Performing the PrenaTest® to exclude or detect a 22q11.2 microdeletion, associated with DiGeorge syndrome and velocardiofacial syndrome (Shprintzen syndrome)

22q11.2 microdeletion[1]

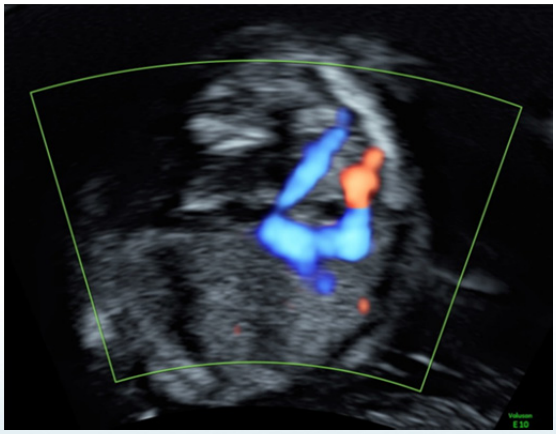
DiGeorge and velocardiofacial syndrome are caused by a microdeletion on the long arm of chromosome 22 in position 11. This 22q11.2 microdeletion is one of the most common chromosomal abnormalities which occurs in one out of approx. every 6000 newborns. This occurs spontaneously in more than 90% of those affected and 6% to 28%[2] of those affected may also have inherited it from a parent. The clinical abnormalities, particularly heart defects, may have different manifestations, depending on the severity of the disease or may not be present at all. Knowledge about DiGeorge or velocardiofacial syndrome may be of importance in the prenatal as well as in the neonatal period.

Medical indication

The exclusion or detection of a 22q11.2 microdeletion may be useful in cases of abnormalities on ultrasound, for example, during organ screening, which may correlate with a DiGeorge or velocardiofacial syndrome:

- Congenital heart defect (as the most important prenatal characteristic of a 22q11.2 microdeletion in ultrasound examinations)
- Detection of an aberrant subclavian artery (see Fig. 1)
- Increased nuchal translucency
- Kidney malformation
- Growth retardation
- Cleft lip and palate
- Small thymus

Fig. 1 | Aberrant subclavian artery in the 13th week of pregnancy*



* Courtesy of PD Dr. med. Michael Entezami,
Center for Prenatal Diagnostics and Human Genetics, Berlin

Validation of the test method

Data from synthetic pooled DNA specimens as well as several specimens from pregnant women whose unborn child had a 22q11.2 microdeletion were investigated. In all cases, a 22q11.2 microdeletion was correctly detected. The validation process was performed in three phases:

Phase 1
Four synthesized specimens, each of which had a different cffDNA level (16%, 8%, 4% and 2%) and which had a 22q11.2 microdeletion, were analyzed several times. The 22q11.2 microdeletion of the two specimens with a cffDNA level of 8% and 16% was correctly determined, while the results of the specimens with a lower cffDNA level were not significant.

Phase 2
Four specimens from pregnant women whose unborn children had a 22q11.2 microdeletion were retrospectively tested and the 22q11.2 microdeletion was correctly detected in each case. In this phase, the 22q11.2 microdeletion was previously confirmed in three of the specimens using invasive diagnostic procedures. In the case of the fourth specimen, a cardiac defect (DORV; double outlet right ventricle) was confirmed by ultrasound. The DiGeorge syndrome was confirmed after birth.

Phase 3
In a final internal blinded study, specimens from phase 2 with a 22q11.2 microdeletion as well as euploidic specimens were investigated. All specimens were correctly classified. Due to the low number of cases, a concrete test sensitivity and specificity cannot currently be derived.

Correctly classified specimens	16/16
Total detection rate	2/2
False-positive rate	0/14

Limits of the test method

cffDNA level ≥ 13%
The detection or exclusion of a 22q11.2 microdeletion is currently only possible for specimens which have a cffDNA level of at least 13%, since in the scope of the validation, the cffDNA level of the patient specimens investigated was between 13% and 27%.

According to Fig. 2, the cffDNA level is highly individual and only increases starting in the 20th week of pregnancy. Therefore it is possible that due to a cffDNA level that is too low, no test result can be achieved. A new blood sample is not requested provided the cffDNA level is sufficient for determining fetal trisomies 13, 18, 21 as well as gonosomal aneuploidies.

