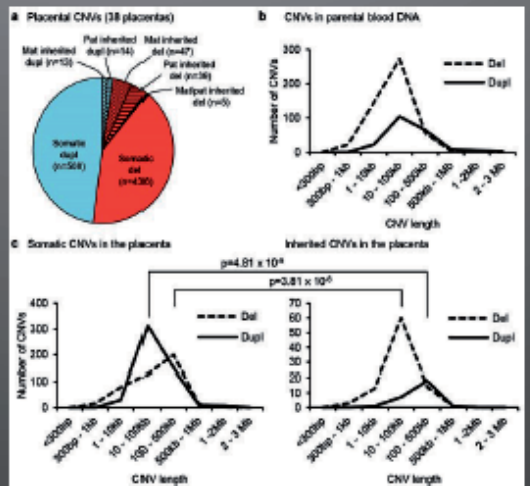
Figure 3. Detection of maternal copy number variations (CNVs)

MATERNAL

PLACENTA – cffDNA

Table 1. Algorithm Improvements and Reductions in False Positive Results.

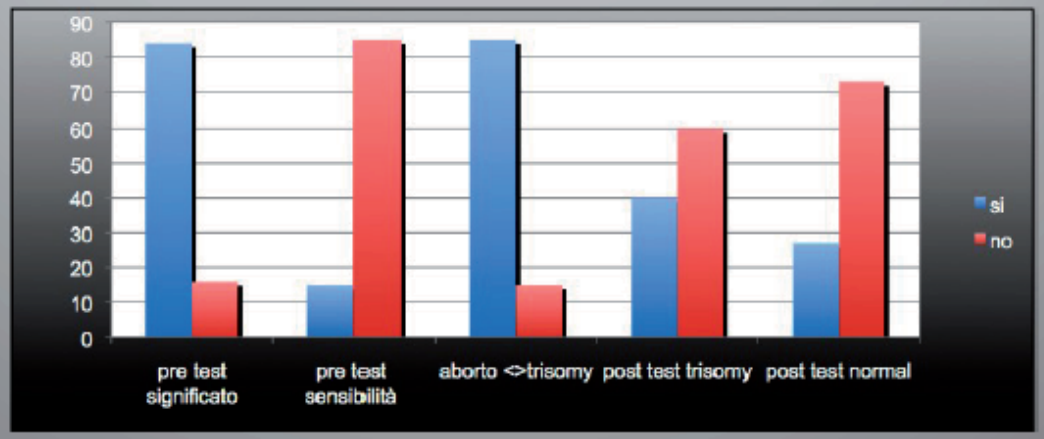
Sample No.	Original Result (as Reported in CARE Study)*	New Result (Current Algorithm)	Explanation
1†	Trisomy 13	Trisomy 13, female infant	Complete chromosome 13 gain confirmed on reanalysis
2†	Trisomy 13	Euploid	Maternal copy-number variant of approximately 5 Mb on chromosome 13q
3†	Trisomy 13	Euploid	Maternal copy-number variant of approximately 8 Mb on chromosome 13q
4†	Trisomy 18	Trisomy 18, male infant	Complete chromosome 18 gain confirmed on reanalysis
			Difference between fetal fraction determined by chromosomes X and 18 suggests chromosome 18 mosaicism‡§
5¶	Trisomy 18	Euploid	Maternal copy-number variant of approximately 2 to 3 Mb on chromosome 18p
6¶	Trisomy 21	Euploid	Reduced coverage variability given additional normalization steps in the current algorithm
7†	Trisomy 21	Euploid	Reduced coverage variability given additional normalization steps in current algorithm
8†	Trisomy 21	Trisomy 21, male infant	Complete chromosome 21 gain confirmed on reanalysis
			Difference between fetal fraction determined by chromosomes X and 21 suggests chromosome 21 mosaicism§
9¶	Trisomy 21	Trisomy 21, male infant	Complete chromosome 21 gain confirmed on reanalysis
			Difference between fetal fraction determined by chromosomes X and 21 suggests chromosome 21 mosaicism‡§
10¶	Trisomy 21	Trisomy 21, female infant	Complete chromosome 21 gain confirmed on reanalysis‡
11¶	Trisomy 21 and 18	Euploid	Reduced coverage variability given additional normalization steps in current algorithm



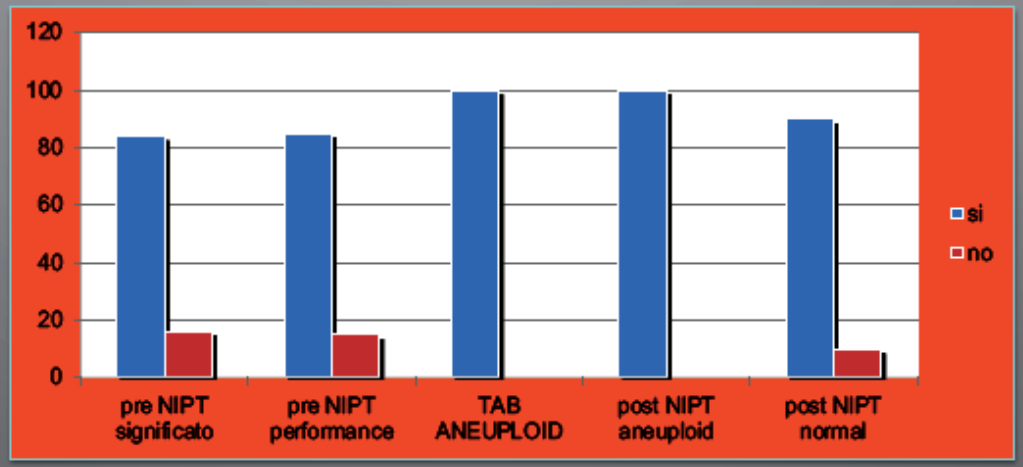
cffDNA inside

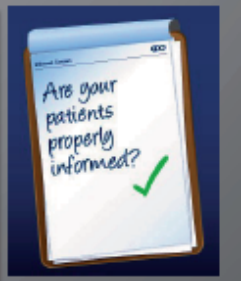
Pazient informations, counselling

Test combined



NIPT cffDNA





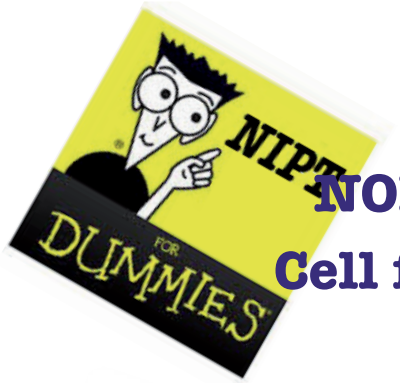
cffDNA inside

Pazient informations, counselling

Casi di Anomalie Cromosomiche	‰ prevalence EU	%	CVS Cytotrophoblast (direct method)		cffDNA	
Totale	4,4		accuracy		accuracy	
T21 T18 T13	3,1	70	99.91 99.93 99.84		99.96 99.97 99.82	
X-Y Trisomies	0,2	5	99.94		99.81	
45.X	0,33	8	99.61		99.75	
Anomalie rare		17				
Anomalie rare >10Mb		10		 	99.8	
Anomalie rare <10Mb		3				

NIPT FOR DUMMIES

The cffDNA NIPT is presented in a synthetic view spanning from the embryology, the various NGS methods who obtained the first validations, the guidelines 2015. It follows the refinement of the methods and accuracy and the entry into the field of whole genome analysis, 2020



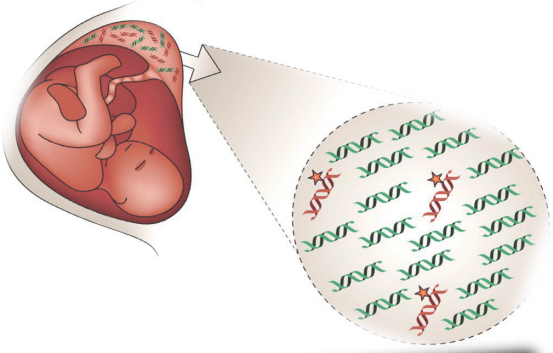
NIPT

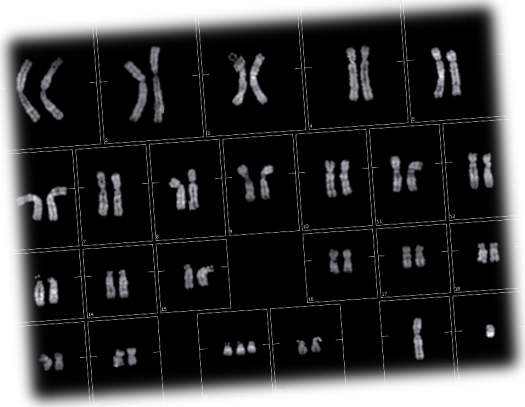
NON INVASIVE PRENATAL TEST

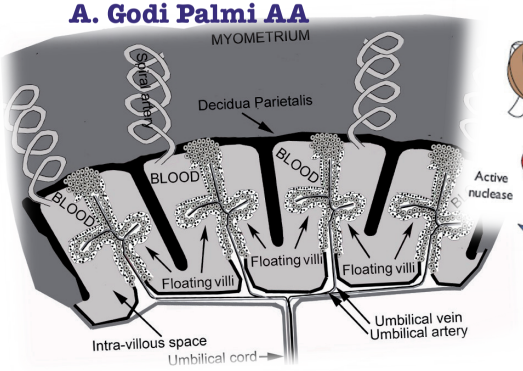
Cell free fetal DNA in Maternal Blood

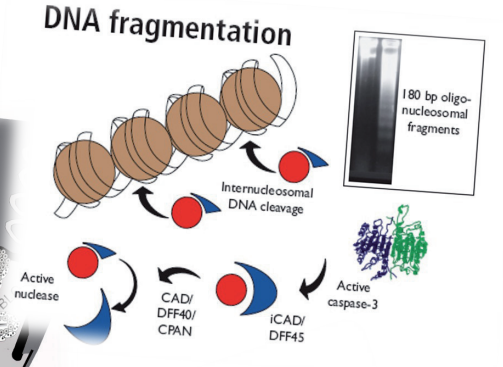
2023

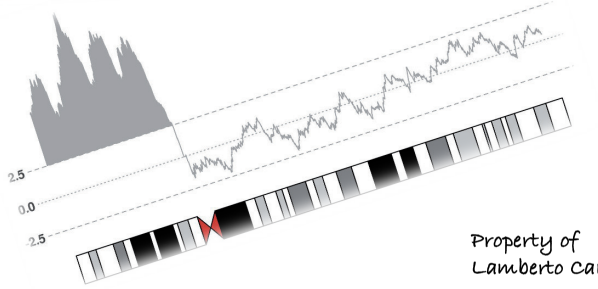
L.Camurri PhD PM
F.Camurri BS PA
G.Camurri BS
A. Godi Palmi AA



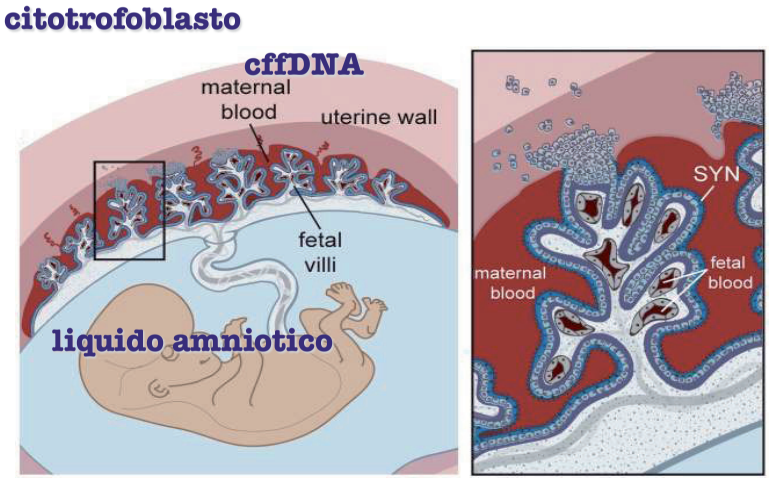






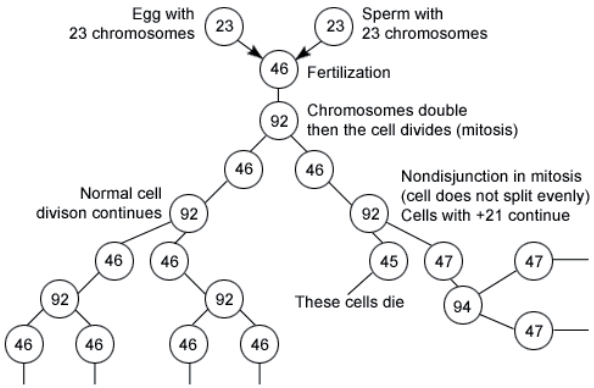


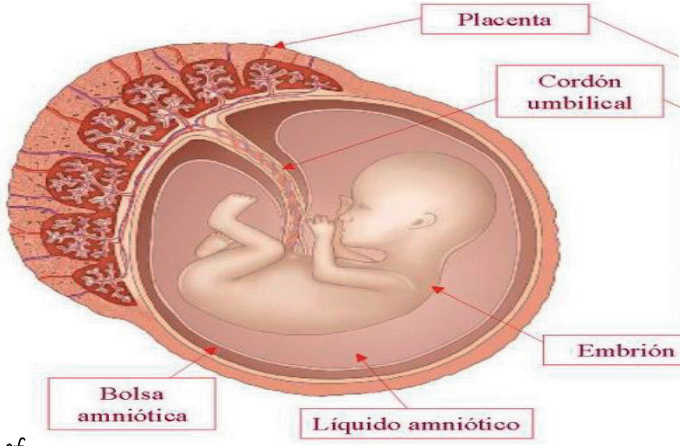
Feto-placental genetic tests use the DNA of the fertilization product: placenta if chorionic villi or free DNA in maternal blood (cytotrophoblast), fetus if amniotic fluid (skin, renal epithelium, amnion). Their aim is to intercept as many causes of fetal congenital malformations as possible

