panorama™
natera prenatal screen

SNP-BASED NONINVASIVE PRENATAL TESTING

THE PANORAMA™ PRENATAL SCREENING TEST

Panorama is a noninvasive prenatal test (NIPT) that analyzes fetal-placental cell-free DNA (cfDNA) isolated from maternal plasma and screens for various fetal chromosomal abnormalities. These abnormalities include the aneuploidies Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), Monosomy X (Turner syndrome), sex chromosome trisomies (XXX, XXY, XYY), triploidy, and five microdeletion syndromes, including 22q11.2 deletion (DiGeorge), Cri-du-chat, Prader-Willi, Angelman, and 1p36 deletion.[1-5] If requested, fetal sex will also be provided. Panorama can be performed with high accuracy as early as nine weeks of gestation.

INCIDENCE OF CHROMOSOMAL ABNORMALITIES

Trisomy 21, Trisomy 18, and Trisomy 13 are the three most common aneuploidies and together occur in approximately 1 in 450 live births. Of these, Trisomy 21 is the most common.[6,7] Clinically relevant microdeletions are more common than previously thought and can occur in pregnancies lacking ultrasound anomalies—the combined at-birth incidence of the five microdeletion syndromes covered by Panorama is approximately 1 in 1,000.[8-12] The most common significant microdeletion condition, 22q11.2 deletion syndrome, is more common than cystic fibrosis **(Figure 1)**.[11,13-15]

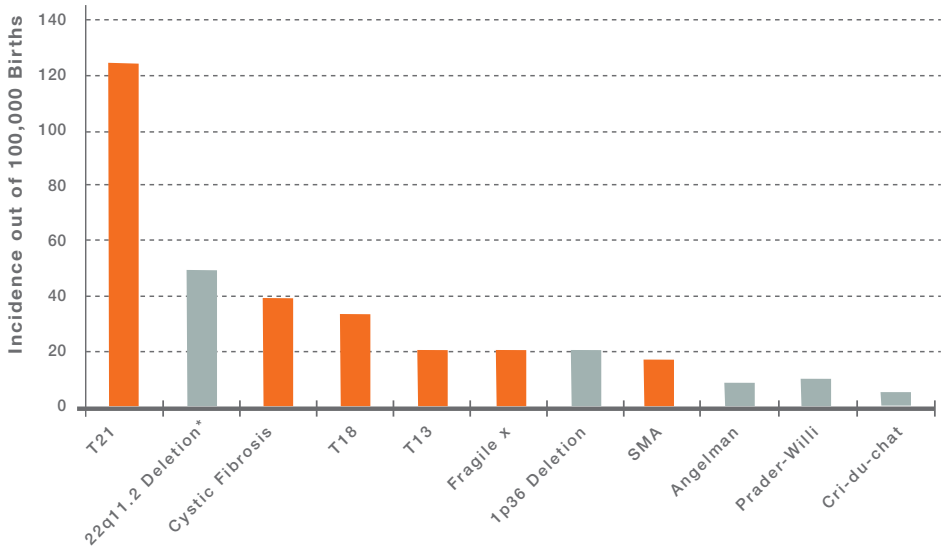

Figure 1. The 22q11.2 deletion is more common than cystic fibrosis*

* Recent studies have shown incidence rates as high as 1/992[15]

procedures. The cfDNA analyzed is a mixture of maternal and fetal DNA, with the percentage of fetal DNA referred to as the "fetal fraction". Fetal fraction has been shown to be positively correlated with gestational age and negatively correlated with maternal weight.[1]

Accurate determination of fetal chromosomal copy number using cfDNA isolated from maternal plasma requires cfDNA amplification and subsequent bioinformatics analysis. To date, there are two major bioinformatics approaches: the first-generation "quantitative" or counting methods used by most cfDNA-based tests and the second-generation approach used by Natera that incorporates genotypic information using single nucleotide polymorphisms (SNPs).

