Prof. Dr. med.
Christian T. Thiel-Hirschmann, MBA
GUTACHTER & SACHVERSTÄNDIGER

HUMAN GENETIC EXPERT OPINION

Case Study | Prenatal Assessment

Suspected fetal trisomy 21 based on an abnormal NIPT result, sonographic markers, and mosaic findings in amniocentesis

Case Number	Court / Insurance [Sample Case]
Client	Court / Health Insurance / Social Services
Expert	Prof. Dr. med. Christian T. Thiel, MBA
Qualifications	Board-certified specialist in human genetics; University Hospital Erlangen; 30 years of clinical and scientific experience; expert witness for courts and insurance providers
Date	March 28, 2026
Subject	Pregnant woman [Name, Date of Birth] and fetus [gestational age] (fictitious case study)
Key Question	How should an abnormal NIPT result be evaluated from a human genetic perspective in conjunction with ultrasound markers and evidence of mosaicism in amniocentesis? Distinction between CPM, fetal mosaicism, and combined fetoplacental mosaicism.

Declaration pursuant to § 407a ZPO

The undersigned declares that the expert opinion has been prepared impartially and to the best of his knowledge and belief. He affirms that he has no personal or financial relationship with the parties to the proceedings that could compromise his impartiality. The expert opinion is based exclusively on the documents submitted and the current state of medical science. The assessment is based on the principles of evidence-based medicine as well as the guidelines of the German Society of Human Genetics (GfHev), the American College of Medical Genetics and Genomics (ACMG), and the American College of Obstetricians and Gynecologists (ACOG).

1. Assignment and Research Question

In the present case, a human genetic evaluation is requested to determine whether the available prenatal findings indicate, with sufficient medical and scientific probability, the presence of fetal trisomy 21. In addition, it must be clarified how an abnormal NIPT result should be interpreted in conjunction with sonographic abnormalities and a mosaic-like finding from amniotic fluid. Finally, the remaining diagnostic uncertainties must be identified, and the specific further medically necessary measures must be determined.

In particular, the following must be assessed:

1. The diagnostic hierarchy and significance of the available findings (NIPT, ultrasound, amniocentesis)

2. The distinction between exclusively placental mosaicism (CPM), true fetal mosaicism, and a combined fetoplacental mosaic pattern
3. The question of whether and to what extent fetal chromosomal involvement should be assumed
4. Which further diagnostic and clinical measures are medically indicated
5. The socio-medical and healthcare-legal classification of the constellation of findings

2. Test materials and methodology

2.1 Submitted findings

- NIPT report: abnormal findings with a high risk of trisomy 21
- Ultrasound findings: increased nuchal translucency, additional soft markers (e.g., shortened femur, echogenic intracardiac focus, and/or pyelectatic configuration)
- Amniocentesis report: Evidence of mosaicism Trisomy 21 in one proportion of the amniocytes examined
- Maternal health record and pregnancy progress documentation

2.2 Methodological principles

The evaluation takes into account the current ACMG guidelines on the use of NIPT in the general population (Dungan et al. 2023 [1]), the current ACOG recommendations on cell-free DNA diagnostics, published data on the positive predictive value (PPV) of NIPT results, and the scientific literature on confined placental mosaicism (CPM) and fetal mosaicism. Causal attributions are made according to the criteria of scientific causality assessment.

3. Facts and Findings

In the present case, the NIPT revealed an abnormal result with a high risk for trisomy 21. Additionally, sonographic abnormalities were observed: increased nuchal translucency as well as other soft markers such as shortened femur, echogenic intracardiac focus, or pyelectatic constellation. During the course of the diagnostic process, amniocentesis was performed, revealing an additional copy of chromosome 21 in a portion of the examined amniocytes, while another portion of the cells was normal. This results in a mosaic finding.

Such a constellation poses a greater diagnostic challenge than the simple sequence of positive non-invasive prenatal testing (NIPT) – free trisomy in amniocentesis. This is due to the fact that an explicit assessment of the relationship between the placenta, amniotic fluid cells, and actual fetal tissue distribution is required.

4. Family history and medical history

In the present case study, no relevant family history of predisposing conditions is documented. In the context of the maternal age history and consideration of any

Previous findings (including abnormal first-trimester tests and prior affected pregnancies) must be factored into the individual pre-test probability. This should be taken into account when interpreting the positive predictive value of NIPT.