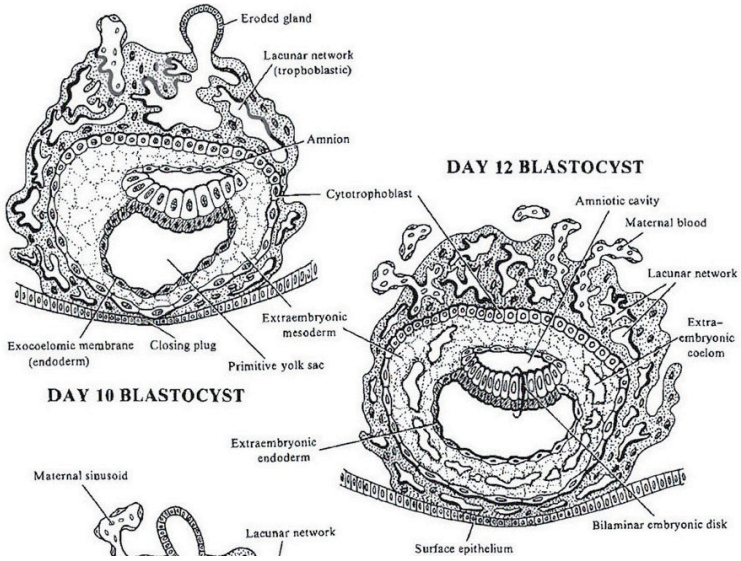


Numero casi (nati + IVG)	6849333	207225
Prevalenza anomalie congenite	2,37 % (1:42)	2,27 % (1:44)
Prevalenza anomalie cromosomiche	0,35 % (1:270)	0,32 % (1:312)

The chromosomal anomalies that arise at fertilization affect both the placenta (chorionic villi, free DNA in the maternal blood) and the fetus (amniotic fluid) and have the same fate. Chromosomal anomalies that arise more than 10 days after fertilization when the placenta and fetus separate affect only one of the tissues and give rise to feto-placental discordance (<0.1%)



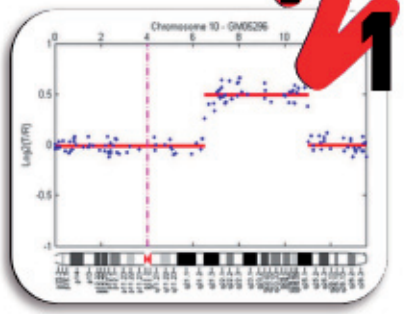


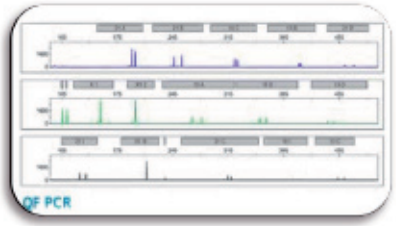


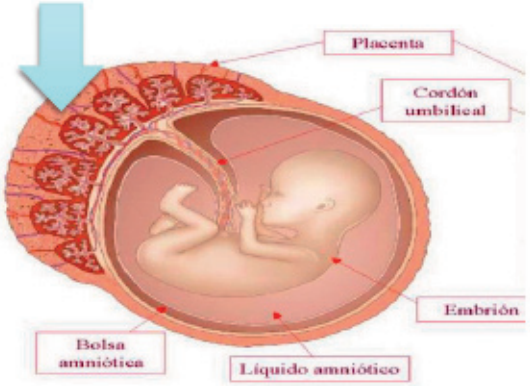
Anomalie Cromosomiche	‰ prevalence EU	CVS Cyto trophoblast direct method	ccfDNA
Totale	4,4	accuracy	accuracy
T21 T18 T13	3,1	99.91 99.93 99.84	99.96 99.97 99.82

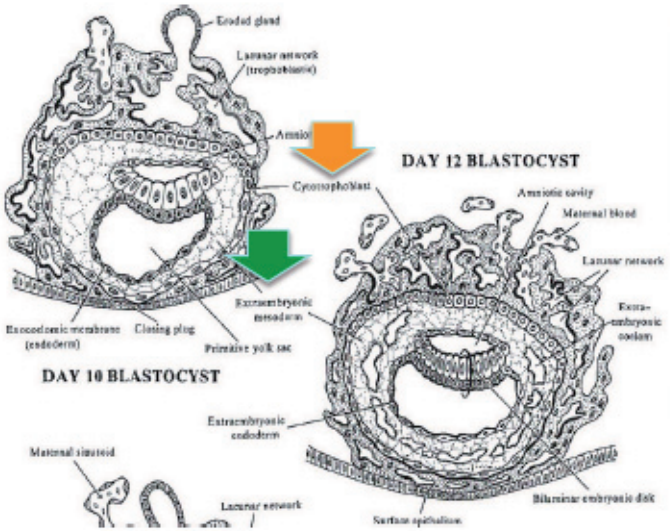
La **accuracy** di un test è: (veri positivi + veri negativi) / tutto il campione

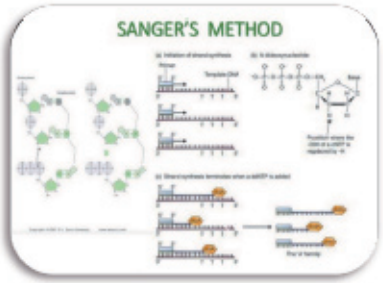
Feto-placental diagnostic genetic tests in the first trimester of pregnancy: cytogenetic and molecular techniques with chorionic villus sampling

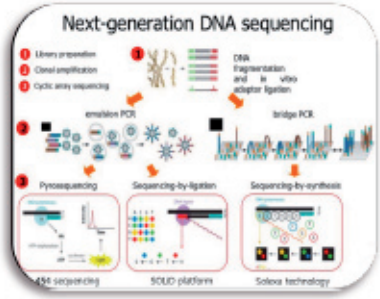


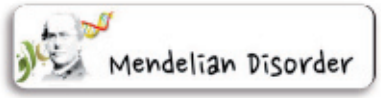




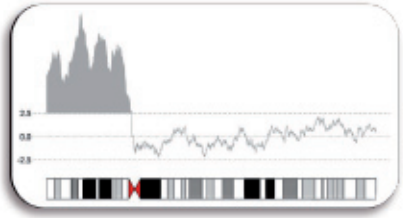




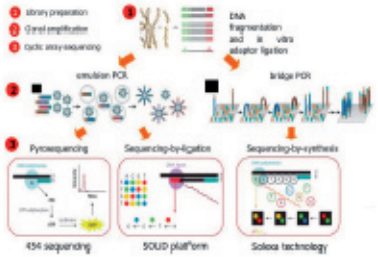


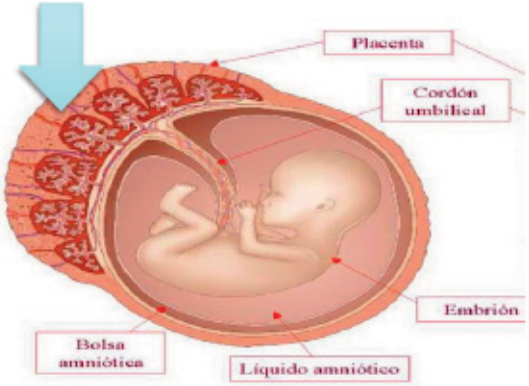


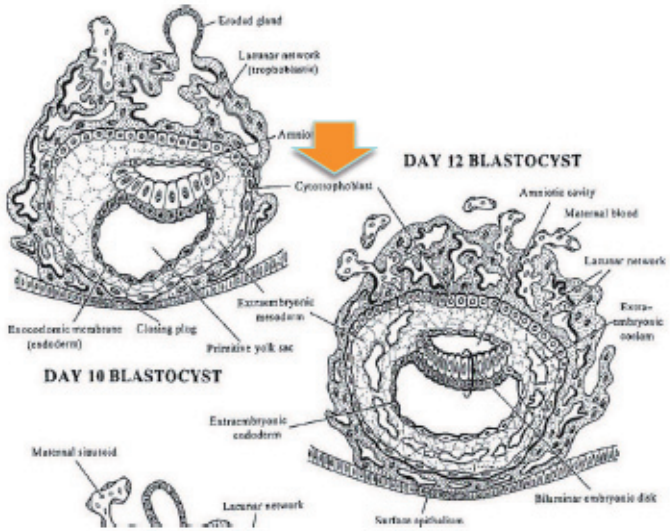
Non-diagnostic feto-placental genetic tests in the first trimester of pregnancy. Free fetal DNA in maternal plasma with maternal blood sampling.



Next-generation DNA sequencing

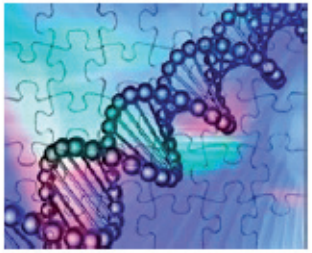






Perché non diagnostici?

1. La apoptosi delle cellule del trofoblasto nel plasma frammenta il DNA che necessita un rimodellamento con NGS.



2. La analisi è condotta su un mix di DNA materno e fetale con possibili effetti di confondimento

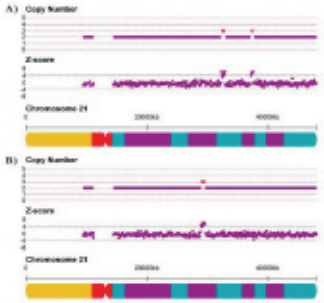
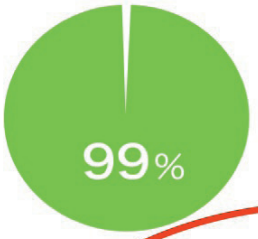


Figure 3. Detection of maternal copy number variations (CNVs)

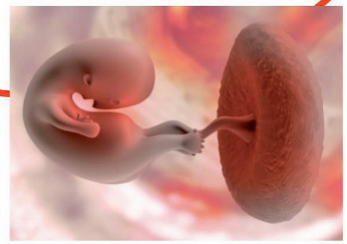


Why?

Fecondazione e errori Cromosomici:

Falsi positivi > anomalia che non c'è
Falsi negativi > tutto bene ma non è vero

Anomalie Cromosomiche	‰ prevalence EU	Liquido Amniotico	Placenta DNA Fetale plasma
Totale	4,4	accuracy	accuracy
T21 T18 T13	3,1	100 100 100	99.96 99.97 99.82



ccfDNA NIPTest. The origin. The fetal DNA present in maternal plasma comes from the placenta

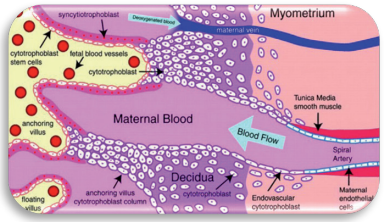
In maternal blood during pregnancy there are nucleated fetal cells and free non-cellular DNA suspended in the plasma.

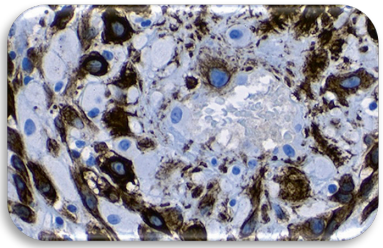
Non-cell free DNA (cffDNA) comes from cells of the placenta. The placental cytotrophoblast anchors to the parietal decidua.

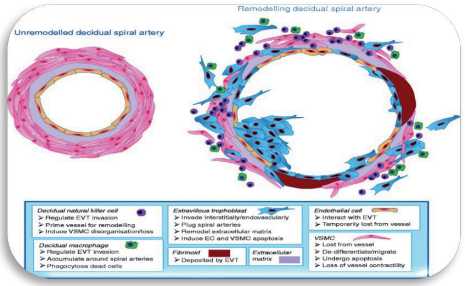
The spiral arteries of the decidua supply the gaps between the decidua and the placenta. The cytotrophoblast invades and lines the walls of the spiral arteries and causes their remodeling.
The turnover of the trophoblast cells that cover the walls of the spiral arteries by cell death or apoptosis (cytokine-mediated) determines the fragmentation of the degenerating DNA.

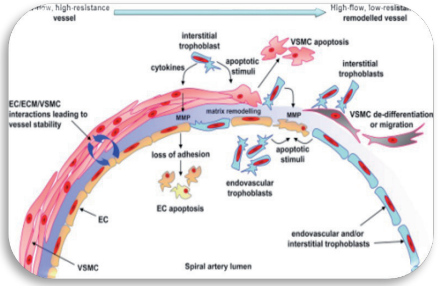
The DNA fragments are approximately 180 bp (base pairs) in size and are suspended in the arterial plasma. The presence of free trophoblast (fetal) DNA in the plasma (cffDNA) is found starting from the 5th week of pregnancy, but the quantity is sufficient for testing starting from the 10th week.

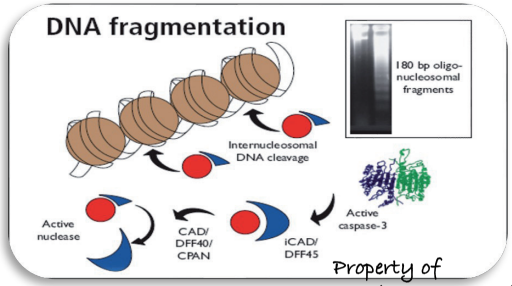
The degeneration of maternal epithelia also releases fragments of DNA suspended in the plasma to generate a mixture of mother and placenta-fetus DNA.











