“POSITIVE” NIPTs: INTERPRETATIONS, LIMITATIONS, & THE 2024 RECOMMENDATIONS from ACOG.

The ability to sequence an entire genome raises several challenging questions for the clinician, including:

●When would NGS be considered clinically?

●What is the best choice among several types of genetic testing available?

●What is the clinical significance of findings from sequencing of an entire genome?

●Which findings should be acted upon and/or conveyed to the patient?

Only Non-Invasive Prenatal Testing/Screening (NIPT/S) will be reviewed in this expose. To make it less confusing, we will use the term “NIPT”.

We’ll talk about genetic testing, counseling, and reporting of incidental findings from genome sequencing separately.

Alternative methods for evaluating genetic / genomic disorders such as Sanger sequencing, polymerase chain reaction (PCR), classical & molecular cytogenetics, e.g., karyotyping, fluorescence in situ hybridization (FISH), aCGH are also presented separately.

1. EXAMPLES OF “POSITIVE” NIPT:

1.1. Trisomy 21 (Down Syndrome; Trisomy G):

Trisomy 21, commonly known as **Down syndrome**, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. It is the most common chromosomal anomaly and is associated with developmental delays, mild to moderate intellectual disability, and characteristic physical features.

1.1.1. Prevalence:

Overall incidence in the United States is approximately 1/700 live births, and the risk increases gradually with increasing maternal age. Based on a large study, at 20 years of maternal age, the risk is 1/1466 births; at 35, it is 1/343; and at 40, it is 1/85. However, because most births occur among younger women, the majority of children with Down

syndrome are born to women < 35 years; only approximately 20% of infants with Down syndrome are born to mothers > 35 years.

1.1.2. Etiology:

i. In 95% of cases, Down syndrome is caused by nondisjunction resulting in an extra chromosome 21 (trisomy 21), which is typically maternally derived. Such people have 47 chromosomes instead of the normal 46.

ii. Approximately 4% of Down syndrome cases are due to a translocation. In a balanced translocation, genetic material is exchanged with material from another nonhomologous chromosome, and the chromosome count is maintained at 46.

ii.1. The most common translocation is t(14;21), in which chromosome 21 is attached to chromosome 14; this is an unbalanced translocation, resulting in a chromosome count of 45. In about half of people with the t(14;21) translocation, both parents have normal karyotypes, indicating a *de novo* translocation. In the other half, one parent (*almost always* the mother), who does *not* have Down syndrome, has only *45* chromosomes, one of which is t(14;21). The theoretical chance that a carrier mother will have a child with Down syndrome is 1:3, but the actual risk is lower (approximately 1:10). If the father is the carrier, the risk is only 1:20.

ii.2. The next most common translocation is t(21;22) with the same risk figures for the mother.

ii.3. t(21q;21q) is much less common. It is particularly important to determine whether a parent is a carrier of, or a mosaic for, t(21q;21q).

> ii.3.i. Each child of a carrier of the translocation will have Down syndrome or monosomy 21. Because monosomy 21 is not typically compatible with life, the risk of having a viable child with Down syndrome is 100%.
>
> ii.3.ii. If the parent is mosaic, that parent has some normal cells and some 45-chromosome cells with t(21q;21q), consequently the risk having Down syndrome children is markedly higher, but this parent may also have normal children with normal chromosomes.

iii. Mosaic Down syndrome results from nondisjunction (when chromosomes do not pass to separated cells) during cell division in the embryo. People with mosaic Down syndrome have two cell lines, one with the normal 46 chromosomes and another with 47 chromosomes, with the extra 21. The prognosis for intelligence and risk of medical

complications probably depends on the proportion of trisomy 21 cells in each different tissue, including the brain. However, in practice, risk cannot be predicted because it is not feasible to determine the karyotype in every single cell in the body. Some mosaic Down syndrome have very subtle clinical signs with normal intelligence; however, very variable findings may be found.

1.1.3. Signs & Complications:

Approximately 50% of affected neonates have congenital heart disease; ventricular septal defect and atrioventricular canal defect (endocardial cushion defect) are most common.

60% of people have eye problems, including congenital cataracts, glaucoma, strabismus, and refractive errors.

Most people have hearing loss, and ear infections are very common.

