Title of the guideline:

Whole Exome Sequencing in Prenatal Diagnostics

Members of the guideline group:

Naja Becher, MD, PhD, Dept. of Clinical Genetics, Aarhus University Hospital (AUH)
Caroline B Miltoft, MD, MD, Dept. of Obstetrics and Gynecology, Copenhagen University Hospital, Rigshospitalet
Lars H Pedersen, MD, PhD, Dept of Obstetrics and Gynecology, AUH
Olav Bjørn Petersen, MD, PhD, Dept of Obstetrics and Gynecology, AUH
Ida Vogel, MD, PhD, D. MSc, Department of Clinical Genetics, AUH

Korrespondance:

Olav Bjørn Petersen, olavpete@rm.dk

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Problem

Most pregnant couples are left without a specific diagnosis for the detected fetal malformations, and therefore in a very uncertain situation considering the outcome of the pregnancy and the prognosis for the child.

Evidence/strength	*evidence*
There is limited knowledge of Whole exome sequencing (WES) in fetal disease. The consequences of implementing WES in prenatal diagnostics are yet to be evaluated.	-

Prenatal WES can be considered in cases where pregnant couples after CMA are left without a specific diagnosis for the detected fetal malformations and when there is a wish for further diagnostics.	-

Prenatal WES should be preceded by consultation with a clinical genetics specialist including written consent.	-

Background:

Since 2004, a national prenatal screening program has been offered to all pregnant women including a combined first trimester screening risk assessment for certain fetal chromosomal anomalies and a second trimester ultrasound scan for fetal anomalies[1]. The prevalence of a fetal anomaly imposing increased risk of intrauterine or neonatal death, chronic disease and increased infant mortality, or mental or physical disabilities is about 1%[1]. Compared to other North European countries, the Danish prenatal screening program has a very high and consistent participation of pregnant women (> 90%)[2].

Studies have shown that replacing conventional chromosome analyses with chromosomal microarray (CMA) additionally identifies a wide range of microdeletion- and microduplication syndromes. The likelihood of detecting a significant chromosomal aberration when applying CMA is around 16 % in fetuses with malformations [3-5]. Though this has been a diagnostic breakthrough, it still leaves most pregnant couples without a specific diagnosis for the detected fetal malformations, and therefore in a very uncertain situation considering the outcome or prognosis for the child.

These years, the diagnostic techniques change enormously. The implementation of Whole Exome Sequencing (WES) in diagnosing child diseases has shown significant diagnostic strength over CMA and panels[6].

In the prenatal setting, Fu et al. recently described that a specific genetic diagnosis was reached retrospectively in 24 % (47/196) of fetuses with malformations when applying WES after a normal karyotyping and a normal CMA-result[7]. The likelihood of finding a significant genetic variant depended on the number of malformations (single; 22.3 % versus multiple; 30.8 %) and the organ system involved (skeletal (30 %), urogenital (23.1 %), dysmorphic (23.5 %), CNS (23.1 %), cardiovascular (20.6 %) and gastrointestinal (none)). Fetuses with sonographic soft markers were not included. In the majority of cases (80 %) the detected genetic variants had occured *de novo* in the fetus (none of the parents carried the variant). Fu et al. conclude that WES-based exome analysis is a promising method for the identification of genetic variants that cause structural abnormalities in fetuses with normal results on karyotype and CMA.

Best et al. summarized that an underlying genetic etiology is identified in up to 40 % of fetuses with malformations by a combination of conventional genetic testing including

quantitative fluorescence polymerase chain reaction, fluorescence in situ hybridization, karyotyping and CMA[8]. They then reviewed studies on WES for prenatal diagnosis from 2014 to May 2017. Sixteen studies included five or more cases of WES with a wide range of inclusion criteria, different methods of WES (proband-only or trio-WES) and were not always preceded by karyotyping or CMA. The overall diagnostic rate ranged from 6.2-80 %. However, a higher diagnostic rate was reported among fetuses with multiple anomalies. The two largest studies (N = 168 (Wapner et al[9]) and N = 259 (Mullan et al[10])) reported a diagnostic rate of 14.3 % and 16.0 %, respectively, among fetuses with multiple anomalies.