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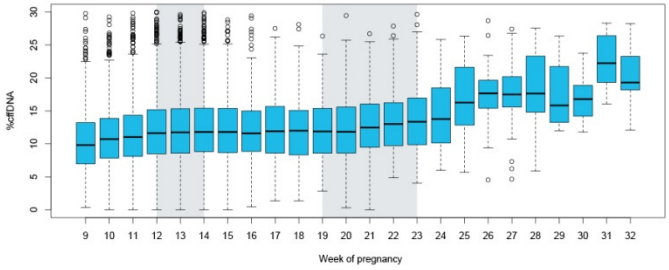
Fig. 2 | Correlation between cffDNA level and week of pregnancy based on data from LifeCodexx AG from routine clinical practice in Germany (8/2012-12/2015)



Week of pregnancy 12–14	Week of pregnancy 19–23	
39%	45%	Samples with cffDNA ≥ 13%
61%	55%	Samples with cffDNA < 13%

It is possible that the mother is the carrier of the 22q11.2 microdeletion but not the unborn child. This can lead to discordant (false-positive) test results.

Size of the microdeletion
In more than 85% of the affected persons, the deletion includes a region measuring approx. 2.5 megabases in the 22q11.2 region of chromosome 22. This region is investigated with the PrenaTest®. A small percentage of affected persons has an even smaller deletion or point mutation in the affected region which cannot be detected with the PrenaTest®. This can lead to discordant (false-negative) test results.

Microduplication in the investigated gene region
In the investigated gene region of chromosome 22, microduplications can also occur. These are not determined with the PrenaTest®.

In combination with PrenaTest® option 3

Due to process requirements, this examination to exclude or detect a 22q11.2 microdeletion can be selected only in combination with the PrenaTest® option 3, however not with PrenaTest® option 1 or option 2. It is free of charge for the patient.

The order is placed as follows:

a) In combination with the initial order for the PrenaTest® option 3. The duration of the test does not change. The completed and signed order is sent together with the initial order and the blood samples in the return box.

b) As a supplement to an already performed PrenaTest® (option 3) from an earlier week of pregnancy in which the test results were inconspicuous for trisomies 13, 18 and 21 and the cffDNA level was ≥ 13%. In general, the test result for this supplemental biocomputational analysis will be available after one workday. If the cffDNA level of the previously performed PrenaTest® analysis (option 3) was too low, you can provide us with a new blood sample for a second PrenaTest® analysis, which will be charged separately.

This is not a screening test

At present, professional associations recommend determining microdeletion syndromes via NIPT either only on a limited basis and also only for clinically relevant syndromes with a significant prevalence as well as a defined phenotype, such as DiGeorge syndrome[3], or else they have a critical attitude towards it[4] or even reject it[5]. The reason is that there are still only very limited data.

For the PrenaTest® as well, only four patient specimens in Germany in which the unborn child was actually affected by a 22q11.2 microdeletion have been able to be recruited and tested to date. Nonetheless, we have decided to offer this test free of charge on the basis of the data that are currently available since we are receiving more requests for it from physicians.

However, we ask for prudent use of this new test option only when there is a corresponding abnormality in the pregnancy. In addition, we would like to request your feedback in the event of a correct or discordant result.

1 OMIM# of the disease: 188400 (DGS), 192430 (VCFS)

2 Sandrin-Garcia P, Macedo C, Martelli LR, et al. Recurrent 22q11.2 deletion in a sibship suggestive of parental germline mosaicism in velocardiofacial syndrome. Clin Genet. 2002;61(5):380–383

3 Schmid M et al. Cell-Free DNA Testing for Fetal Chromosomal Anomalies in clinical practice: Austrian-German-Swiss Recommendations for non-invasive prenatal tests (NIPT). Ultraschall in Med 2015; 36: 507-510

4 European Journal of Human Genetics (2015) 23, 1438–1450; doi:10.1038/ejhg.2015.57; published online 18 March 2015

5 Cell-free DNA screening for fetal aneuploidy. Committee Opinion No. 640. American College of Obstetricians and Gynecologists. Obstet Gynecol 2015;126:e31–7.