Lamberto Camurri Ph.D.

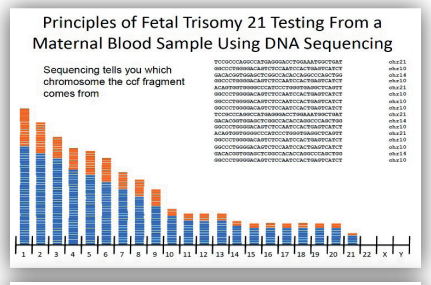
Capturing fetal DNA: sequencing the genome

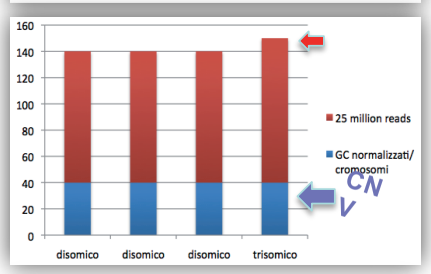
MPSS, massive parallel shotgun sequencing (Verinata Verifi, Sequenom MateriT21, BGI Nifty)
Analyze all chromosomes.

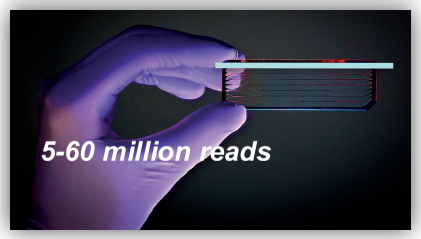
The massive analysis of all chromosomes highlights the differences in GC base density for which it is necessary to introduce a Normalization Factor (CNV) which allows for comparable the sequence reading blocks.

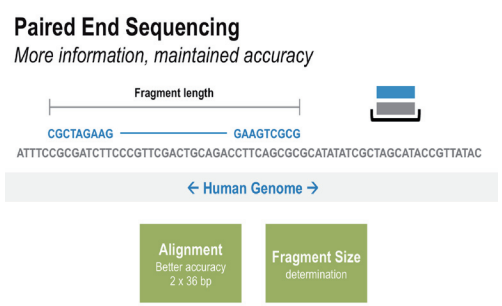
No PCR The massive sequencing technique allows the analysis of the entire genome. New techniques sequence DNA fragments from both sides with more information and accuracy.

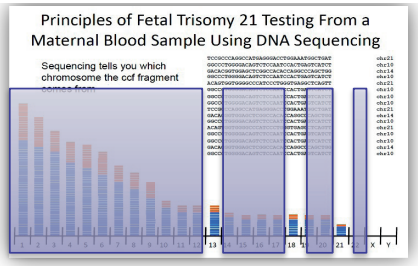
The techniques can be of different precision depending on the coverage of the genome with the reads. 5 million reads for standard techniques, up to 60 million reads for the "whole genome".

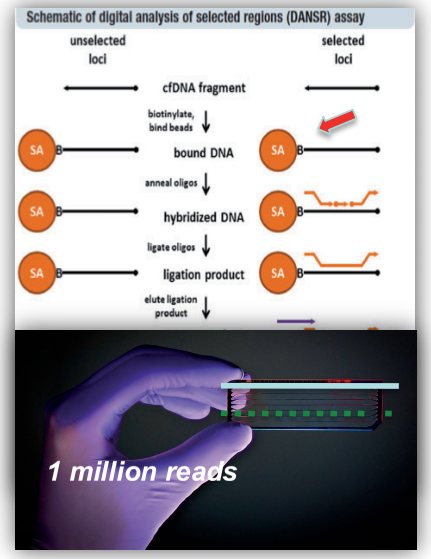


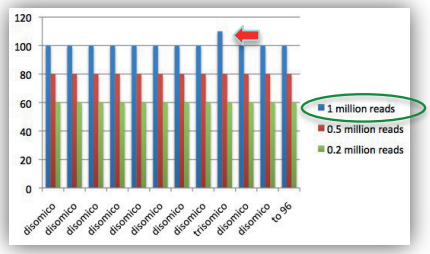










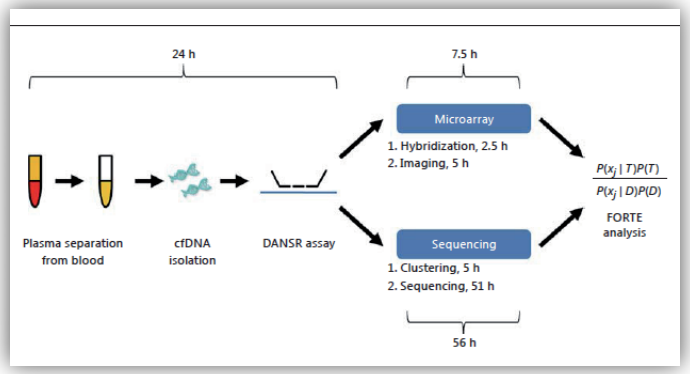


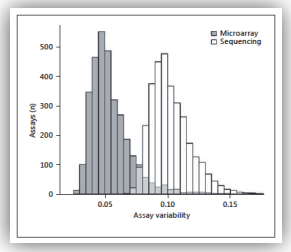
The capture of fetal DNA: sequence only chromosomes 21, 18, 13

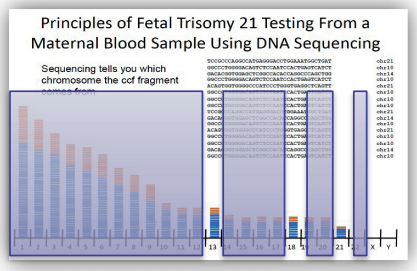
To identify a trisomy 21 (or 13 or 18) you can select the DNA fragments of the chromosomes, eliminating the rest of the genome. DANSR (Ariosa Harmony) The sample is amplified with PCR 1. Sequencing: Performs selective (high multiplexed) sequencing of the DNA fragments of chromosomes 21, 18, 13 only (clustering, sequencing). 2. Microarrays: Perform the analysis on an array platform (hybridization, imaging). Reduces variability between samples. The selection of fragments occurs by hybridizing fluorescent probes to: 576 non-polymorphic STR markers of chromosomes (21, 18, 13) for the search for trisomies 192 polymorphic STR markers of chromosomes between 1 and 12 to define the fetal fraction of each sample. Only fragments attached to the fluorescent probes will be sequenced for assay and processing. The technique performs approximately 1 million readings.

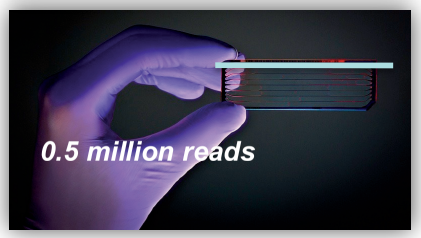
Second generation Techniques

Microarrays (*Ariosa Harmony*)









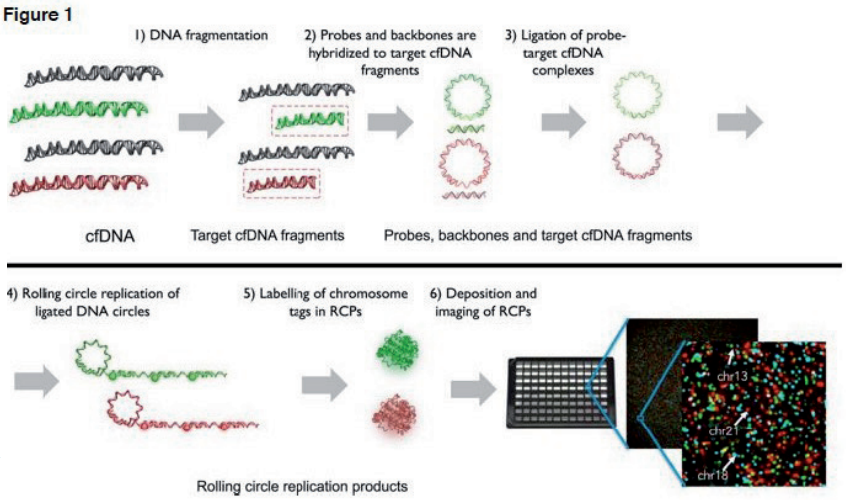
The capture of fetal DNA: intercept only chromosomes 21, 18, 13

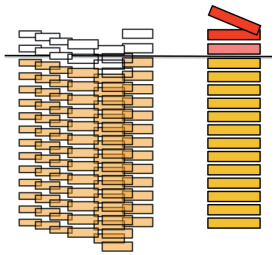
Enzymatic and chemiluminescent technique

To identify a trisomy 21 (or 13 or 18) you can select the DNA fragments of the chromosomes, eliminating the rest of the genome. Fragment selection occurs without sequencing No initial PCR By digesting the fragments with restriction enzymes, hybridizing fluorescent probes to: 3500 non-polymorphic loci of chromosome 21 for the search for trisomies. Only the fragments attached to the fluorescent probes will be amplified with PCR and used The technique performs approximately 0.5 million readings.

A sequencing and PCR-free method was developed to enable a cost efficient and high precision measurement of chromosomal aneuploidies. Thereby, we were able to eliminate expensive sequencing, complicated sample preparation protocols, PCR bias and bioinformatics. The maximum precision of digitally-enabled assays is dictated by the number of molecules analysed. To enable quantification based on high molecular count numbers without PCR, two strategies were used. Firstly, probes were designed to capture and generate labelled rolling circle replication products (RCPs) from ~3500 loci on chromosome 21, thereby increasing the number of counts per chromosome equivalent in the sample. Secondly, an optically transparent nanofilter 96-well plate was developed to capture RCPs with high yield by mechanical filtering prior to imaging, thereby increasing the number of molecules analysed from the sample.

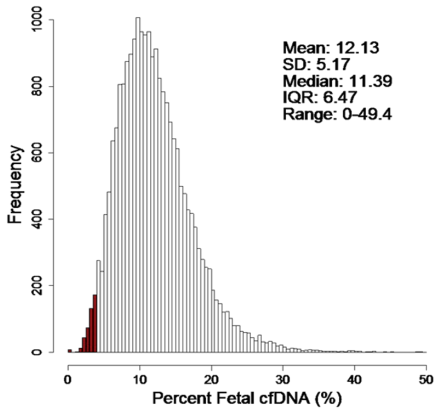
The Vanadis NIPT assay (Fig. 1) is based on four consecutive enzymatic steps that specifically generate labelled RCPs from chromosomal DNA targets. The specificity of the DNA labelling approach eliminates the need for DNA sequencing and advanced bioinformatics data analysis. First, target chromosomes are digested into defined target cfDNA fragments using a restriction enzyme. Secondly, the digested target cfDNA fragments are mixed with a probe set where each probe carries a complementary sequence motif to the target cfDNA fragments of interest. The mixture also contains backbone oligonucleotides carrying a chromosome-specific sequence motif ("chromosomal tag") used for subsequent labelling and identification. The probes are designed to specifically guide hybridization of target cfDNA fragments, thereby allowing subsequent DNA ligation of the target cfDNA fragments to the backbone oligo, such that a single stranded DNA circle can be formed. For this to occur, the selected cfDNA fragments need to hybridize perfectly to the probe and ligate both the 3´and 5´ends to the backbone. The chromosomal tags within the backbones





The quantity of fetal DNA: the test must indicate the fetal fraction of the sample

The fetal fraction of each chromosome to identify a trisomy must allow an expected ratio >1. Harmony test is based on SNP polymorphisms and indicates 4%. Other methods are based on DNA fragment size, CNV, Y chromosome, and indicate 2% as the operating limit.



Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10

SNP
(DANSR)

ABRSJA5517	Maternal (buffy coat)	A/C	G/G	C/T	A/T	A/A	A/G	C/T	C/C	A/C	A/G
	Fetal (cfDNA)					A/G			C/C		

Fragment size, #X CNV & #Y (Tscore e altri)

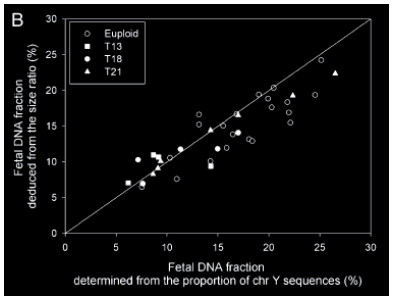
Normal
Pregnancies
.4% fail

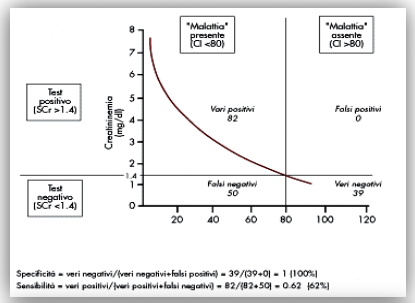
Fragment size distribution

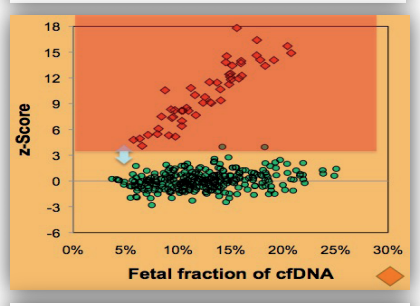
Coverage profile

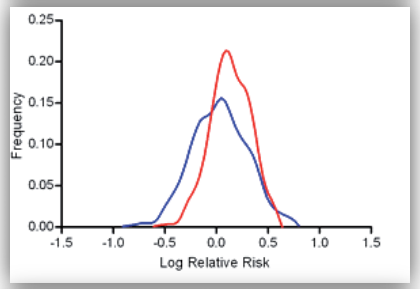
Copy number changes on X

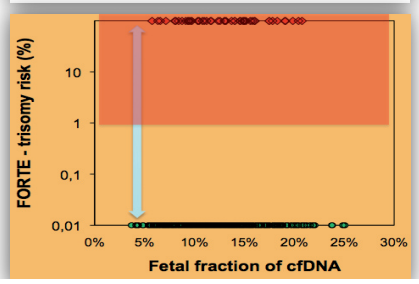
Presence of Y









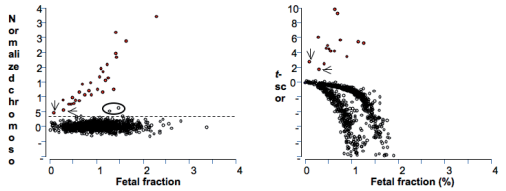


Data Analysis

The test calculates the likelihood ratio between the probabilities that the samples contained in a sequence line are euploid