Several other studies have evaluated the use of WES in a prenatal setting[11-14], and the number of studies in this field is rapidly rising. Recently, a Joint Position Statement from the International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis was published[15]. This statement recommends fetal sequencing in a current pregnancy with a single fetal major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic etiology with no genetic diagnosis after CMA (including pregnancies with recurrence of similar anomalies) or following a multidisciplinary review and consensus. Furthermore, parental sequencing is recommended in case of preconception counselling with a history of an undiagnosed fetus (or child) affected with a major single or multiple anomalies suggestive of a genetic aetiology to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype. Fetal sequencing is also recommended in families with a history of recurrent stillbirths of unknown etiology after karyotype and/or CMA, where the fetus in the current pregnancy has a recurrent pattern of anomalies. To date, no evidence supports routine testing on fetal tissue obtained from an invasive prenatal procedure for indications other than fetal anomalies. The authors recognize that scientific and clinical knowledge about the use for prenatal diagnostic evaluation for fetal disease and malformations is still incomplete and constantly changing. Evolving research results are likely to inform further refinement of the recommendations.

Significance of WES:

Although, knowledge of WES in fetal disease is limited, WES gives promises of diagnosis, informed decision-making and treatments[8]. The side effects of implementing WES in prenatal diagnosis are increased need for counseling, secondary findings (genetic variants unrelated to the primary presentation) and incidental findings (potential clinical relevance but unrelated to the purpose of the test) and dealing with uncertain results in difficult situations[8]. Also, WES can imply difficulties in establishing the pathogenicity of detected variants which accentuates the importance of accurate fetal phenotyping.

Over the last two years, AUH have implemented fetal WES for a limited number of cases, and especially after termination of pregnancy. In AUH 4 out of 12 (33 %) fetal exome

analyses provided a diagnosis for the fetus in cases not solved by CMA. WES analysis revealed unexpected fetal diagnoses in a fetus thereby completely altering the premises for decision-making regarding future pregnancies – now based on a specific diagnosis and knowledge of recurrence risk.

The ultimate objective of implementing prenatal WES is to cost-effectively improve the treatment in pregnancies and in newborns through an accurate diagnosis provided by this new genetic technology. In a pediatric setting with infants with suspected monogenic disorders, use of WES early in the diagnostic pathway has shown to more than triple the diagnostic rate for one-third the cost per diagnosis[16 17].
Hereby we ensure the highest quality in treatment and care and, not least, shorten the process of diagnosing disease. This is a change in paradigm while shifting the postnatal diagnostic effort into the management of an on-going pregnancy enabling better treatment decisions in the neonatal period.

WES facts:

Prenatal WES is performed on DNA from the placenta after CVS or DNA from amniocytes after amniocentesis. The local clinical genetic department can specify the amount of material needed. DNA from the placenta/fetus is analyzed together with DNA from both parents ("Trio-analysis"). Parental samples are used in the filtering of fetal data according to expected inheritance. Parental samples are *not* analyzed individually for genetic disease and a separate result/report on the parents is not provided. Time to get a result is around 15 working days.

WES can detect single nucleotide variants (SNV) but not deletions and duplications. Therefore, CMA should always be performed before WES. Also, technical limitations imply that repeat expansions, 1-2 exon deletions within genes and SNVs in poorly covered regions are not detected. Intronic variants are only revealed by Whole Genome Sequencing (WGS). This implies that a normal result after prenatal WES reduces, but does not eliminate the risk of a genetic disease in a fetus with malformations.

Diagnostic yield

CMA and karyotyping detects aberrations in about 20 % of fetuses with malformations. Additional diagnostic yield after WES is around 30 % depending on the malformation. CMA together with WES may therefore provide a diagnosis in around 50 % of the cases.