FIRST GENERATION: COUNTING METHODOLOGY

Quantitative counting methods compare the relative number of sequence reads from a chromosome-of-interest such as chromosome 21 (in which trisomy results in Down syndrome), to a reference chromosome or set of chromosomes presumed to be euploid. Although this approach is effective at detecting Trisomy 21 and Trisomy 18, it is not as successful in the detection of Trisomy 13 or sex chromosome aneuploidies.[20-24] The counting approach is not able to detect triploidy, a condition that is estimated to occur in approximately 1:2,000 pregnancies at 12 weeks of gestation,[25,26] and that can result in severe placental and fetal abnormalities along with increased risks for spontaneous abortion, pre-eclampsia, and gestational trophoblastic neoplasia. The counting method is also unable to distinguish between maternal and fetal genotypes, which can be a problem in the event of maternal duplications. This method also precludes the detection of a vanishing twin; both maternal abnormalities and vanishing twin pregnancies lead to false-positive results for the fetus.[27-30] As the fetal fraction of cfDNA actually originates from the placenta, there are occasions where the fetal and placental DNA differ due to confined placental mosaicism. This type of mosaicism occurs in 1–2% of pregnancies[31-33] and can contribute to false-negative and false-positive results for both the counting methodology and the SNP-based methodology.

SECOND GENERATION: SNP-BASED METHODOLOGY

Panorama is the only commercially available NIPT approach that specifically targets SNPs to determine ploidy. This approach isolates the mother's DNA from her white blood cells, sequences it, and uses that information to "subtract out" the maternal genotype from the plasma sample. This provides more robust data about the fetal genotype and higher accuracy even at fetal fractions as low as 2.8%. Panorama targets 13,392 SNPs covering chromosomes 21, 18, 13, X, and Y; additional sets of SNPs are targeted for detection of microdeletions. A patented algorithm is then used to screen for chromosomal fetal aneuploidy and fetal sex. The ability to differentiate between maternal and fetal cfDNA also allows Panorama to identify the presence of a vanishing twin and maternal duplications and can lead to identification of a previously undetected microdeletion such as 22q11.2 deletion syndrome in the mother. Because Panorama does not require a reference chromosome, it is uniquely able to detect triploidy and full molar pregnancies.

A validation study for chromosomes 13, 18, 21, X, and Y revealed sensitivities of 100% for Trisomy 21 and Trisomy 13, 96% for Trisomy 18, and 90% for Monosomy X; specificities of at least 99.9% were observed for each indication.[2] The PPVs for a high-risk result were determined following a large clinical validation study of Panorama.[1] The PPVs in that study were 90.9% for Trisomy 21, 93.1% for Trisomy 18, 38.1% for Trisomy 13, and 50.0% for Monosomy X.[1] In the microdeletion validation study,[34] sensitivities of greater than 97% and specificities of greater than 99% were reported for each microdeletion condition. Clinical validation of microdeletion detection is currently ongoing.[35]

CLINICAL UTILIZATION

Panorama can be ordered as early as nine weeks gestation and requires a simple blood draw from the mother. The father can also provide a DNA sample via a buccal swab, which maximizes the chances that a sample will return a result, but is not required. For sample collection, special blood collection tubes that protect the cfDNA are used. Samples are sent at room temperature to Natera's CLIA- and CAP-certified laboratory in San Carlos, California. After samples are processed, a report containing personalized risk scores for each of the chromosomes evaluated is generated. Fetal sex is reported if requested. If ordered, risk scores are also provided for microdeletion syndromes. For pregnancies identified as high risk by Panorama, follow-up testing and genetic counseling are recommended.

Furthermore, unlike whole chromosome aneuploidies (e.g., Trisomy 21) that are more prevalent in women of advanced maternal age, the risk for microdeletions is independent of maternal age. For pregnant women under the age of 28, this means they are more likely to have a pregnancy with one of these five microdeletions than with Down syndrome **(Figure 2)**. Most concerning is that these microdeletion syndromes can be severe and result in serious physical and/or intellectual impairment that may otherwise go undiagnosed until childhood.