MPSS (Verinata Verifi SAFER, Sequenom MaterniT21, BGI Nifty) – 2012 Algorithm to define the trisomy risk threshold value based on: One sample set The calculation on a single sample is made necessary by the small number of tests per sequence line. positive-negative binary hypothesis with t-Student (z-score) and Likelyhood Odds Ratio (likelihood ratio). 2) CNV sequence normalization factor. 3) Run variations between the various sequencing lines corrected with a z-score algorithm. 4) Definition of a threshold value for trisomy (z-score value between 3 and 4) The algorithm does not consider the fetal fraction. Therefore, with low fetal fraction, the score +/- values are very close, increasing the possibility of false positives and negatives. DANSR – FORTE (Ariosa Harmony) – 2012 Algorithm to define the trisomy risk threshold value based on: Multiple sample set The calculation on multiple samples is made possible by the small size of the sequence reading blocks (1 million reads) which allows the analysis in a sequence line of 96 samples which are compared with each other. 1) Percentage hypothesis with Odds ratio (likelihood ratio) between disomic/trisomic models, (normal distribution curves) 2) Calculation of the fetal fraction 3) Montecarlo Simulation which also includes maternal age and gestational age in the calculation of the FORTE algorithm. The algorithm considers the fetal fraction. Therefore the score values with low fetal fraction are normalized allowing a risk assessment independent of the quantity of fetal DNA.

T-SCORE (Labco Neobona/Illumina) – 2015 Compound z-score like algorithm Fetal fraction calculation
Fragment distribution by size Batch-sample comparison / sequence depth LIFE CIRCLE (Perkin Elmer)-2018



Identification of Fetal Trisomies with cffDNA: validation

Company	Sequenom	Verinata	Ariosa	Natera	BGI
NASDAQ	SQNM	ILMN			
Prodotto	MaterniT21plus	Verifi	Harmony	Panorama	Nifty
sequenziamento	MPSS	MPSS	TPS	TPS-SNPbased	MPSS
tecnologia	SEQureDx		DANSR	NATUS	
algoritmo		SAFeR	FORTE		
statistic	z-score binary	z-score binary	OR multiple	z-score binary	z-score GC correct
weeks	10	10	10	10	12
condizioni	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX
accreditamento	CAP CLIA	CAP CLIA	CAP CLIA	CAP CLIA	
statements	ACMG ACOG ISPD	ACMG ACOG ISPD	ACMG ACOG ISPD	ACMG ACOG ISPD	
clinic-patients	2500	650	6000	300	3500/11100
failure			2%	5%	<1%
specificity 21	99,4-99,9	99,1-100	99,8-99,9	98,2-100	99,60-99,97
sensibility 21	95,9-99,7	95,9-100	95,5-100	86,3-100	100-100

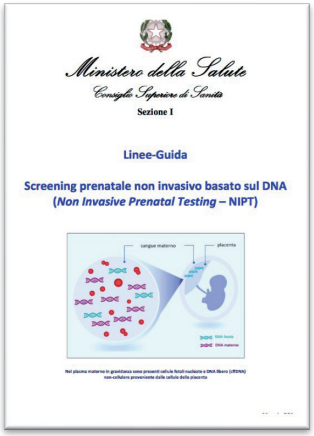
Table 1 Results from four published clinical trials that measured NIPT's sensitivity and specificity in detecting common aneuploidies

	Trisomy 21		Trisomy 18		Trisomy 13	
	Sensitivity (95 % CI)	Specificity (95 % CI)	Sensitivity (95 % CI)	Specificity (95 % CI)	Sensitivity (95 % CI)	Specificity (95 % CI)
Palomaki et al. 2011	98.6 % (95.9 - 99.7)	99.8 % (99.4 - 99.9)	———	———	———	———
Palomaki et al. 2012	———	———	100 % (93.9 -100)	99.7 % (99.3 - 99.9)	91.7 % (61-99)	99.1 % (98.5 - 99.5)
Bianchi et al. 2012	100 % (95.9 – 100)	100 % (99.1 – 100)	97.2 % (85.5 – 99.9)	100 % (99.2 – 100)	78.6 % (49.2 – 99.9)	100 % (99.2 – 100)
Norton et al. 2012	100 % (95.5-100)	99.97 % (99.8 -99.99)	97.4 % (86.5-99.9)	99.93 % (99.75 - 99.98)	———	———
Ashoor et al. 2012	———	———	———	———	80 % (49-94.3)	99.95 % (99.71-99.99)

2012. The international agreements are configured on the tests of the companies indicated and on the scientific production linked to the clinical trials that have demonstrated the sensitivity and specificity data of NIPT.

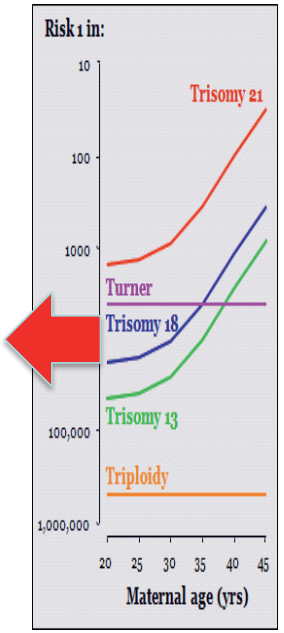
2015. The company Illumina, manufacturer of NGS sequencers, and owner of Verinata, agrees with Sequenom for "sharing revenues". Roche acquires Ariosa and Illumina launches second generation NGS test (Paired end sequencing) with LabcoEU (NeoBona)

2015. Test validation guidelines are drawn up on this data.


Ministero della Salute
Consiglio Superiore di Sanità
Sezione I
Linee-Guida
Screening prenatale non invasivo basato sul DNA
(*Non Invasive Prenatal Testing* – NIPT)

Identification of Fetal Trisomies with cffDNA: European population prevalence

Casi di Anomalie Cromosomiche		Popolazione EU ‰	% Anomalie Cromosomiche
Totale	10323	4,4	100
Trisomie T21 T18 T13	7335	3,1	70 (48<77)
X0,XXX,XXY,XYY	1251	0,6	13



European Journal of Human Genetics (2012) 20, 521–526

www.nature.com/ejhg

ARTICLE

Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe

Identification of Fetal Trisomies with cffDNA: Published case series and performances: comparison with cytotrophoblast from chorionic villi. The methods do not differ in clinical specificity and sensitivity, determined by the biology of the placenta and its trophoblast.

Casi di Anomalie Cromosomiche	‰ prevalence EU	% Anomalie Cromosomiche	CVS Cytotrophoblast (direct method)	CVS Cytotrophoblast (direct method)	cffDNA	cffDNA
Totale	4,4		Specificità%	Sensibilità%	Specificità%	Sensibilità%
T21 T18 T13	3,1	70 (48<77)	99,9 99,9 99,8	99,5 98,4 98,4	99,9 99,9 99,7	99,5 98,4 98,4
X/Y Trisomies	0,2	5	99,9	99,0	99,9	100
45,X	0,33	8	99,7	99,1	99,8	100

Totale	4,4		PPV%	NPV%	PPV%	NPV%
T21 T18 T13	3,1	70 (48<77)	96 92 62	99,98 99,99 99,99	97,7-92,2 88,7-76,6 82,0-32,8	99,99 99,99 100
X/Y Trisomies	0,2	5	85	99,00	73,40	100
45,X	0,33	8	43	99,10	61,60	100

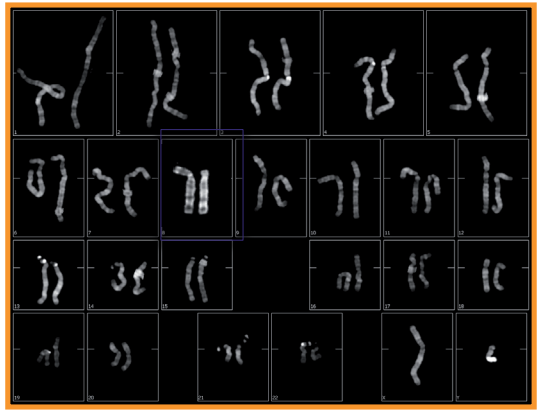
La **specificità** di un test è: N° FP/N° TN (falsi positivi/veri negativi)

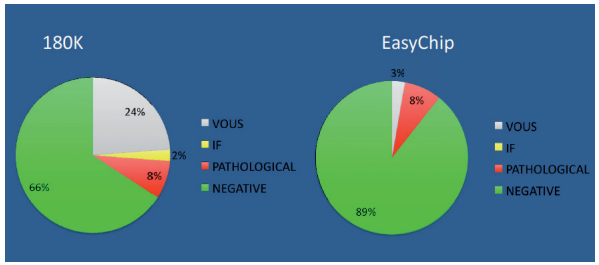
La **sensibilità** (detection) di un test è: N° FN/N° TP (falsi negativi/veri positivi)

Identification of Fetal Chromosomal Anomalies with cffDNA: European population prevalence. Genome analysis:

Conventional karyotyping identifies structural anomalies larger than 10-15 Mb. An aCGH or NGS molecular analysis calibrated to >10Mb identifies genomic structural anomalies, limiting findings of dubious significance to a minimum. A molecular genomic analysis at >10Mb is able to identify chromosomal anomalies (CNVs, deletions, duplications) equal to the conventional karyotype

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche strutt.	1737	0,7	17 (40<10)
Anomalie > 10Mb		tutto genoma	10





Identification of Fetal Chromosomal Anomalies with cffDNA: European population prevalence. Genome analysis: Illumina validates and markets the Veriseq2 methodology, 2019

NIPT DNA WHOLE GENOME ACCURACY

2017 WHOLE GENOME APPROACH 12000 CASES

PrenatalSafe®

Prenatal Diagnosis 2017

Table 3 Performance of the genome-wide cfDNA screening approach

	Trisomy 21 (*n* = 12 114)	Trisomy 18 (*n* = 12 114)	Trisomy 13 (*n* = 12 114)	Sex chromosome aneuploidies (*n* = 12 114)	Rare aneuploidies (*n* = 12 114)	Segmental imbalances (*n* = 12 114)
False positive—no.	1	1	1	12	7	5
False negative—no.	0	0	0	0	0	0
True positive—no.	88	15	12	36	10	8
True negative—no.	12.025	12.098	12.101	12.066	12.097	12.101
Sensitivity (95% CI)—%	100.00% (95.89%–100.00%)	100.00% (78.20%–100.00%)	100.00% (73.54%–100.00%)	100.00% (90.26%–100.00%)	100.00% (69.15%–100.00%)	100.00% (63.06%–100.00%)
Specificity (95% CI)—%	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.90% (99.83%–99.95%)	99.94% (99.88%–99.98%)	99.96% (99.90%–99.99%)
Positive predictive value (95% CI)—%	98.88% (92.54% to 99.84%)	93.75% (67.88%–99.07%)	92.31% (62.83%–98.84%)	75.00% (63.02%–84.08%)	58.82% (40.52%–74.97%)	61.54% (39.98%–79.35%)
Negative predictive value (95% CI)—%	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100,00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)

The clinical utility of genomewide cfDNA screening

2019 WHOLE GENOME APPROACH 20000 CASES

Noninvasive prenatal testing for fetal subchromosomal copy number variations and chromosomal aneuploidy by low-pass whole-genome sequencing

Dongyi Yu, Kai Zhang, [...], and Yang Du

TABLE 4 Evaluation of the NIPSCCD method in detecting CNVs

CNV size	TP	FP	FN	Sensitivity (%)	PPV (%)	FNR (%)
>10 Mb	11	2	1	91.67	84.62	9.33
5 Mb–10 Mb	5	2	0	100.00	71.43	NA
<5 Mb	13	3	6	68.42	81.25	31.58
Total CNVs	29	7	7	80.56	80.56	19.44

Note. TP, true positive NIPSCCD-detected CNVs that were confirmed by amniocytes testing; FP, inconsistent CNVs that were detected by NIPSCCD while not detected by amniocytes testing were classified as false positive; FN, amniocytes testing-characterized CNVs that were not detected by the NIPSCCD method; PPV, positive predictive value; FNR, false negative rate; NA, not applicable.