For trisomy 21, maternal age is the most important known risk factor. The risk increases from approximately 1 in 1,500 for 20-year-olds to approximately 1 in 100 for 40-year-old pregnant women. The individual pre-test probability is decisive for determining the positive predictive value (PPV) of the NIPT result.

5. Methodological Preface and Diagnostic Hierarchy

5.1 NIPT as a screening procedure—not a definitive diagnosis

It should first be noted that NIPT is not a diagnostic test, but a screening procedure. The analysis is based on cell-free DNA (cfDNA) found in maternal plasma. This DNA does not originate directly from fetal organs, but predominantly from apoptotic trophoblast cells of the placenta. A positive NIPT result therefore indicates a risk profile; however, it should not be interpreted on its own as proof of fetal aneuploidy.

For this reason, ACOG and ACMG recommend genetic counseling, a qualified ultrasound evaluation, and an offer of invasive confirmatory testing in the event of a positive cfDNA/NIPT result. Although NIPT is the most sensitive and specific screening test for common trisomies, it remains, by definition, a probabilistic rather than a definitive procedure.

5.2 Performance parameters of NIPT for trisomy 21

A systematic review (Iwarsson et al. 2017 [3]) reports a pooled sensitivity of 99.3% and a specificity of 99.9% for trisomy 21 in the general population; in high-risk cohorts, the pooled sensitivity is 99.8%. **These figures demonstrate the high clinical performance of the procedure but do not constitute a diagnostic confirmation.** In the assessment, therefore, it is not merely the technical quality of the test that is decisive, but the post-test probability in the specific individual case.

The positive predictive value (PPV) of NIPT for trisomy 21 is highly dependent on the pre-test probability. In a large, genetically confirmed cohort (Dar et al. 2022 [4]), a prevalence ratio of 85.7% for trisomy 21 was observed in the low-risk group and 97.5% in the high-risk group. In an Italian multicenter analysis (Grati et al. 2022 [5]), a positive predictive value (PPV) of 93% was determined for trisomy 21. It can therefore be concluded that a positive NIPT result for trisomy 21 should be taken seriously; however, its significance must always be interpreted in the context of the specific population and case. It must not be treated in isolation, as a karyotype would be.

6. Molecular genetic and cytogenetic findings

6.1 Biological basis: Why NIPT and the fetus do not always reflect the same thing

The key point is that NIPT primarily analyzes the DNA of the placenta. In biology, it is entirely conceivable that the placenta exhibits a chromosomal abnormality while the fetus is unaffected, or that both are affected, but to varying degrees. The phenomenon described is referred to as confined placental mosaicism (CPM) when the chromosomally abnormal cell line is confined to the placenta.

In the relevant literature (see Kalousek & Vekemans 1996 [6]), CPM is described in approximately 1–2% of viable pregnancies examined by chorionic villus sampling. More recent data (see Rosenblum et al. 2024 [7]) confirm that CPM is a major cause of false-positive cfDNA/NIPT results. In a recent series, CPM was confirmed by postnatal placental analysis in 32.3% of cases with positive cfDNA screening and negative amniocentesis.

Furthermore, it must be taken into account that chromosomally abnormal cell lines may be unevenly distributed within the placenta. Recent studies (see Eggenhuizen et al. 2024 [8]) demonstrate a patchy distribution, i.e., a spotty or regionally variable occurrence of abnormal cells in the placental tissue. Even a targeted placental analysis can only partially represent the extent of the mosaic pattern. This heterogeneity explains why discrepant results between NIPT, chorionic villus sampling, amniocentesis, and postnatal examination are biologically plausible and should not be hastily interpreted as laboratory errors.

6.2 Findings in the sample case

Findings – Sample Case

NIPT: Positive for trisomy 21 (high risk)

Ultrasound: increased nuchal translucency + additional soft markers (e.g., shortened femur, echogenic intracardiac focus, pyelectasis)

Amniocentesis: Mosaic finding – detection of trisomy 21 cells in a portion of the amniocytes, remaining cells unremarkable

Diagnostic classification: Strong suspicion of fetal mosaic trisomy 21

7. Assessment and genetic classification

7.1 Differential diagnosis: Placental mosaicism versus fetal mosaicism

A purely placental mosaic can explain a positive NIPT result in the presence of an unremarkable fetus. However, if Trisomy cells are detected during amniocentesis, there is a significant shift in the diagnostic assessment. Amniocentesis is used to examine fetal cells or cell populations derived from the fetus in the amniotic fluid and therefore, in cases of conflict, has a significantly higher evidential value regarding fetal involvement than NIPT alone.