Many Down people develop thyroid disease (most often hypothyroidism) and diabetes.

There is an increased risk of Alzheimer disease at an early age.

1.1.4. Next Step (after NIPT "positive" result):

Always confirm NIPT "positive" findings, with a DIAGNOSTIC Test (e.g., amniocentesis for Karyotyping, FISH, or CGH Microarray).
Genetic Counseling is warranted.

1.2. Trisomy 18 (Edwards syndrome):

Trisomy 18 is a genetic disorder in which a person has a third copy of material from chromosome 18, instead of the usual 2 copies. Rarely, the extra material may be attached to another chromosome (translocation). Most cases are not passed down through families (not inherited). Instead, the problems that lead to this condition occur in either the sperm or the egg that forms the fetus.

Trisomy 18 occurs in 1 in 6000 live births. It is 3 times more common in girls than boys.

The syndrome occurs when there is extra material from chromosome 18. The extra material affects normal development.

- Trisomy 18: the presence of an extra (third) chromosome 18 in all of the cells.
- Mosaic trisomy 18: the presence of an extra chromosome 18 in some of the cells.

- Partial trisomy 18: the presence of a part of an extra chromosome 18 in the cells.

Symptoms may include:

Clenched hands, Crossed legs, Feet with a rounded bottom (rocker-bottom feet), Low birth weight, Low-set ears, Mental delay, Poorly developed fingernails, Small head (microcephaly), Small jaw (micrognathia), Undescended testicle, Unusual shaped chest (pectus carinatum), Hole, split, or cleft in the iris of the eye (coloboma), Separation between the left and right side of the abdominal muscle (diastasis recti),Umbilical hernia or inguinal hernia, Congenital heart disease, such as: Atrial septal defect (ASD), Patent ductus arteriosus (PDA), Ventricular septal defect (VSD), Kidney problems, including: Horseshoe kidney, Hydronephrosis, Polycystic kidney.

There are no specific treatments for trisomy 18. Only individual treatments depending on the person's individual condition.

Prognosis (outlook):
One half of infants with this condition do not survive beyond the first week of life. Nine out of ten children will die by 1 year of age. Rarely did they survive to the teenage years, but with serious medical and developmental issues.

Next Step (after NIPT “positive” result):
Always confirm “positive” NIPT findings, with a DIAGNOSTIC Test (e.g., amniocentesis for Karyotyping, FISH, or CGH Microarray).
Genetic Counseling is warranted.

1.3. Trisomy 13 (Patau Syndrome; Trisomy D):

Trisomy 13 is caused by an extra chromosome 13 and causes abnormal forebrain, midface, and eye development; severe intellectual disability; heart defects; and small birth size. Trisomy 13 occurs in approximately 1.7/10,000 pregnancies (based on data from induced abortion for fetal anomalies, stillbirths, and live births); approximately 80% of cases are complete trisomy 13. Advanced maternal age increases the likelihood, and the extra chromosome is usually maternally derived.

These Small-for-Gestation-Age (SGA) infants may present holoprosencephaly (failure of the forebrain to divide properly), facial anomalies such as cleft lip and cleft palate, microphthalmia, colobomas (fissures) of the iris, and retinal dysplasia. Supraorbital ridges are shallow, and palpebral fissures usually are slanted. Ears usually low-set. Hearing loss is common. Loose folds of skin often are present over the back of the neck. A single transverse palmar crease, polydactyly, and hyperconvex narrow fingernails are also common. Approximately 80% of cases have severe congenital

cardiovascular anomalies; dextrocardia is common. an abnormal scrotum occurs in boys, and a bicornuate uterus occurs in girls. Intellectual disability is severe.

Diagnosis of trisomy 13 may be suspected postnatally by appearance or prenatally by abnormalities on ultrasonography (e.g., intrauterine growth restriction), or by increased risk noted on multiple marker screening or NIPT using cell-free fetal DNA analysis on a maternal blood sample.

Next Step (after NIPT "positive" result):
Management decisions, including termination of pregnancy, should not be made based on NIPT alone.
Prenatally, confirmation is by karyotyping, FISH analysis, or chromosomal microarray analysis of samples obtained by chorionic villus sampling or amniocentesis.
Postnatally, confirmation is by classical cytogenetic / molecular cytogenetic testing usually of the newborn blood sample.
Prognosis:
In the past, most infants died during the neonatal period; however, the 5-year survival has improved in recent times. The underlying genetic abnormality cannot be cured. Only supportive care and support for the family.