Indications for prenatal WES

WES can be considered in fetuses with a likely genetic disorder, where a diagnosis has not been obtained by other genetic tests[18]. At the moment, offering WES to patients who carry a chromosomally normal fetus with malformations on ultrasound is suggested to best balance diagnostic yield, ethical considerations and costs[12]. This is in correspondence with the recently published Joint Position Statement from the International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal

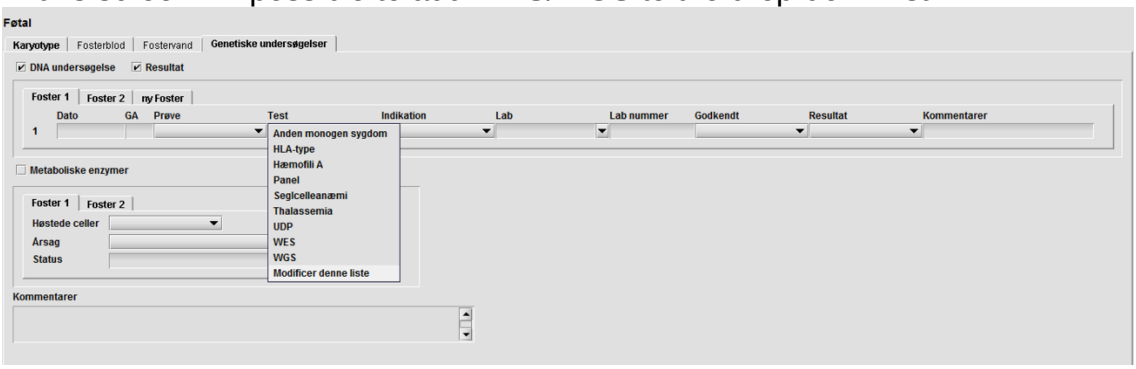
Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis[15].

Counseling issues

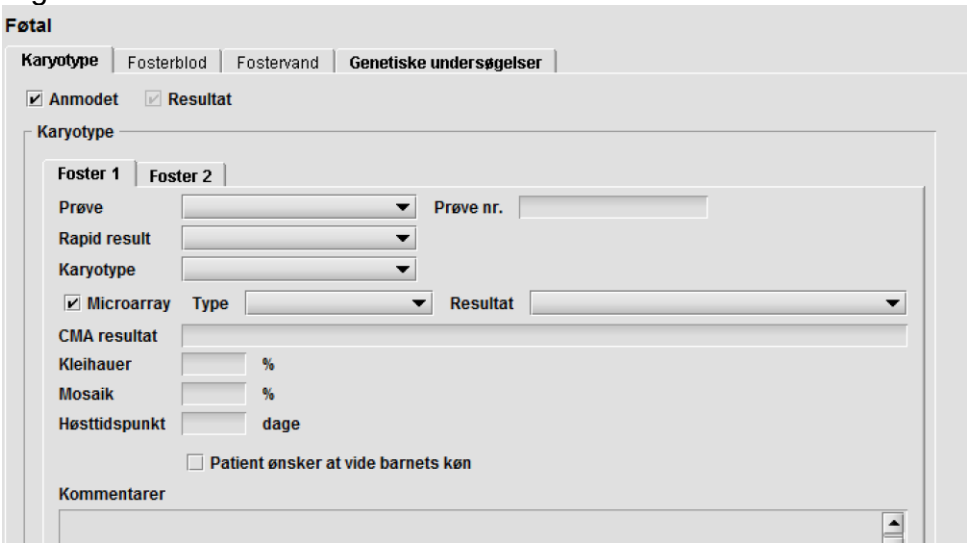
It is recommended that prenatal WES is offered in collaboration between the referring fetal medicine unit and the local clinical genetic department. WES should be preceded by a pre-test consultation by a clinical genetics specialist including written consent (appendix 1) and written patient information regarding the possible outcomes of a WES analysis (appendix 2). Also the WES-result should be communicated by a clinical genetics specialist. The recently published Joint Position Statement strongly recommends thorough pre-test counseling, and multidisciplinary post-test counseling[15].

Registration of WES results in Astraia

Today, registration of WES/WGS results is possible through the Fetal Karyotype/Genetic testing screen - see below
In this screen it's possible to add WES/WGS to the drop-down list "Test"



For future registrations, we have requested a change/add on in Astraia, with addition of a checkbox "Sequencing" that opens up two drop down boxes: "Type" (here WES/WGS could be added), and "Result", and a test line "Sequencing results" similar to the CMA registration seen below:



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Appendices:

Appendiks 1: Consent form in Danish

Samtykke til udvidet gen-undersøgelse af fosteret

	Gravide	**Partner**
Cpr.nr.		
Navn		

Jeg ønsker, at DNA fra **graviditet, min partner og jeg** opbevares og analyseres med udvidet gen-undersøgelse (trio exomsekventering) for følgende sygdom/fund:

_____

Jeg er informeret omkring resultater og begrænsninger ved udvidet gen-undersøgelse.