A Trisomy 21 mosaic detected by amniocentesis can therefore no longer be adequately explained by an exclusively placental mosaic. Rather, three possibilities must be considered in the differential diagnosis:

1. True fetal mosaic (the most likely explanation given the current findings)
2. Combined fetoplacental mosaic constellation
3. Technical or culture-related pseudomosaic constellation (significantly rarer; particularly relevant for discussion when the mosaic proportion is very low, the aberrant cell line appears only in a single culture or colony, and there are no converging additional findings)

In the present case, there is no isolated borderline situation, but rather a convergent chain of findings: positive NIPT, sonographic markers, and detection of trisomic cells in the

amniocentesis. This combination substantially increases the likelihood of true fetal involvement.

7.2 Interpretation of the mosaicism rate from amniocentesis

A common interpretive error is to interpret the percentage of trisomic cells in the amniotic fluid directly as a marker of severity. This approach is not justifiable from a biological perspective. Mosaics arise postzygotically; therefore, the subsequent tissue distribution depends on the time at which missegregation or trisomic rescue occurred in the embryonic or extraembryonic tissue and on the resulting cell line.

Accordingly, a moderate mosaic proportion in cultured amniocytes may be associated with a clinically relatively mild presentation; conversely, however, it cannot rule out relevant organ manifestations. This applies in particular to the heart, the CNS, and growth patterns. A numerical percentage based on a single sample cannot therefore be used as a valid surrogate marker for the subsequent neurological or somatic phenotype.

7.3 Specific Classification for Trisomy 21

In the case of trisomy 21, NIPT proves to be particularly effective as a screening tool. This results in a significant shift from pre-test to post-test. If mosaicism is detected during amniocentesis, the appropriate conclusion is no longer merely a suspicion, but a **strong suspicion of fetal mosaic trisomy 21 with remaining uncertainty regarding the extent and organ distribution.**

In other words: The existence of a relevant chromosomal constellation is much better established in such a case than its final phenotypic expression. It is crucial that this distinction be explicitly stated in the report to avoid misinterpreting the remaining prognostic uncertainty as diagnostic ambiguity.

7.4 Sonographic Correlation and Evidential Value

Sonographic markers are usually not conclusive on their own. However, in combination with a positive NIPT and amniotic mosaicism, they offer significant interpretive value. They serve as independent clinical convergent findings. In particular, increased nuchal translucency, cardiac abnormalities, growth abnormalities, or multiple soft markers increase the likelihood that the chromosomal abnormality is not limited to the placenta.

An erroneous assessment arises from viewing ultrasound and genetics in isolation as separate entities. An integrative evaluation of both disciplines is, however, necessary. ACOG explicitly recommends a comprehensive ultrasound evaluation and the offer of diagnostic confirmation in the event of a positive cfDNA result.

8. Causality Assessment

8.1 Convergent chain of findings and causality

The causal relationship between the detected trisomy 21 constellation and the clinical findings can be established with a high degree of medical and scientific probability based on the available chain of findings:

– Positive NIPT for trisomy 21 (sensitivity 99.3%, specificity 99.9% in the general population) [3]

- Independent convergent ultrasound findings (nuchal translucency, soft markers) that increase the plausibility of fetal involvement
- Detection of trisomic cells in amniocentesis (higher diagnostic quality than NIPT alone regarding fetal involvement)
- Biological plausibility of the constellation of findings consistent with the clinical picture of fetal mosaic trisomy 21

8.2 Remaining uncertainty: extent, not existence

It is crucial for the assessment to consider the following aspects: The remaining uncertainty no longer focuses on the fundamental question of whether fetal involvement exists at all, but rather on the quantitative distribution of the abnormal cell line in various fetal tissues and thus on the prognosis of severity. This distinction is of considerable relevance from both a legal and a medical-ethical perspective.