1.4. 47,XXY Chromosome Disorder:

47,XXY chromosome disorder, also known as Klinefelter syndrome, is a genetic condition that affects males. It occurs when a boy is born with an extra copy of the X chromosome. Symptoms can vary and may include reduced muscle mass, reduced body and facial hair, and enlarged breast tissue. It can also lead to learning difficulties and issues with fertility. Treatment often involves testosterone replacement therapy and fertility treatment if necessary.
Klinefelter syndrome (47,XXY chromosome disorder) is not rare; it occurs in approximately **1 in 500 male births**. However, only about 25% of boys with Klinefelter syndrome are diagnosed, and this diagnosis usually occurs in adulthood.

Next Step (after NIPT "positive" result):
Always confirm "positive" NIPT findings, with a DIAGNOSTIC Test (e.g., amniocentesis for Karyotyping, FISH or CGH Microarray).
Genetic Counseling is warranted.

1.5. 45,X Chromosome Disorder:

45,X chromosome disorder, also known as Turner syndrome, affects females and occurs when one of the X chromosomes is missing or partially missing. It can cause a variety of medical and developmental problems, including short stature, failure of the ovaries to develop, heart defects, and certain learning disabilities. While there's no cure for Turner syndrome, some treatments can help manage its symptoms, such as hormone therapies and fertility treatments.

Turner syndrome is caused by a missing or partially missing X chromosome in some or all of the cells. The condition can result from several different genetic alterations:

- **Monosomy**: Complete absence of one X chromosome in all cells.
- **Mosaicism**: Some cells have only one X chromosome or an altered second X chromosome, while other cells have two normal X chromosomes.
- **X chromosome abnormalities**: Cells have one complete and one altered or missing X chromosome.

These genetic changes are usually random and not inherited from the parents. The loss or alteration of the X chromosome can occur either in the father's sperm, the mother's egg, or very early during fetal development.

No specific data on the prevalence of Turner syndrome in Vietnam. However, globally, Turner syndrome occurs in approximately 1 in 2000 to 1 in 3000 live female births. This rate is based on epidemiological and newborn genetic screening data from Europe, Japan, and the United States.

Next Step (after NIPT "positive" result):
Always confirm "positive" NIPT findings, with a DIAGNOSTIC Test (e.g., amniocentesis for Karyotyping, FISH or CGH Microarray).
Genetic Counseling is warranted.

2. NIPT LIMITATIONS & ACOG RECOMMENDATIONS:

2.1. LIMITATIONS:

Several factors may render NIPT less accurate. They may include but are not limited to: maternal, fetal and/or placental mosaicism, low fetal fraction, blood transfusion, transplant surgery, stem cell therapy, heparin therapy and the abnormal karyotype of biological parents or surrogate. The detection accuracy may be reduced to some extent for severely obese pregnant women (BMI >40), twin (or triple…) pregnancies.

2.1.1. Let's talk about placental mosaicism or confined placental mosaicism (CPM):
Confined placental mosaicism (CPM) is a condition where there is a discrepancy between the chromosomal makeup of the cells in the placenta and the cells in the fetus. Here are some key points about CPM:

i. Definition: CPM occurs when some cells in the placenta have a different number of chromosomes compared to the cells in the fetus. In other words, while the fetus may have a normal chromosomal makeup, the placenta may have cells with an abnormal number of chromosomes.

ii. Types of CPM:

- **Type 1**: The error occurs in trophoblast cells (cells that form the outer layer of the placenta). This type is often associated with normal pregnancy outcomes.
- **Type 2**: The error occurs in non-fetal cells of the inner cell mass, confined to the chorionic villus stroma. This type can sometimes be associated with delayed fetal growth.
- **Type 3**: Trisomic cells are present in both trophoblast cells and the villus stroma but are absent in the embryo. This type is more commonly associated with delayed fetal growth.

iii. Detection, prevalence: CPM is often detected during prenatal testing, such as chorionic villus sampling (CVS). CPM is relatively uncommon**.** The prevalence of CPM in twin pregnancies is estimated to be around **2-4%**. This is slightly higher than the prevalence in singleton pregnancies, which is approximately 1-2%. If trisomic cells are found in the placenta but not in the fetus, CPM is diagnosed.