PPV	Sen
61	100
71	100
83	74

2019 WHOLE GENOME VALIDATION 2000 CASES

illumina®

	Trisomy 21	Trisomy 18	Trisomy 13	RAA[d]	CNV ≥ 7 Mb	Any anomaly[e]
Sensitivity[b]	> 99.9% (130/130)	> 99.9% (41/41)	> 99.9% (26/26)	96.4% (27/28)	74.1% (20/27)	95.5% (318/333)
2-sided 95% CI[c]	97.1%, 100%	91.4%, 100%	87.1%, 100%	82.3%, 99.4%	55.3%, 86.8%	92.7%, 97.3%
Specificity	99.90% (1982/1984)	99.90% (1995/1997)	99.90 (2000/2002)	99.80% (2001/2005)	99.80% (2000/2004)	99.34% (1954/1967)
2-sided 95% CI[c]	99.63%, 99.97%	99.64%, 99.97%	99.64%, 99.97%	99.49%, 99.92%	99.49%, 99.92%	98.87%, 99.61%

a. Seven twin pregnancies reported correctly as T21 not shown in table

b. Basic screen performance is reported for T21, T18, and T13 and excludes 16 samples with known mosaics and an additional 49 samples affected with anomalies for the genome-wide screen only; genome-wide screen performance is reported for RAAs and CNVs

c. CI based on Wilson's score method

d. RAA excludes chromosomes 21, 18, and 13

e. Any anomaly includes samples from SCA basic and genome-wide screens

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Casi positivi *n*= 1011 Totale follow-up *n*=868	Trisomia 21	Trisomia 18	Trisomia 13	SCA	Altre anomalie*	Performance complessive (71883 gravidanze)
Veri positivi	437	93	37	156	58	781
Falsi positivi	3	1	8	17	54	83
Veri negativi	70870	70872	70872	70871	70871	71015
Falsi negativi	2	0	0	1	1	4
Sensibilità (95% CI)	**99.54%** (98.36%-99.94%)	**100%** (96.11% - 100.00%)	**100%** (90.51%-100.00%)	**99.36%** (96.50%-99.98%)	**98.31%** (90.91% - 99.96%)	**99.49%** (98.70%-99.86%)
Specificità (95% CI)	**100%** (99.99% - 100.00%)	**100%** (99.99% - 100.00%)	**99.99%** (99.98% - 100.00%)	**99.96%** (99.96%-99.99%)	**99.92%** (99.90% - 99.94%)	**99.88%** (99.86%-99.91%)
PPV (95% CI)	**99.32%** (97.92% - 99.78%)	**98.94%** (92.91% - 99.85%)	**82.22%** (69.82% - 90.24%)	**90.17%** (85.08%-93.66%)	**51.79%** (45.08% - 58.42%)	**90.39%** (88.36%-92.11%)
NPV (95% CI)	**100%** (99.99%-100.00%)	**100%** (99.99%-100.00%)	**100%** (99.99%-100.00%)	**100%** (99.99%-100.00%)	**100%** (99.99% - 100.00%)	**99.99%** (99.99%-100.00%)

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Aneuploidie dei cromosomi sessuali	X0	XXX	XXY	XYY
Veri positivi	52	27	51	26
Falsi positivi	13	0	3	1
Veri negativi	70871	70872	70872	70872
Falsi negativi	1	0	0	0
Sensibilità (95% CI)	98.11% (89.93% -99.95%)	100% (87.23%-100.00%	100% (93.02%-100.00%)	100% (86.77%-100.00%)
Specificità (95% CI)	99.98% (99.97% -99.99%)	100% (99.99%- 100.00%)	99.99% (99.99% -100.00%)	99.99% (99.99% -100.00%)
PPV (95% CI)	80% (69.88% -87.34%)	100% (99.99%-100.00%)	99.44% (84.57%- 98.14%)	96.3% (78.55% -99.46%)
NPV (95% CI)	100% (99.99%-100.00%)	100% (99.99%-100.00%)	100% (99.99%-100.00%)	100% (99.99%-100.00%)

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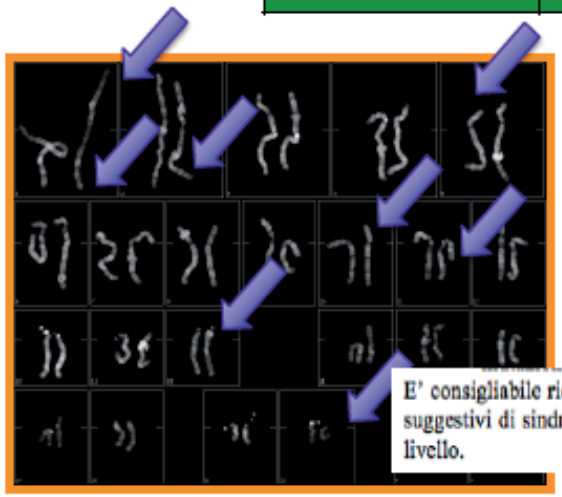
Altre anomalie	RAA	Anomalie segmentali (>7 Mb)	Microdelezioni* (anomalie segmentali <7 Mb)
Veri positivi	33	20	5
Falsi positivi	36	16	2
Veri negativi	70872	70872	70871
Falsi negativi	0	0	1
Sensibilità (95%CI)	**100%** (89.42%-100.00%)	**100%** (83.16%-100.00%)	**83.33%** (35.88% -99.58%)
Specificità (95%CI)	**99.95%** (99.93%- 99.96%)	**99.98%** (99.96%- 99.99%)	**100%** (99.99%- 100.00%)
PPV (95%CI)	**47.83%** (39.81%- 55.96%)	**55.56%** (43.37% - 67.11%)	**71.43%** (37.40% -91.27%)
NPV (95%CI)	**100%** (99.99%- 100.00%)	**100%** (99.99%- 100.00%)	**100%** (99.99%- 100.00%)

Identification of Fetal Chromosomal Anomalies with cffDNA: European population prevalence. Microdeletion analysis:

The cffDNA analysis of microdeletions (<10Mb) searches for a series of rare syndromes with a clinical sensitivity between 50 and 95%. The analysis is recommended as a second level investigation in conjunction with ultrasound findings and genetic counseling.

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche strutt.	1737	0,7	17 (40<10)
Anomalie < 10Mb			3

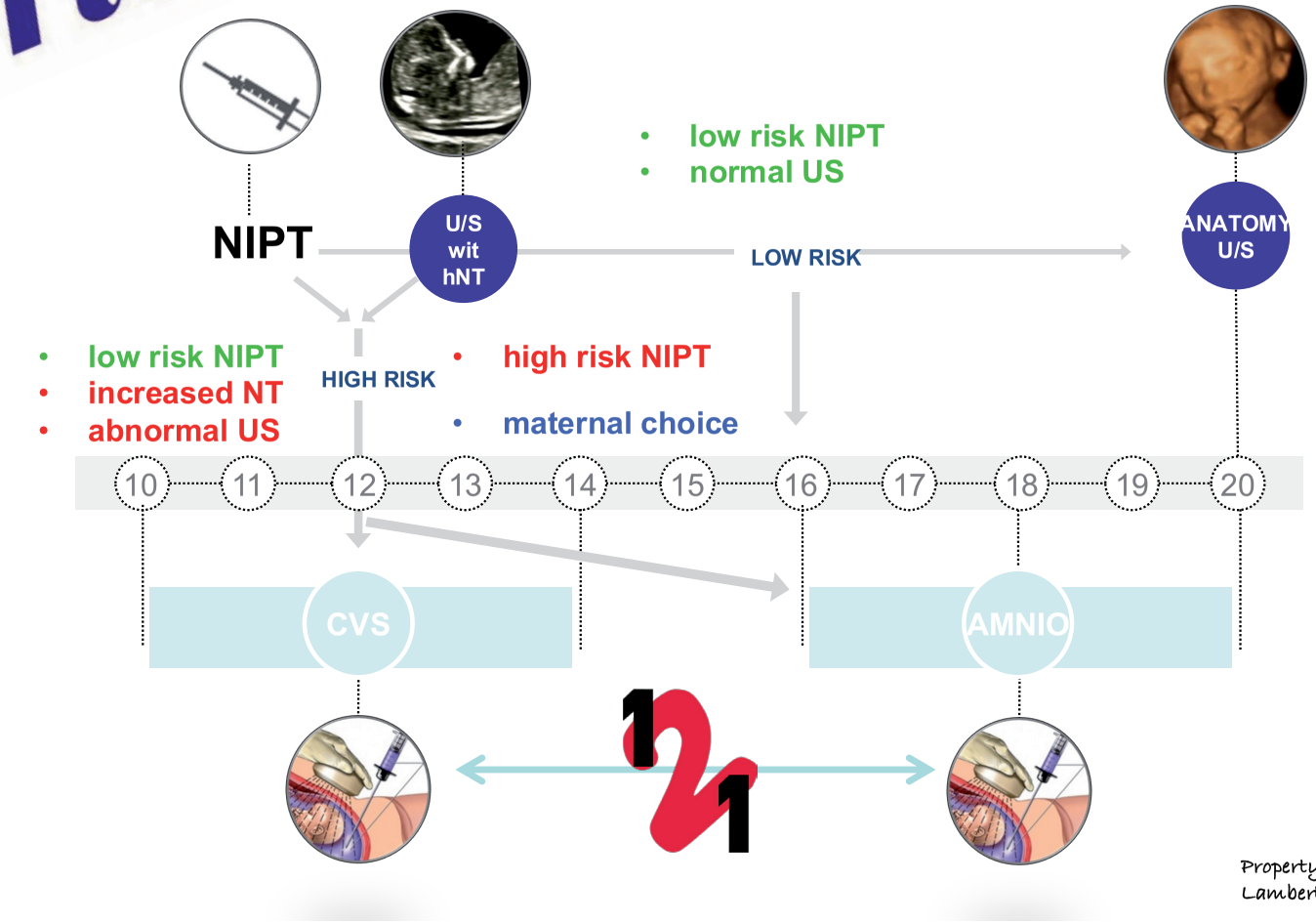


Sindrome da microdelezione	Regione cromosomica	Prevalenza (alla nascita)
Sindrome di DiGeorge	delezione 22q11.2	1/2.000 - 1/4.000
Sindrome Cri-du-chat	delezione 5p15.3	1/15.000 - 1/50.000
Sindrome di Prader-Willi	delezione 15q11.2	1/25.000
Sindrome Angelman	delezione 15q11.2	1/10.000 - 1/20.000
Sindrome da delezione 1p36	delezione 1p36	1/5.000 – 1/10.000
Sindrome di Wolf-Hirschhorn	delezione 4p16.3	1/20.000 -1/50.000
Sindrome di Jacobsen	delezione 11q23-q24.3	1/100.000
Sindrome di Langer-Giedion	delezione 8q24.11-q24.13	1/200.000
Sindrome di Smith-Magenis	delezione 17p11.2	1/15.000 - 1/25.000

E' consigliabile ricorrere all'utilizzo del **PrenatalSafe® Karyo Plus solo in determinati contesti clinici** (esempio dubbi ecografici suggestivi di sindrome da microdelezione cromosomica) per i quali risulta giustificato un approfondimento diagnostico di secondo livello.

flow

Identification of Fetal Chromosomal Anomalies with cffDNA: Flow screen



Identification of Fetal Chromosomal Anomalies with cffDNA: Flow screen

flow

- high risk NIPT
- increased NT
- abnormal US

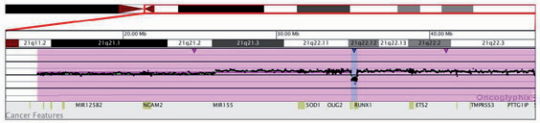
- high risk NIPT
- normal US

- abnormal US

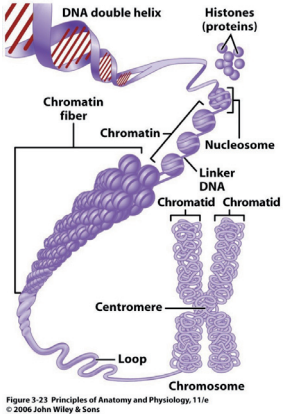
10 11 12 13 14 15 16 17 18 19 20

CVS

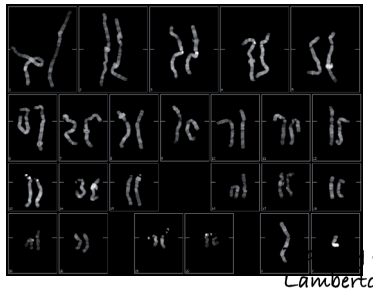
AMNIO



- Deletion/duplication
- 500 kbases DNA



- Deletion/duplication
- 15 Mbases DNA



of
Lamberto Camurri Ph.D.

CFF DNA BASIC GUIDELINES

In the prenatal period, various screening tests and diagnostic tests are available which differ from each other in terms of type of information offered and performance, as well as benefits and limitations. When considering a screening test, it should not be forgotten that there are no tests that are superior to others absolute terms and in all circumstances; therefore the choice of test is the result of an interaction between the operator which develops a consultation aimed at the characteristics of the patient and the patient with her process articulated decision-making.

Each patient should receive an informational interview focused on all available tests, both for the screening and for the diagnosis of fetal aneuploidies. It is essential that the doctor who manages the pregnancy be prepared to discuss not only the risk of chromosomal abnormalities, but also the benefits and limitations relating to the different tests available, whether diagnostic or screening.

Otherwise it is necessary to consult a specialist in fetal medicine.

The test that should be performed is ideally the result of a conscious choice by the patient based on the information received and the clinical context of the pregnancy, on the healthcare resources available, but also on the values, interests and specific objectives of the patient. Furthermore, each patient should be given the opportunity to choose between tests screening and diagnostic ones, and, similarly, every patient should be put in a position to freely accept or decline, after consultation, the proposed tests.

The same articulation is valid for both public structures as well as private ones. As regards the international literature on the subject, the path of information on the tests of screening has been outlined since 2007 by specific guidelines drawn up by the scientific societies of American (ACOG), Canadian (SCOG) and English (NICE, RCOG) obstetricians and gynecologists and, since 2009, by the lines- "Physiological Pregnancy" guide of the Istituto Superiore di Sanità (SNLG-ISS), in which it is clearly indicated that "the path for prenatal diagnosis of Down syndrome must be offered to all women".