9. Conclusion / Expert Assessment

Summary of the expert findings

1. **Diagnostic classification:** The present constellation of findings is to be classified as highly consistent with fetal mosaic trisomy 21, although the possibility of a combined fetoplacental mosaic situation must also be considered. A purely placental mosaic therefore represents only one of several possible explanations. This becomes significantly less likely, particularly if trisomic cells are additionally detected in amniocentesis and there is sonographic convergence. The present diagnosis should be formulated as a high degree of suspicion of fetal mosaic trisomy 21, whereby an open constellation of suspicion must be ruled out.
2. **Causal relationship:** Based on the convergent chain of findings (NIPT, ultrasound, amniocentesis), the causal relationship between the identified chromosomal configuration and the clinical presentation is evident with a high degree of medical and scientific probability.
3. **Remaining uncertainty:** The remaining uncertainty no longer concerns the question of fetal involvement per se, but rather the extent to which tissue distribution, organ manifestation, and the subsequent developmental profile will change. This prognostic uncertainty must not be misinterpreted as diagnostic ambiguity.
4. **Social-medical relevance:** From a human genetic perspective, there is significant chromosomal evidence that necessitates structured further evaluation as well as differentiated prenatal and postnatal care planning.

The above expert findings are provided to the best of my knowledge and belief, impartially and conscientiously, in accordance with § 407a of the German Code of Civil Procedure (ZPO). The undersigned certifies that he has no relationship of dependency with the parties and no financial interest in the outcome of the proceedings.

9a. Specific Recommendations for Action

From a human genetic and prenatal medical perspective, the following measures are indicated:

1. First, a methodological validation of the mosaic finding from the amniocentesis is required. This should ideally be performed by testing an independent sample or using an orthogonal method from the same diagnostic setting. In this way, culture-related artifacts can be ruled out as far as possible.
2. As part of the examination, a qualified fetal echocardiogram is performed, since cardiac malformations are among the clinically significant organ manifestations associated with trisomy 21. This fact influences the prognosis and care planning.
3. Close monitoring via ultrasound, including growth assessment, as CPM and fetoplacental mosaics may also be associated with placental dysfunction and growth abnormalities.
4. During the follow-up human genetic and prenatal medical counseling, the crucial difference between the diagnostic confirmation of a chromosomal abnormality and the uncertainty regarding the severity of the prognosis is explained. ACOG and ACMG explicitly emphasize the importance of qualified pre- and post-test counseling as well as the availability of diagnostic confirmation following abnormal cfDNA findings.
5. Postnatal confirmatory testing using peripheral blood. If postnatal clinical discrepancies exist between the test results and the phenotype, examination of additional tissues may be considered, as mosaicism can be distributed in a tissue-specific manner.
6. Placental histology or cytogenetic analysis of the placenta as a supplementary measure if the question of placental involvement remains relevant for the final interpretation of findings.

9b. Medical-Social-Legal Classification

Based on the diagnostic hierarchy (NIPT, ultrasound, amniocentesis), the present constellation of findings indicates a diagnostically significant chromosomal abnormality. From a legal and socio-medical perspective, it is crucial to distinguish the diagnostic certainty of chromosomal involvement from the uncertainty regarding the clinical severity of the condition.

The present constellation of findings necessitates structured care planning that includes fetal echocardiography as well as close follow-up monitoring. Furthermore, it includes qualified human genetic counseling that clarifies the difference between diagnostic probability and phenotypic prognosis. Finally, the constellation necessitates interdisciplinary prenatal-postnatal care coordination. From a legal and insurance medicine perspective, the constellation of findings is to be classified as sufficiently substantiated to justify specific medical measures and entitlements to care.

10. References

1. Dungan JS, Klugman S, Darilek S, et al. Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2023.

2. ACOG. Prenatal Diagnostic Testing for Genetic Disorders. Practice Bulletin No. 162. Obstet Gynecol. 2016 (reaffirmed 2023). [Current ACOG guidance: cell-free DNA is the most sensitive and specific screening test for common fetal aneuploidies, but is not equivalent to diagnostic testing; positive results should prompt counseling, ultrasound, and an offer of diagnostic testing.]

3. Iwarsson E, Jacobsson B, Dagerhamn J, et al. Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18, and 13 in a general pregnant population and in a high-risk population—a systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2017.

4. Dar P, et al. Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation. Am J Obstet Gynecol. 2022.

5. Grati FR, et al. Positive predictive values and outcomes for uninformative cell-free DNA tests: ICARO study. Prenat Diagn. 2022.

6. Kalousek DK, Vekemans M. Confined placental mosaicism. J Med Genet. 1996.

7. Rosenblum J, Blaumeiser B, Janssens K. The impact of confined placental mosaicism on prenatal cell-free DNA screening. Placenta. 2024.

8. Eggenhuizen GM, et al. Confined placental mosaicism: Distribution of chromosomally abnormal cells across the term placenta. Placenta. 2024.

Erlangen, March 28, 2026

Prof. Christian T. Thiel, M.D., MBA

Board-certified specialist in human genetics