iv. Mechanism: CPM can occur through mitotic non-disjunction (an error in cell division) or trisomic rescue (where a trisomic conception undergoes correction in some cells, leaving trisomic cells confined to the placenta)[1].

v. Impact on Pregnancy: Most pregnancies with CPM continue to term without complications, and the children develop normally. However, in some cases, CPM can lead to prenatal or perinatal complications, including a higher rate of pregnancy loss.

vi. Risk factors for developing CPM:

The exact causes of CPM are not fully understood, but several risk factors exist:

. Advanced Maternal Age.

. In Vitro Fertilization (IVF): Pregnancies conceived through assisted reproductive technologies, such as IVF, have a higher incidence of CPM.
. Previous Pregnancy Complications: A history of pregnancy complications, such as intrauterine growth restriction (IUGR) or stillbirth may increase the risk of CPM.
. Genetic Factors: specific genetic markers have not been clearly identified.
. Placental Factors: Abnormalities in placental development or function can lead to CPM. This includes issues like placental insufficiency or abnormal placental attachment.
. Environmental Factors: Exposure to certain environmental factors, such as toxins or infections, during early pregnancy may increase the risk of CPM.

Understanding CPM is important, especially when interpreting results from prenatal tests like CVS. If CPM is suspected, further testing, such as amniocentesis, may be recommended to confirm the chromosomal makeup of the fetus.

2.1.2. Limitations in Twin/Triplet Pregnancies:

i. Reduced Sensitivity and Specificity: NIPT is generally less accurate in twin pregnancies compared to singleton pregnancies. The combined positive predictive value (PPV) is lower, meaning there is a higher chance of false positives/negatives. NIPT is not typically offered for triplet or higher-order multiple pregnancies due to the complexity and reduced accuracy.

ii. Confined Placental Mosaicism: This condition, where the placenta has a different genetic makeup than the fetus, can lead to inaccurate results.

iii. **Difficulty in Identifying Affected Twin**: If an abnormality is detected, it can be challenging to determine which twin is affected.

Despite these limitations, NIPT remains a valuable tool for prenatal screening, especially when combined with other diagnostic methods.

2.2. ACOG 2024 RECOMMENDATIONS:

- *Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to* **all** ***pregnant patients regardless of maternal age or risk of chromosomal abnormality****. After review and discussion,*

every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.

- If screening is accepted, **patients should have one prenatal screening approach**, and should not have multiple screening tests performed simultaneously.
- Cell-free DNA is the **most** sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, **cell-free DNA testing is not equivalent to diagnostic testing**.
- **All patients should be offered a second-trimester ultrasound for fetal structural defects**, since these may occur with or without fetal aneuploidy; ideally this procedure is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein).
- Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.
- **Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected.** The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings such as fetal anomalies identified on ultrasound examination become evident.
- Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) **should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic**

counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.

- **If an enlarged nuchal translucency or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions and a comprehensive ultrasound evaluation** including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities.
- **The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test.** However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- **In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding and cell-free DNA, quad screen testing, or amniocentesis should be offered.** If aneuploidy testing is performed and is low risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended.
- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations.
- **Cell-free DNA screening can be performed in twin pregnancies.** Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the

small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13.

- Because preimplantation genetic testing is not uniformly accurate, **prenatal screening and prenatal diagnosis should be offered to all patients regardless of previous preimplantation genetic testing.**
- **The use of multiple serum screening approaches performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended** because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.
- Patients with unusual or multiple aneuploidies detected by cell-free DNA **should be referred for genetic counseling and maternal–fetal medicine consultation.**

NGUỒN THAM KHẢO:

1. Mai CT, Isenburg JL, Canfield MA, et al: National population-based estimates for major birth defects, 2010-2014. *Birth Defects Res* 111(18):1420-1435, 2019. doi: 10.1002/bdr2.1589
2. Morris JK, Mutton DE, Alberman E: Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. *J Med Screen* 9(1):2-6, 2002. doi: 10.1136/jms.9.1.2
3. Antonarakis SE: Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. Down Syndrome Collaborative Group. *N Engl J Med* 324(13):872-876, 1991. doi: 10.1056/NEJM199103283241302
4. Mutton D, Alberman E, Hook EB: Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. *J Med Genet* 33(5):387-394,