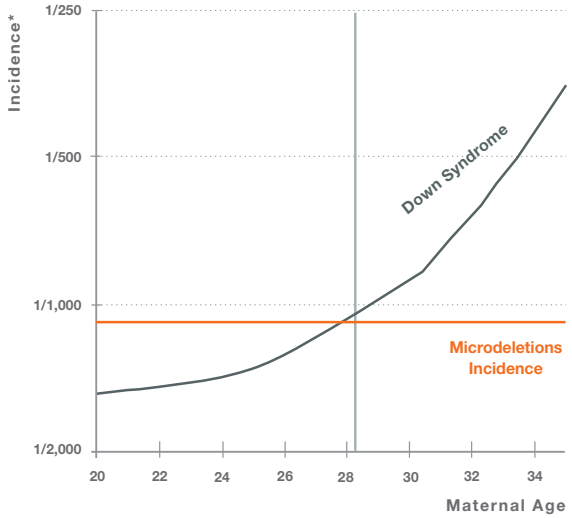

Figure 2. In younger women, the five microdeletions screened for using Panorama are more common than Down syndrome.

* The combined at-birth incidence of these five microdeletion syndromes is approximately 1 in 1,000.[8-12]

TRADITIONAL SCREENING METHODS

Until recently, the primary screening modality for aneuploidy involved measurement of maternal serum biochemical markers during the first and/or second trimesters, sometimes in combination with first trimester ultrasound to measure nuchal translucency (NT). Although designed to detect Trisomy 21, this integrated screening approach can also detect Trisomy 18 and Trisomy 13. Increased NT (as well as cystic hygroma and other indicators) is also associated with certain sex chromosome aneuploidies (and indirectly associated with the 22q11.2 deletion syndrome). Large studies using traditional screening methods reported detection rates for Trisomy 21 of 79-90% with a false-positive rate of 4-5%.[16] The positive predictive value (PPV)—i.e., the likelihood that a high-risk result on a test indicates a true positive result in the patient—for Trisomy 21 using traditional screening methods is <5%.[17] This means that for every twenty women who receive a screen positive result, at least nineteen will be false positives.

Traditional screening methods are not designed to detect microdeletions. Unlike aneuploidies, microdeletion syndromes do not have well defined markers that can be detected using serum screening. Many of these syndromes also lack structural abnormalities that can be detected on ultrasound. Prenatal diagnosis of microdeletion syndromes requires an invasive procedure, i.e., amniocentesis or chorionic villus sampling (CVS), to provide cells that are subsequently analyzed using fluorescence in-situ hybridization (FISH) or chromosomal microarray. CVS and amniocentesis procedures that only involve karyotyping (and not FISH or microarray) cannot detect most microdeletions. Recent guidelines[18] specifically state that microarray testing is appropriate for pregnant women who are undergoing invasive testing, regardless of maternal age. However, due to the risk of pregnancy loss associated with invasive procedures (1/450–1/900),[19] many women do not routinely undergo invasive testing unless they have another high-risk indication. In the absence of effective noninvasive prenatal screening methods, many affected babies have gone undiagnosed until after birth, or even well into adulthood; as a result, opportunities for early intervention were missed. Thus, there is a clear need for a prenatal screening approach that is both accurate and noninvasive in all patients.

NONINVASIVE DETECTION OF FETAL CHROMOSOMAL ABNORMALITIES USING CELL-FREE DNA

Prenatal screening for fetal aneuploidies has been revolutionized by noninvasive prenatal tests that analyze cfDNA in maternal plasma. Because fetal cfDNA traverses the blood-placental barrier and enters maternal circulation, a simple maternal blood draw provides the means to detect fetal chromosomal copy numbers without exposing the fetus to the risks associated with invasive

Both the validation study[2] and the clinical validation study[1] for Panorama contained a large cohort of women from the general pregnancy population, and equivalent performance and PPVs were observed for the low-risk and high-risk cohorts. In addition, because microdeletions occur independently of maternal age and other traditional risk factors, Panorama could be a useful screening tool for the general pregnancy population.

CONCLUSIONS

Panorama offers high sensitivity and specificity for detection of Trisomy 21, Trisomy 18, Trisomy 13, Monosomy X, triploidy, and fetal sex, and offers early reassurance. This screening test can also detect the presence of sex chromosome trisomies. Compared with conventional screening methods, Panorama's low false-positive rate may reduce the number of invasive diagnostic procedures that women choose to undergo. Panorama has also been validated as a screening tool for the most common microdeletions, including 22q11.2 deletion (DiGeorge), 1p36 deletion, Cri-du-chat, Prader-Willi, and Angelman syndromes. For expecting parents wanting to learn more about the health of their baby, Panorama offers a highly accurate, noninvasive way to screen for the most common chromosomal abnormalities in early pregnancy.

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