It is also reported that information about screening tests should be offered to the woman at the first contact with the professional who assists you, in a context capable of offering the possibility of starting a discussion. Information on tests, whether screening or diagnostic, must include the characteristics of the test (reliability), the methods of execution and a comprehensive description of the condition being investigated and, finally, the need to clarify "the woman's right to accept or refuse the test".

The cfDNA/NIPT pre-test interview is critical to providing up-to-date and clear benefit information and about the limitations of this screening test and the implications of the results, both high- and low-risk, even in relation to the type of anomaly identified. Since it is a screening test and consider the different biological causes underlying the possible discordance between the NIPT result and the real genomic structure of the fetus (e.g. feto-placental mosaicisms, reabsorbed twin and maternal chromosome arrangement), high-risk results must be confirmed through an appropriate diagnostic test, in the prenatal or postnatal period.

In the case of a low-risk result, the woman must be informed that the test does not

provide certainty that the fetus is healthy, given the risk of a false negative due to the conditions investigated and the impossibility of analysis conditions that are not investigated by the test.
In cases where NIPT provides a non-informative result, even after the analysis of a second sample, yes also recommends offering genetic counseling to the pregnant woman to evaluate the most appropriate path based on the results of ultrasound investigations and any screening tests performed previously, the gestational age, the clinical history, the a priori risk and the pregnant woman's willingness to undergo to an invasive sampling
It is possible that in rare cases cfDNA/NIPT will provide a fetal sex result discordant with that determined by ultrasound. In these cases it is appropriate for the laboratory to verify the raw data and the process analysis and, if the discordance is not resolved, proceed with a further ultrasound investigation to confirm the phenotypic sex, review the patient's medical history and exclude the presence of any reabsorbed twins, a maternal transplant/transfusion, or a known disease-related genetic condition of sexual development.
If the problem is not resolved it is necessary to evaluate during the consultancy genetic, the opportunity to perform an amniocentesis to investigate the cause of the discrepancy.

CffDNA UPDATED PERFORMANCE FOR PRE TEST COUNSEL

At the beginning of 2022 Eurofins Genome Rome Italy completed the retrospective analysis of the data collected since 2019 on over 70,000 pregnancies, applying Illuminain's CE-IVD VeriSeq protocol association with a proprietary analysis algorithm developed internally. The results were compared with those obtained by the invasive or birth follow up, obtaining the data of 85% of the total cases. The results demonstrate that the already excellent performances validated by Illumina are further improved if associated with the private analysis algorithm allowing to achieve an overall very high sensitivity (99.49%) and specificity (99.88%)..
In particular, with regard to common aneuploidies such as trisomy 21, 18 and 13, the study showed an overall sensitivity and specificity of 99.65% and 99.98%. Sex chromosome aneuploidies showed a high reliability for the anomalies XXY (Klinefelter syndrome), XYY (Jacobs syndrome) and XXX (trisomy X) and slightly lower for monosomy X (Turner syndrome), confirming overall excellent sensitivity (99.36%) and specificity (99.96%)
The data obtained for rare trisomies reveal high sensitivity and specificity (99.99%, 99.95%), although with a limited positive predictive value due to a rate of relevant feto-placental mosaicism and the risk of spontaneous interruption of the first quarter found in these cases.
The clinical utility of researching rare trisomies is confirmed in relation to the possible effects of feto-placental mosaicism on growth fetal especially in the 3rd trimester of gestation, but it is also particularly useful for the identification of uniparental disomy of chromosomes subject to imprinting, as in case of trisomy 15 whose rescue led to the

Fetal Chromosomal Anomalies in cffDNA: performances

	Falsi +		Falsi -		Prev-popolaz /10000	Casi coorte
T21,18,13	12	1 6000	3	1 24000		72000
⇧ X, Y	16	1 4500	1	1 72000	99,99/100	72000
⇩ Deletions	16	1 4500	1	1 72000	99,99/100	72000

False positives >

False negatives >

cffDNA PERFORMANCE 72000 CASES SYNTHESIS

Prof. Dr. Lamberto Camurri Ph.D.

identification of two cases of syndrome Prader-Willi.
This cffDNA NIPT analysis has excellent performance also for segmentals anomalies with dimensions greater than 7 Mb (sensitivity 99.99%; specificity 99.98%) where the presence of false positives is attributable to fetoplacental mosaicisms, as well as the presence of maternal benign tumors such as uterine fibroids.
Regarding the finding of false negative results, the data are in line with the data of the international scientific literature on the limits of NIPT.
TOOLS FOR PRE TEST GENETIC COUNSELLING

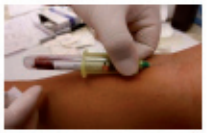
Each patient should receive an informational interview focused on all available tests, both for the screening and for the diagnosis of fetal aneuploidies.

It is essential that the doctor who manages the pregnancy be prepared to discuss not only the risk of chromosomal abnormalities, but also the benefits and limitations relating to the different tests available, whether diagnostic or screening.

1) Tutorial

This presentation replaces the reading of the information notes attached to the form that will be submitted to the patient for acceptance. The essential points are indicated with:

PRENATAL GENETIC TEST

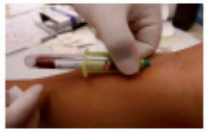


NOT INVASIVE

INVASIVE

This presentation replaces the reading of the information notes attached to the form that will be submitted to the patient for acceptance. The essential points are indicated with:

PRENATAL GENETIC TEST

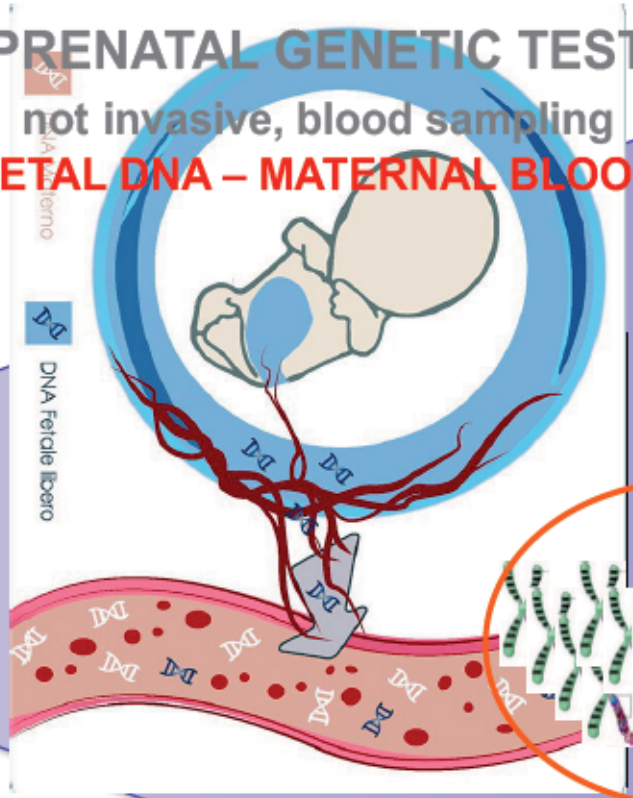


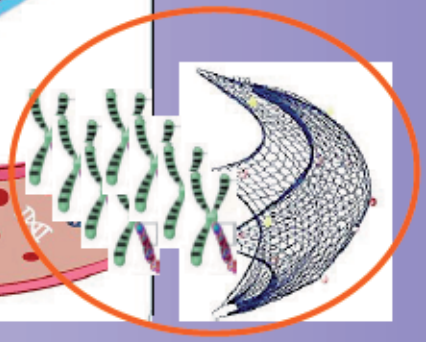
PRENATAL GENETIC TEST

not invasive, blood sampling

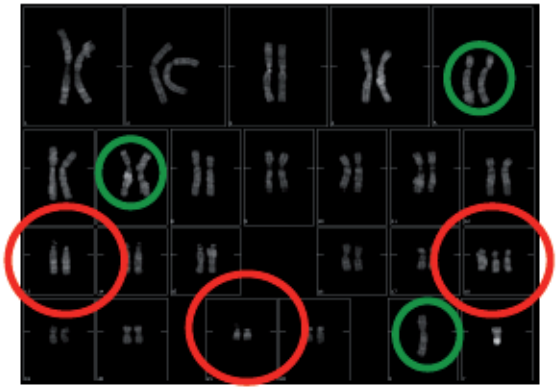
FETAL DNA – MATERNAL BLOOD

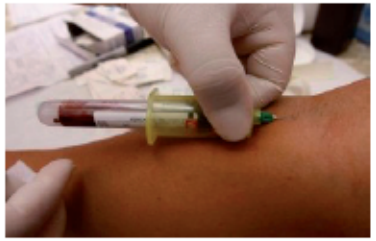
Fetal Fraction	Expected ratio for Trisomy
2-4%	1.02
10%	1.05
20%	1.10
40%	1.20





Not invasive prenatal test.
Genetic Diseases:
Frequent and serious diseases.





PrenatalSafe®
Harmony™ PRENATAL TEST

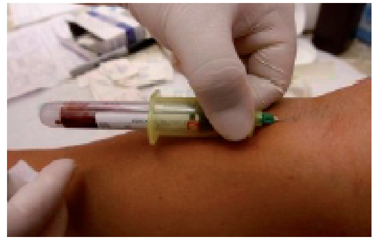
Fetal DNA:
Frequent and serious Trisomies

		Sopravvivenza.	
TRISOMIA 21	**1/750**	1a 88%	10a 82%
TRISOMIA 18	**1/2000**	1a 5%	1a 1%
TRISOMIA 13	**1/5000**	1a 10%	10a 0
XXX,X0,XXY,XYY			

Frequent and serious diseases:
Maternal DNA

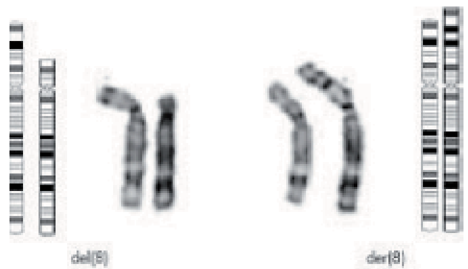
FIBROSI CISTICA	**1/2500**	40a 50%	
ATROFIA MUSCOLARE SPINALE	**1/10000**	SMA1 2a 5%	SMA2 20° 70% SMA3 adulta
RITARDO MENTALE FRAXA	**1/5000**	adulta	

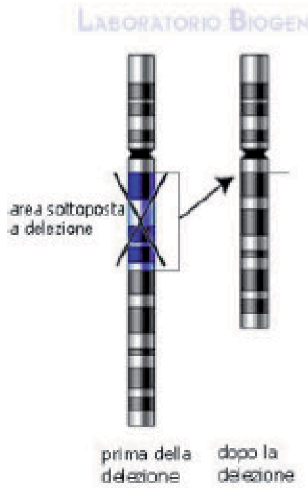
Fetal DNA
In maternal
Plasma



Chromosomal anomalies: trisomies e structural aberrations
Molecular Karyotype. 46 chromosomes

DELEZIONI	0,30‰ (1/3000)	1° 90%	10° >50%
DUPLICAZIONI	0,15 ‰ (1/6000)	1° 90%	10° >50%





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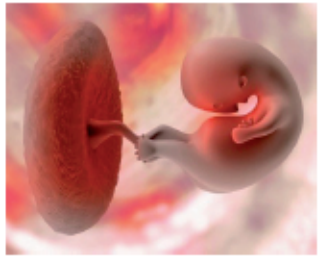
Property of
Lamberto Camurri Ph.D.

NIPT
Fetal DNA in maternal blood



Casi di Anomalie Cromosomiche	‰ prevalenza EU	Validazione clinica	cffDNA Sangue materno		cffDNA Sangue materno		DNA fetale placenta	Liquido Amniotico
Totale	4,4		SP	PPV	SN	NPV	Accuratezza%	Accuratezza%
T21 T18 T13	3,1	casi > 72000	99,9 99,9 99,9	99 99 82	99,5 99,9 99,9	99 99 99	99,96 99,87 99,92	100
XXX,XXY,XYY,X	0,6	casi > 72000	99,8-99,9	99-80	100 - 98	100		
			SP	PPV	SE	NPV		
Anomalie > 10Mb	0,4	casi > 72000	99,9	55,6	99-100	100		

False positives >

False negatives >

Fetal Chromosomal Anomalies in cffDNA: performances

	Falsi +		Falsi -		Prev-popolaz /10000	Casi coorte
T21,18,13	12	1 6000	3	1 24000		72000
⇧ X, Y	16	1 4500	1	1 72000	99,99/100	72000
⇩ Deletions	16	1 4500	1	1 72000	99,99/100	72000

False positives >

False negatives >

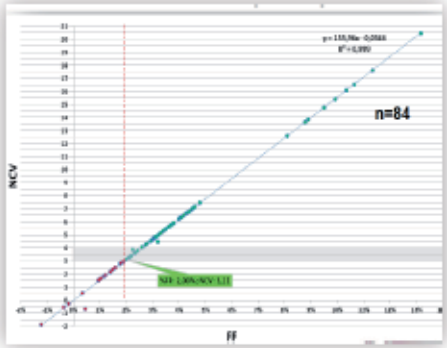
Fetal Chromosomal Anomalies in cffDNA

PrenatalSafe®

Trisomies & Karyo

Direct analysis of fetal molecular karyotype

SAFER SCORE



RISULTATO NEGATIVO.
L'analisi del DNA fetale libero (cffDNA) eseguita sul campione biologico in esame **NON HA RILEVATO** aneuploidie o alterazioni cromosomiche strutturali, nei limiti di risoluzione della metodica.