1996. doi: 10.1136/jmg.33.5.387
5. de Groot-van der Mooren MD, Scheerman BC, Rammeloo LAJ, et al: Neonatal mortality and morbidity in Down syndrome in the time of prenatal aneuploidy testing: A retrospective cohort study. *Eur J Pediatr* 182(1):319-328, 2023. doi: 10.1007/s00431-022-04686-3

6. Davidson YS, Robinson A, Prasher VP, Mann DMA: The age of onset and evolution of Braak tangle stage and Thal amyloid pathology of Alzheimer's disease in individuals with Down syndrome. *Acta Neuropathol Commun* 6(1):56, 2018. doi: 10.1186/s40478-018-0559-4
7. Kucik JE, Shin M, Siffel C, Marengo L, Correa A; Congenital Anomaly Multistate Prevalence and Survival Collaborative: Trends in survival among children with Down syndrome in 10 regions of the United States. *Pediatrics* 131(1):e27-e36, 2013. doi: 10.1542/peds.2012-1616
8. Madan-Khetarpal S, Arnold G. Genetic disorders and dysmorphic conditions. In: Zitelli BJ, McIntire SC, Nowalk AJ, eds. *Zitelli and Davis' Atlas of Pediatric Physical Diagnosis.* 8th ed. Philadelphia, PA: Elsevier; 2023:chap 1.
9. National Center for Advancing Translational Sciences. Genetic and Rare Diseases Information Center website. Trisomy
18. rarediseases.info.nih.gov/diseases/6321/trisomy-18. Updated February 2023. Accessed October 17, 2023.
10. Bacino CA, Lee B. Cytogenetics. In: Kliegman RM, St. Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM, eds. *Nelson Textbook of Pediatrics.* 21st ed. Philadelphia, PA: Elsevier; 2020:chap 98.
11. Goel N, Morris JK, Tucker D, et al: Trisomy 13 and 18-Prevalence and mortality-A multi-registry population based analysis. *Am J Med Genet A* 179(12):2382-2392, 2019. doi: 10.1002/ajmg.a.61365
12. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics; Committee on Genetics; Society for Maternal-Fetal Medicine: Screening for fetal chromosomal abnormalities: ACOG Practice Bulletin, Number 226. *Obstet Gynecol* 136(4):e48-e69, 2020. doi: 10.1097/AOG.0000000000004084.
13. Meyer RE, Liu G, Gilboa SM, et al: Survival of children with trisomy 13 and trisomy 18: A multi-state population-based study. *Am J Med Genet A* 170A(4):825-837, 2016. doi: 10.1002/ajmg.a.37495.
14. Samango-Sprouse C, Lasutschinkow PC, Mitchell F, et al. 49,XXXXY syndrome: A study of neurological function in this uncommon X and Y chromosomal disorder. Am J Med Genet A. 2021;185(12):3557-3566. doi:10.1002/ajmg.a.61742
15. Samango-Sprouse, CA, Counts DR, Tran SL, Lasutschinkow PC, Porter GF, Gropman

