Belgian guidelines for fetal genome-wide sequencing in ongoing pregnancies.

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Genome-wide sequencing, including (clinical) exome or whole genome sequencing, is a valuable diagnostic tool for ongoing pregnancies*, when a fetal condition of unknown etiology is suspected to be genetic in origin.

*Diagnostic testing in a fetus with a suspected genetic condition, but for whom the pregnancy was interrupted based on the clinical findings, is outside the scope of these guidelines. In this situation, the standard postnatal approach is followed.

INDICATIONS

Genome-wide sequencing can be offered for fetuses with ultrasound anomalies for whom a genetic diagnosis is essential for further pregnancy or early neonatal management. The following conditions must be met:

- All cases should be reviewed in a multidisciplinary team including a clinical geneticist.
- In all instances, expert fetal ultrasound examinations are required to provide the best possible phenotypic evaluation. When beneficial, fetal MRI may be performed.
- Fetal chromosomal imbalances should have been excluded by means of chromosomal microarray.
- Single-gene sequencing can be performed if ultrasound examinations are highly suggestive of a specific monogenic disorder.

Guidelines should be regularly reviewed as we will gather more knowledge on which ultrasound anomalies have the highest diagnostic rate in prenatal genome-wide approaches.

PRE-TEST COUNSELING

Pre-test counseling by a clinical geneticist is mandatory. The counseling should at least include:

- Explanation of the test, including the possibilities and limitations.

  NOTE: a normal genome-wide analysis does not exclude a genetic cause, since certain genetic defects are not detected, e.g. imprinting disorders, unstable trinucleotide repeats, some cases of mosaicism, deep intronic and non-coding mutations.

- Information on the different possible test results.

This information is summarized in an information leaflet.

DATA ANALYSIS

Trio analysis, i.e. simultaneous analysis of the fetus and both parents, **is required*** for genome-wide sequencing methods. This approach promotes speed and accuracy of variant classification and interpretation.

Detailed phenotypic information must be communicated to the laboratory, preferably in HPO terms (https://hpo.jax.org/app/).

Fetal DNA can be extracted from villous trophoblast cells, amniotic fluid cells or cord blood. Before proceeding with fetal genome-wide sequencing, maternal contamination has to be excluded.

*duo analysis (fetus and mother) can be considered if the father is not available.

Filtering strategy:

- For certain recognizable phenotypes, a predefined *in silico* phenotype-driven panel of genes can be analyzed. The indication of a restricted gene panel versus an unbiased approach should be discussed in a multidisciplinary team guided by the clinical geneticist.
- When the expert ultrasound findings do not allow the choice of a gene panel or when the results of this panel are normal, genome-wide trio filtering is performed exploring different modes of inheritance depending on the context.
- In all instances, genotype-phenotype correlation is essential for variant interpretation.

TURNAROUND TIME

The turnaround time should not exceed **8 weeks** for an ongoing pregnancy. Each laboratory should optimize its procedures to achieve the shortest TAT possible.

COMMUNICATION OF PRIMARY RESULTS

- **Pathogenic (class V) and likely pathogenic (class IV) variants** with known effect on gene function and which fit with the fetal phenotype and the inheritance mode are communicated.
- Benign (class I) and likely benign (class II) variants are not communicated.
- **Variants of uncertain significance (class III)** are in principle not communicated. However, laboratories should consider reporting variants in known disease genes that a) fit the fetal phenotype, b) are expected to show the same pathomechanism as known pathogenic variants and c) arose as *de novo* events or are detected *in trans* with a pathogenic or likely pathogenic variant (Monaghan et al., 2020) and for which further clinical exams (ultrasound, MRI, etc) are recommended to refine variant classification, possibly leading to a genetic diagnosis (upgrade of the variant to class IV/V).

In case of uncertainty, the *ad hoc* committee, consisting of clinical and laboratory geneticists of the BeSHG prenatal working group (http://www.beshg.be/index.php?page=workgroups), can be consulted for advice before the final protocol is issued.

SECONDARY FINDINGS

Secondary findings are defined as class V and class IV variants in genes which are not related to the test indication, but are intentionally analyzed, i.e. the American College of Medical Genetics (ACMG) gene list (Kalia et al., 2016).

No systematic search for secondary findings, unrelated to the fetal phenotype, will be performed.

INCIDENTAL FINDINGS

Incidental findings are defined as class V and class IV variants identified incidentally in genes unrelated to the primary test indication. Detection of these variants will depend on the filtering strategy performed by each laboratory. Settings should be optimized to minimize the detection of these variants without jeopardizing the detection of primary results.

Incidental findings should be managed according to the guidelines outlined below.

De novo fetal incidental findings

Highly penetrant class V and class IV variants detected in genes unrelated to the primary test indication, but known to cause moderate or severe childhood onset disorders, should be reported.

Inherited incidental findings

Four categories of inherited incidental findings can be distinguished:

- Late-onset disorder with clinical utility: class V and IV variants causing late onset disorders, typically cancer caused by mutations of a tumor suppressor gene, will be communicated if undeniable health benefit can be expected (see the latest guidelines from the ACMG, Kalia et al., 2016).
- Late onset disease without actionable possibilities will not be reported.
- Carriership for X-linked recessive disorder will be communicated.
- Carriership for autosomal recessive disorders will not be communicated.

POST-TEST COUNSELING

Post-test counselling by a clinical geneticist is highly recommended and mandatory in case of an abnormal result.

REFERENCES

Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020 Apr;22(4):675-680. doi: 10.1038/s41436-019-0731-7. Epub 2020 Jan 8. PMID: 31911674.

Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017 Feb;19(2):249-255. doi: 10.1038/gim.2016.190. Epub 2016 Nov 17. Erratum in: Genet Med. 2017 Apr;19(4):484. PMID: 27854360.

Version history

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V2021	10.12.2020	05.02.2021	New document: no history available