Sesso fetale: **Maschio.**
Frazione fetale: 10%

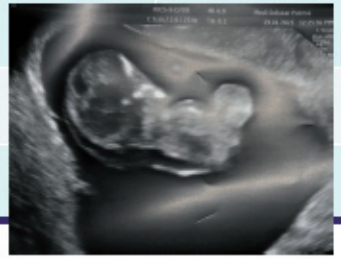
Rischio di risultati Falsi Negativi: <0.1%

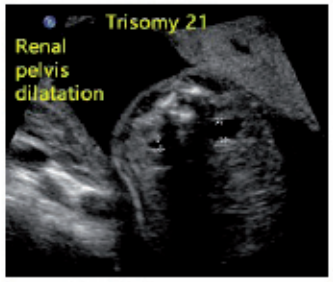
L'analisi del DNA fetale libero (cffDNA) eseguita sul campione biologico in esame **HA RILEVATO una SOSPETTA DUPLICAZIONE del braccio corto del cromosoma 12.**
Non sono state rilevate aneuploidie o alterazioni cromosomiche strutturali a carico di altri cromosomi, nei limiti di risoluzione della metodica.
Sesso fetale: Femmina.
Frazione fetale: 11%
Valore Predittivo Positivo (PPV): 61.54%

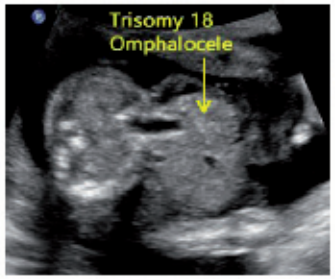
PrenatalSafe®

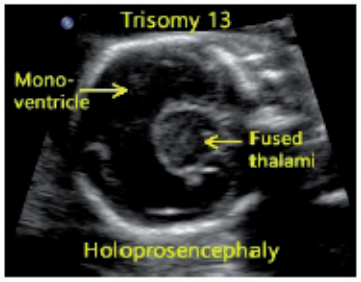
Fetal Chromosomal Anomalies in cffDNA: performances

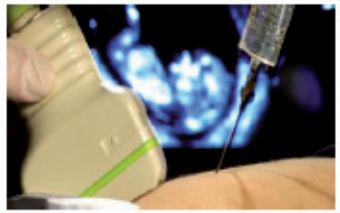
	Falsi +	Veri +	Falsi -	Prev-coorte /10000	Prev-popolaz /10000	Casi coorte
T21			2	120	15	72000
T18			1	30		72000
T13			0	13	0,6	7

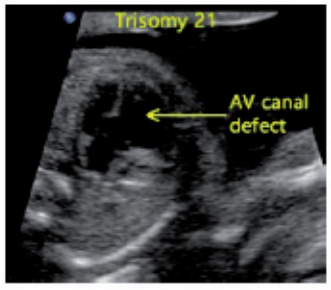


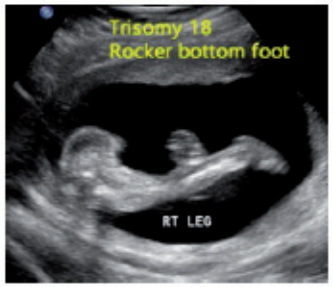


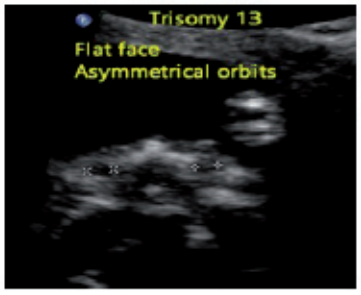








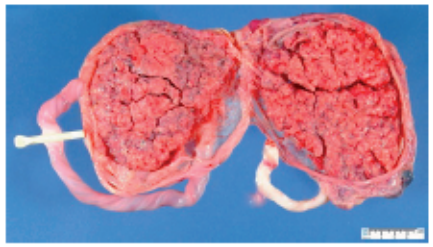




Fetal Aneuploidy Detection with cffDNA
Multiple Pregnancies

DNA Fetale nel Plasma Materno

Twins/multiple	
2 max	Valore di rischio distribuito
regular	No sex, no identificazione feto affetto
IVF	No sex, no identificazione feto affetto
Oocytes etero	No sex, no identificazione feto affetto
Sensitivity T21*	93.7% *(FN/tested popul. 0.6%)*
Specificity T21*	99.8% *(FPR 0.23%)*
DNA fetal fraction	m 7,4% (12% single)
**Gil M et al 2015*	*Ultrasound Obstet Gynecol 45, 249*



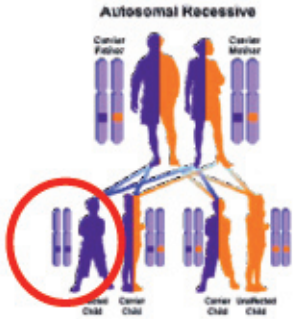
PluriGenTEST™

Test: Non Invasive Prenatal. Genetic diseases: frequenti, serious.

Parental DNA > heterozigous healty carrier. Diagnostic test

Ricerca portatori malattie mendeliane ereditarie

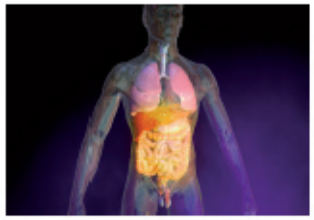
Fibrosi Cistica	port 1/25 malati 1 /2500	sensib.	90%
Ritardo mentale FRAXA	port. 1/260 1/1250M		99%
Atrofia Muscolare Spinale	port. 1/50 malati 1/10000		93%
	port. 1/40 ER malati 1/6400		



Rischio residuo feto affetto

	FC	SMA
???? / ????	1/2500	1/10000
Neg 1 / ????	1/10000	1/100 000
Neg 1 / Neg 1	1/40000	1/1 000 000

FC
Fibrosi Cistica



SMA
Atrofia Muscolare Spinale

FRAXA
Ritardo Mentale

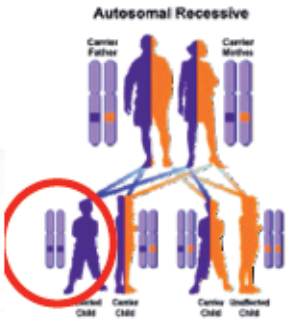
ritardo mentale: IQ tra 20 e 70

- deficit di memoria a breve termine di informazioni complesse
- ritardo nel linguaggio
- ridotte abilità visuo-spaziali
- ipersensibilità agli stimoli
- iperattività con deficit di attenzione
- comportamento autistico

- Macrocefalia con fronte, mento e orecchie sporgenti
- Macroorchidismo (<30ml) dopo la pubertà
- Anomalie connettivali: prolasso della mitrale, lassità articolare, piede piatto
- Disfunzioni ipotalamiche?

PluriGenTEST™

Test: Non Invasive Prenatal.
Genetic diseases: 300 genes



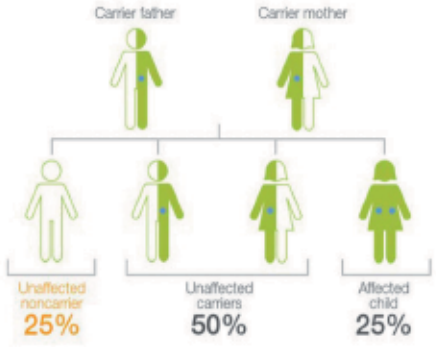
genescreen®

La genomica di nuova generazione

Negli ultimi anni, gli straordinari progressi conseguiti nel settore della genomica e delle biotecnologie hanno posto le basi per leggere e comprendere le informazioni contenute nel nostro DNA, il **genoma**. In particolare **le nuove tecnologie di sequenziamento, *Next Generation Sequencing* (NGS)**, ci permettono oggi di accedere alla sequenza del nostro DNA in modo più facile ed efficace, fornendo una valutazione approfondita dell'informazione genetica di ogni singolo individuo.

Ogni persona nasce, infatti, con caratteristiche genetiche che la differenziano dagli altri e che la rendono unica. Mentre la maggior parte delle differenze nella sequenza del DNA tra persone diverse è innocua, alcuni cambiamenti, definiti **mutazioni genetiche**, possono alterare la funzionalità genomica e rendere quella persona portatrice di una specifica malattia genetica trasmissibile ai propri figli.

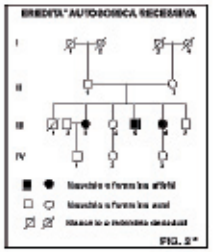
I portatori di malattie genetiche sono tipicamente individui sani, completamente privi di sintomi ed inconsapevoli di essere a rischio di trasmettere tale "errore" del DNA ai figli.



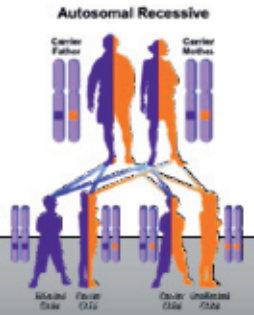
Il test GeneScreen® Easy

GeneScreen® Easy è un test diagnostico, sviluppato da GENOMA Group, che permette di eseguire un'analisi multipla di **oltre 600 malattie genetiche ereditarie**, tra cui quelle più frequenti nella popolazione italiana, come la Fibrosi Cistica, l'Anemia Falciforme, la Talassemia, la Sordità Ereditaria.

Test
Prenatal
Non
Invasive



PluriGenTEST™



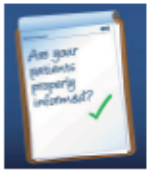
Ricerca portatori malattie mendeliane

Fibrosi Cistica	90% sensibilità (rischio residuo 1/95)
Atrofia muscolare spinale	93% sensibilità
Sordità NS	90% sensibilià (rischio residuo 1/350)
Ritardo mentale FRAXA	99% sensibilità

Probabilità feto affetto malattie mendeliane recessive

	Fibrosi Cistica	SMA
???? / ????	(1/25 x 1/25 x ¼) 1/2500	(1/50 x 1/50 x ¼) 1/10000
Neg 1 / ????	(1/25 x ¼ x 1/25 x ¼) 1/10000	(1/50 x 1/10 x 1/50 x ¼) 1/100 000
Neg 1 / Neg 1	(1/25 x ¼ x 1/25 x ¼ x ¼) 1/40000	(1/50 x 1/10 x 1/50 x 1/10 x ¼) 1/1 000 000
Etero / ???	(1 x 1/25 x ¼) 1/100	(1 x 1/50 x ¼) 1/200
Etero / Neg 1(75)	(1 x 1/25 x ¼x ¼) 1/400	Etero /Neg 1(93) (1 x 1/50 x 1/12 x ¼) 1/2400
Etero / Neg 2(90)	(1 x 1/25 x 1/10 x ¼) 1/1000	
Etero / Neg 3(95)	(1 x 1/25 x 1/20 x ¼) 1/2000	
Etero / Neg 4(99)	(1 x 1/25 x 1/100 x ¼) 1/10000	
Etero / Etero	¼	¼

Mutazioni: Neg 1(37), Neg3 (139-152), Neg4 (intero gene)



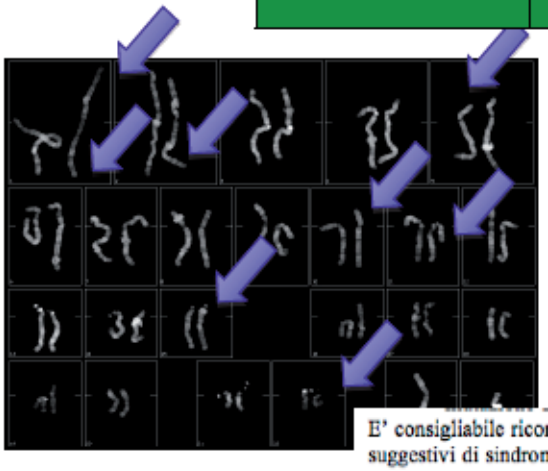
Prenatal screening

La analisi cffDNA delle microdelezioni (<10Mb) ricerca una serie di sindromi rare con una sensibilità clinica fra il 50 e il 95%.
L'analisi è consigliata come indagine di secondo livello in concomitanza con finding ecografici e consulenza genetica.

plus

PrenatalSafe®

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche strutt.	1737	0,7	17 (40<10)
Anomalie < 10Mb			3



Sindrome da microdelezione	Regione cromosomica	Prevalenza (alla nascita)
Sindrome di DiGeorge	delezione 22q11.2	1/2.000 - 1/4.000
Sindrome Cri-du-chat	delezione 5p15.3	1/15.000 - 1/50.000
Sindrome di Prader-Willi	delezione 15q11.2	1/25.000
Sindrome Angelman	delezione 15q11.2	1/10.000 - 1/20.000
Sindrome da delezione 1p36	delezione 1p36	1/5.000 – 1/10.000
Sindrome di Wolf-Hirschhorn	delezione 4p16.3	1/20.000 -1/50.000
Sindrome di Jacobsen	delezione 11q23-q24.3	1/100.000
Sindrome di Langer-Giedion	delezione 8q24.11-q24.13	1/200.000
Sindrome di Smith-Magenis	delezione 17p11.2	1/15.000 - 1/25.000

E' consigliabile ricorrere all'utilizzo del **PrenatalSafe® Karyo Plus solo in determinati contesti clinici** (esempio dubbi ecografici suggestivi di sindrome da microdelezione cromosomica) per i quali risulta giustificato un approfondimento diagnostico di secondo livello.