AL. Update on the clinical perspectives and care of the child with 47,XXY (Klinefelter syndrome). Appl Clin Genet. 2019;12:191-202. Published 2019 Oct 23. Doi:10.2147/TACG.S180450
16. Samango-Sprouse, C, Hall, MP, Kırkızlar, E, Curnow, K, Demko, Z, Lawson, P, Gross,S, Gropman, A. Incidence of X and Y chromosomal aneuploidy in a large childbearing population. Public Library of Science. Plos ONE. 2016; April 11(8), 1-11. doi:10.1371/journal.pone.0161045.
17. Clinical manifestations and diagnosis of Turner syndrome – UpToDate.com
18. https://www.nichd.nih.gov/health/topics/turner/conditioninfo/risk
19. https://stacks.cdc.gov/view/cdc/131789
20. https://www.eymj.org/pdf/10.3349/ymj.2022.0143
21. The test performance of the non-invasive prenatal test in multiple gestations and vanishing twin pregnancies. 21st WORLD CONGRESS IN FETAL MEDICINE Lisbon, Portugal, 23-27 June 2024
22. Vanishing twin syndrome affects NIPT results (contemporaryobgyn.net)
23. Landy HJ, Keith LG. The vanishing twin: a review. Hum Reprod Update. 1998;4(2):177-183. https://doi.org/10.1093/humupd/4.2.177
24. Zamani Z, Parekh U. Vanishing Twin Syndrome; 2022.
25. Soler A, Morales C, Mademont-Soler I, et al. Overview of chromosome abnormalities in first trimester miscarriages: a series of 1,011 consecutive chorionic villi sample karyotypes. Cytogenet Genome Res. 2017;152(2):81-89. https://doi.org/10.1159/000477707
26. Rose NC, Kaimal AJ, Dugoff L, Norton ME; American College of Obstetricians and Gynecologists. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. Obstetrics & Gynecol. 2020;136(4):e48-e69.
27. Mackay, D., Bliek, J., Kagami, M. *et al.* First step towards a consensus strategy for multi-locus diagnostic testing of imprinting disorders. *Clin Epigenet* **14**, 143 (2022). https://doi.org/10.1186/s13148-022-01358-9
28. Jasmin Beygo, Deniz Kanber, Thomas Eggermann, Matthias Begemann; Molecular testing for imprinting disorders**. Medizinische Genetik.** https://doi.org/10.1515/medgen-2020-2048
29. ACOG.org. Policy Priorities. Current ACOG Guidance. NIPT Summary of Recommendations. 2024. https://www.acog.org/advocacy/policy-priorities/non-invasive-prenatal-testing/current-acog-guidance.
30. Bui TH, Iselius L, Lindsten J (1984). "European collaborative study on prenatal diagnosis: mosaicism, pseudomosaicism and single abnormal cells in amniotic fluid

cell cultures". Prenatal Diagnosis. **4** (7): 145–62.
doi:10.1002/pd.1970040710. PMID 6463032. S2CID 6708983.
31. Kalousek DK, Dill FJ (August 1983). "Chromosomal mosaicism confined to the placenta in human conceptions". *Science*. **221** (4611): 665–7.
Bibcode:1983Sci...221..665K. doi:10.1126/science.6867735. PMID 6867735.
32. Robinson WP, Barrett IJ, Bernard L, et al. (April 1997). "Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increased risk of fetal intrauterine growth restriction". *American Journal of Human Genetics*. **60** (4): 917–27.
PMC 1712477. PMID 9106539.
33. Wolstenholme, J. (1996). "Confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16, and 22: Their incidence, likely origins, and mechanisms for cell lineage compartmentalization". *Prenatal Diagnosis*. **16** (6): 511–524.
doi:10.1002/(SICI)1097-0223(199606)16:6<511::AID-PD904>3.0.CO;2-8.
PMID 8809892.
34. Benn, Peter A.; Lillian Y. F. Hsu (2004). "Prenatal Diagnosis of Chromosomal Abnormalities through Amniocentesis". In Aubrey Milunsky (ed.). *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment*. Baltimore: Johns Hopkins University Press. pp. 214–296. ISBN 978-0-8018-7928-9.
35. Gardner, R. J. M.; Grant R. Sutherland (1996). *Chromosome abnormalities and genetic counseling*. Oxford: Oxford University Press. ISBN 978-0-19-510615-2.
36. Tyl H. Taylor, Susan A. Gitlin, et al., The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans, *Human Reproduction Update*, Volume 20, Issue 4, July/August 2014, Pages 571–581,
https://doi.org/10.1093/humupd/dmu016.
37. Francesca Romana Grati, Jose Ferreira, et al., Outcomes in pregnancies with a confined placental mosaicism and implications for prenatal screening using cell-free DNA; GENETICS in MEDICINE | Volume 22 | Number 2, pages 309-316 | February 2020.
38. Sabbagh, Q., Larrieux, M., Schneider, A. *et al.* Confined placental mosaicism is a diagnostic pitfall in dystrophinopathies: a clinical report. *Eur J Hum Genet* (2024).
39. NIPT may not be as effective for twin pregnancies (contemporaryobgyn.net)
40. Non-invasive prenatal test to screen common trisomies in twin pregnancies | Molecular Cytogenetics | Full Text (biomedcentral.com).