Jeg accepterer, at der er en lille risiko for, at der gøres fund, som ikke er relateret til den tilstand, der undersøges for (sæt ét kryds):

_____ Jeg ønsker *kun* svar på den tilstand, som er årsag til analysen

_____ Jeg ønsker *også* information om andre fund end det, der undersøges for, hvis det får betydning for min eller mine nuværende/kommende børns sundhed

Af hensyn til kvalitetssikring vil laboratoriet, der udfører sekventeringen, gerne opbevare data i 10 år. For at gøre det lettere at finde sygdomsdisponerende forandringer i arvemassen hos andre mennesker, vil laboratoriet gerne placere dine data i en anonym database, hvor der samles sekventeringsdata fra mange hundrede danskere.

_____ Mine anonymt opbevarede gendata kan bruges i forskningsprojekter til gavn for andre mennesker. Sådanne forskningsprojekter skal først godkendes af Videnskabsetisk Komité.

_____ _____

Underskrift af patient/dato Navn med blokbogstaver (patient)

_____ _____

Underskrift af læge/dato Navn med blokbogstaver (læge)

Appendiks 2: Written patient information in Danish

Udvidet gen-undersøgelse af fosteret

Hvornår tilbydes du gen-undersøgelse af fosteret?

Gen-undersøgelse af fosteret tilbydes ved fund af misdannelser eller stor nakkefold.

Hvad er gener?

Menneskets arvemateriale indeholder ca. 20.000 gener. Generne bestemmer de arvelige egenskaber, dvs. indeholder de koder, eller "opskrifter", som fortæller cellerne hvordan kroppen skal udvikle sig og fungere. Hvis koden i et gen er ændret, kan det medføre misdannelser eller øget risiko for sygdom.

Hvad er en udvidet gen-undersøgelse?

Med en gen-undersøgelse (også kaldet exom) leder man efter nyopstående eller nedarvede ændringer i fostrets gener, som kan være med til at forklare de tegn på sygdom, som fosteret har.

Selvom alle fosterets 20.000 gener undersøges fra ende til anden, så er det kun de forandringer, der svarer til de fund, der har været beskrevet hos fostret, som søges frem og dermed kommer til vores opmærksomhed.

Hvorfor skal blodprøver fra begge forældre også undersøges?

Forældrenes blodprøver er nødvendige for at kunne fortolke betydningen af eventuelle gen-varianter, som findes hos fosteret: I nogle tilfælde finder vi gen-varianter, som vi endnu ikke kender betydningen af. I sådanne tilfælde undersøger vi, om gen-varianten også findes hos en af forældrene. Hvis en gen-variant med ukendt betydning er nedarvet fra en rask far eller mor, vil der være tale om en normal variant, som ikke forårsager sygdom.

Hvad er fordelene ved en gen-undersøgelse hos fosteret?

Gen-undersøgelsen åbner mulighed for, at vi kan stille en specifik diagnose hos fosteret. Resultatet af undersøgelsen kan derfor hjælpe os, når vi skal rådgive jer om resten af graviditeten, samt i forhold til om vi skal tage særlige hensyn til barnet efter fødslen. Analysen kræver konsultation og rådgivning på en klinisk genetisk afdeling.

Hvilke svar kan gen-undersøgelsen munde ud i?

1) Resultatet af undersøgelsen kan være normalt. Vi har i den situation ikke fundet gen-varianter hos fosteret, som kan forklare fosterets misdannelser eller store nakkefold.

2) Resultatet af undersøgelsen kan vise, at fosteret har en gen-variant, som med sikkerhed kan forklare de tegn på sygdom, som fosteret har.

3) Resultatet af undersøgelsen kan vise, at fosteret har en gen-variant, som vi med vores nuværende lægefaglige viden ikke kender betydningen af.

Hvornår får du svar på prøven?

Når prøven er taget, bliver den sendt til Klinisk Genetisk Afdeling på Aarhus Universitetshospital, som varetager analysen. De fleste svar vil være klar inden for 15 hverdage. Du bliver kontaktet af personalet fra afdelingen, hvor prøven blev udtaget, så snart de modtager svar.

Klinisk Genetisk Afdeling