Prenatal screening

Malattie genetiche	Anomalie ‰	Specificità Sensibilità BIOLOGICA	Specificità Sensibilità TECNICA	Anomalie % tot
Malattie genetiche non ereditarie	<0,01-0,4	>95 >70	>95 >70	

GeneSafe™

GENE	MALATTIE SINDROMICHE
JAG1	Sindrome di Alagille
CHD7	Sindrome di CHARGE
HDAC8	Sindrome di Cornelia de Lange tipo 5
NIPBL	Sindrome di Cornelia de Lange tipo 1
MECP2	Sindrome di Rett
NSD1	Sindrome di Sotos tipo1
ASXL1	Sindrome di Bohring-Opitz
SETBP1	Sindrome di Schinzel-Giedion
	SINDROME DI NOONAN
BRAF	Sindrome Cardio facio cutanea (CFC) tipo 1
CBL	Sindrome di Noonan-simile con comparsa leucemia mielomonocitica giovanile
KRAS	Sindrome di Noonan /cancers
MAP2K1	Sindrome Cardio facio cutanea (CFS) tipo e 3
MAP2K2	Sindrome Cardio facio cutanea (CFS) tipo 4
NRAS	Sindrome di Noonan 6/cancers
PTPN11	Sindrome Noonan 1/ Sindrome di LEOPARD/cancers
PTPN11	Leucemia mielomonocitica giovanile (JMML)
RAF1	Sindrome di Noonan 5/Sindrome di LEOPARD 2
RIT1	Sindrome di Noonan 8
SHOC2	Sindrome Noonan-simile con capelli cadenti in fase anagen
SOS1	Sindrome di Noonan 4

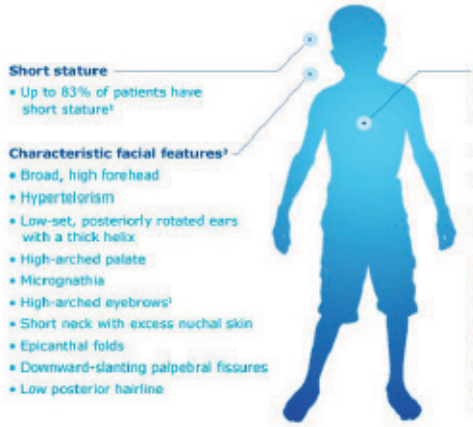
1/70000
1/15000
1/50000 Se 50%
1/30000 Se bassa
1/2500 Se 50-75%

GENE	PATOLOGIE SCHELETRICHE
COL2A1	Acondrogenesi tipo 2
	Acondroplasia
	Sindrome CATSHL
	Sindromedi Crouzon con acanthosis nigricans
FGFR3	Ipocondroplasia
	Sindrome di Muenke
	Displasia tanatofora, tipo I
	Displasia tanatofora, tipo II
	Sindrome di Ehlers-Danlos, classica
	Sindrome di Ehlers-Danlos, tipo VIIA
	Osteogenesi imperfetta, tipo I
COL1A1	Osteogenesi imperfetta, tipo
	Osteogenesi imperfetta, tipo III
	Osteogenesi imperfetta, tipo IV
	Sindrome di Ehlers-Danlos, forma cardiaco-valvolare
	Sindrome di Ehlers-Danlos, tipo VIIB
COL1A2	Osteogenesi imperfetta, tipo II
	Osteogenesi imperfetta, tipo III
	Osteogenesi imperfetta, tipo IV
	CRANIOSINOSTOSI
	Sindrome di Antley-Bixler senza anomalie genitali o disordini della steroidogenesi
	Sindrome di Apert
FGFR2	Sindrome di
	Sindromedi Jackson-Weiss
	Sindrome di Pfeiffer, tipo 1
	Sindrome di Pfeiffer, tipo 2
	Sindrome di Pfeiffer, tipo 3

1/25000
1/20000 Se 95%
1/2500 Se 20%

Prenatal screening

Malattie genetiche	Anomalie ‰

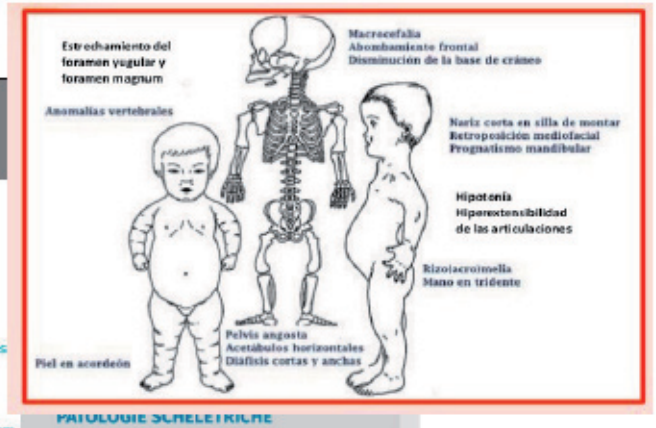


Short stature
- Up to 83% of patients have short stature[1]

Characteristic facial features[2]
- Broad, high forehead
- Hypertelorism
- Low-set, posteriorly rotated ears with a thick helix
- High-arched palate
- Micrognathia
- High-arched eyebrows[1]
- Short neck with excess nuchal skin
- Epicanthal folds
- Downward-slanting palpebral fissures
- Low posterior hairline

Congenital heart defects[3]
- Pulmonary valve stenosis
- Hypertrophic obstructive cardiomyopathy
- Atrial and ventricular septal defects
- Persistent ductus arteriosus

Other clinical manifestations[3-5]
- Pectus carinatum, pectus excavatum
- Scoliosis
- Cryptorchidism
- Lymphatic abnormalities
- Coagulopathy
- Cognitive/learning disabilities
- Ophthalmological issues
- Arnold-Chiari malformation
- Seizures



SETBP1	Sindrome di Schinzel-Giedion
	SINDROME DI NOONAN
BRAF	Sindrome Cardio facio cutanea (CFC) tipo 1
CBL	Sindrome di Noonan-simile con o senza leucemia mielomonocitica giovanile
KRAS	Sindrome di Noonan /cancers
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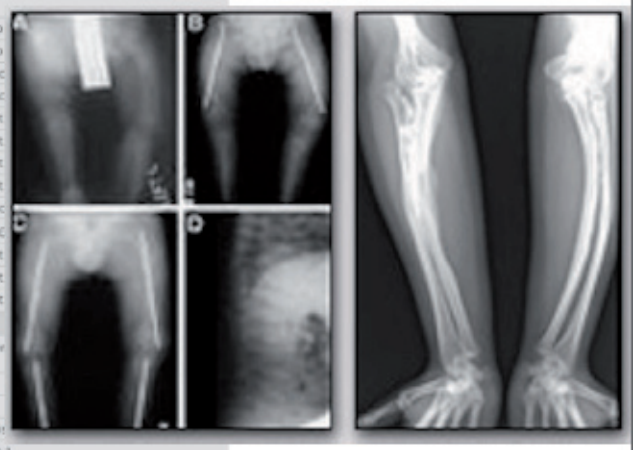
	PATOLOGIE SCHELETRICHE
	Acondrogenesi tipo 2
	Acondroplasia
	Sindrome CATSHL
	Sindromedi Crouzon con acanthosis nigricans
	Ipocondroplasia
	Sindrome di Muenke
	Displasia tanatofora, tipo
	Displasia tanatofora, tipo
	Sindrome di Ehlers-Danlc
	Sindrome di Ehlers-Danlc
COL1A1	Osteogenesi imperfetta, t
	Osteogenesi imperfetta, t
	Osteogenesi imperfetta, t
	Osteogenesi imperfetta, t
	Sindrome di Ehlers-Danlc
	Sindrome di Ehlers-Danlc
COL1A2	Osteogenesi imperfetta, t
	Osteogenesi imperfetta, t
	Osteogenesi imperfetta, t
	CRANIOSINOSTOSI
	Sindrome di Antley-Bixler ni della steroidogenesi
	Sindrome di Apert
	Sindrome di Crouzon
FGFR2	Sindromedi Jackson-Wei
	Sindrome di Pfeiffer, tipo 1
	Sindrome di Pfeiffer, tipo 2
	Sindrome di Pfeiffer, tipo 3



TOOLS FOR PRE TEST GENETIC COUNSELLING

Each patient should receive an informational interview focused on all available tests, both for the screening and for the diagnosis of fetal aneuploidies.

It is essential that the doctor who manages the pregnancy be prepared to discuss not only the risk of chromosomal abnormalities, but also the benefits and limitations relating to the different tests available, whether diagnostic or screening.

1) cffDNA at a Glance

GENETIC DISEASES

Cystic Fibrosis is a common disease as it affects 1:2500 both males and females, it is generally serious, present from birth. It is inherited from parents who are, almost always without knowing it, healthy carriers. The bronchi and lungs suffer the most damage where mucus tends to stagnate, generating infection and inflammation which, over time, leads to respiratory failure. The damage also involves the pancreas, intestine and liver. The severity and type of symptoms may vary from person to person. The median life expectancy is 40 years, that is, half of the patients survive to 40 years of age.
In Italy there is one healthy carrier for approximately every 25 people. Two healthy carriers, in each pregnancy, have a one in four chance of having an affected child. Fetal analysis is performed with chorionic villus sampling or amniotic fluid.

Spinal Muscular Atrophy (SMA) is a neuromuscular pathology characterized by the progressive death of the nerve cells of the spinal cord that give the muscles the movement command; it is very disabling and with reduced life expectancy. SMA affects approximately 1:10,000 births and is the most common genetic cause of infant death. Father and mother are, almost always without knowing it, healthy carriers and have a 25% chance of transmitting the disease to each of their children.
In Italy there is one healthy carrier for approximately every 50 people. The healthy carrier couple, with each pregnancy, has a one in four chance of having an affected child. Fetal analysis is performed with chorionic villus sampling or amniotic fluid.

Fragile X Syndrome (FRAX) or Martin Bell is the most common form of mental retardation after Down syndrome, as it affects 1:1250 males and 1:2000 females.
It is a hereditary disease caused by the alteration of a gene located in the X chromosome (CGG base repeats) usually in the mother which can be transmitted to the fetus.
The main symptoms are medium mental retardation (IQ between 20 and 70) sometimes with autistic behavior,
The mother can have the pre-mutation and therefore be a healthy carrier and cause the disease in the fetus.
Fetal analysis is performed with chorionic villus sampling or amniotic fluid.

PluriGenTEST™

PRENATAL GENETICS SCREENING

NIPT - FETAL DNA IN MATERNAL PLASMA

TRISOMIY 21, 18, 13, SEX KARYOTIPE

GENETIC DISEASES

CYSTIC FIBROSIS
SPINAL MUSCULAR ATROPHY (SMA)
MENTAL RETARDATION FRAXA

GENESCREEN

FETAL DNA IN MATERNAL BLOOD CHROMOSOME ANALYSIS - NIPT

Trisomy 21 Trisomy 13 Trisomy 18
Fetal sex
Molecular karyotype (analysis of the number and structure of all chromosomes)

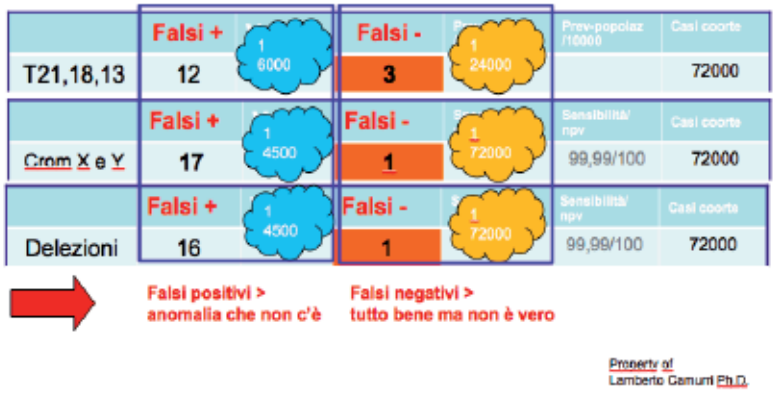
Prenatal genetic diagnosis is a discipline that sees the commitment of various qualified specialists.

A blood sample from the pregnant woman, starting from the tenth week of pregnancy, allows us to analyze all 46 chromosomes (Molecular Karyotype) of the fetus and evaluate the risk of chromosomal anomalies such as trisomy 21, which causes Down syndrome, trisomy 13 causes of Patau syndrome, trisomy 18, responsible for Edwards syndrome, fetal sex and all anomalies of the other chromosomes (X, Y, deletions and duplications).

These tests have the same objectives as those performed with invasive techniques the accuracy exceeding 99% with a positive predictive value between 80 and 99%.
Our tests comply with the Guidelines of the Ministry of Health and the Major Societies of Human Genetics which recommend DNA sequencing with at least 8 million readings and the calculation of the fetal DNA fraction.
The PRENATALEASY - PRENATALSAFE test is based on the validated Illumina-Veriseq2 complete genome sequencing technique with next generation techniques (NGS) implemented by an additional algorithm for Chromosomal Deletions and Fetal Fraction.
In fact, each sample is evaluated for the quantity of fetal DNA present, in the rare case the quantity is not sufficient, the sample is repeated free of charge.

The PRENATALEASY - PRENATALSAFE test is the only one to have been compared with the analysis of amniotic fluid (amniocentesis) in terms of accuracy on 72,000 consecutive cases. False positives for all chromosomal anomalies ((trisomies, X and Y chromosomes, deletions) are approximately 1/5000 (they are neutralized with the amniocentesis control), false negatives are approximately 1/25000.
This direct data makes the accuracy of the PRENATALEASY - PRENATALSAFE test transparent.

The test is not diagnostic as the sample is a mix of maternal and fetal DNA.
The accuracy of the test is strengthened by performing the ultrasound for nuchal translucency and the premorphological ultrasound, thus reducing the frequency of false negatives.



Accuracy of chromosomal analysis on fetal DNA in maternal blood: trisomies, karyotype on all chromosomes. The accuracy is equal to the direct analysis on chorionic villi (chorionic villus sampling) and slightly lower than that of amniotic fluid (amniocentesis) since fetal DNA and villus use placental tissue while amniocentesis uses fetal cells.