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Chapter 2

Introducing WISECONDOR for noninvasive prenatal diagnostics

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Noninvasive prenatal testing is a relatively new screening method for the detection of fetal chromosome abnormalities using next-generation sequencing (NGS) of fetal DNA in maternal blood. Recently, the introduction of a new tool called WIthin SamplE COpy Number aberration DetectOR (WISECONDOR) marked a new era in prenatal screening. WISECONDOR detects copy number aberrations at a resolution that is almost comparable to classic karyotyping and requires only shallow sequencing, making noninvasive prenatal screening cost-effective. This emphasizes the role of NGS in the daily clinical practice of prenatal diagnosis and will require reorganization of clinical genetics laboratories to accommodate NGS. For prenatal diagnostics, WISECONDOR introduces an exciting development that will substantially improve the information provided to pregnant couples regarding their fetus’s wellbeing.

The use of chorionic villus sampling and amniocentesis for fetal chromosome analysis provides reliable results but is associated with a risk for miscarriage and pain [1]. While it has been known for years that maternal plasma contains fetal cell-free DNA [2, 3], methods to use this source of fetal DNA have only recently become available due to the development of next-generation sequencing (NGS) [4]. Today, several NGS-based approaches are available to identify fetuses with chromosomal aberrations by sequencing a maternal plasma sample [5]. This has drastically changed current prenatal diagnostics, and consequently noninvasive prenatal testing (NIPT) already resulted in a substantial decrease in the number of invasive prenatal procedures performed.

To keep costs of the noninvasive prenatal test in line with current practice, several attempts have been made to adapt current NGS-based approaches to comply with low sequence coverage (number of sequence reads per genomic location). Until now, sequencing variability across the genome and between samples complicated the accurate detection of chromosomal abnormalities, especially for cases other than Down’s syndrome cases such as trisomy 13 and 18 [6]. Recently, with the introduction of WIthin SamplE COpy Number aberration DetectOR (WISECONDOR) [7], an improvement in the detection accuracy of trisomy 13, 18 and 21 cases with low sequence coverage was realized. Moreover, WISECONDOR can detect such copy number changes without re-sequencing (normal) reference samples with every run. Smaller unbalanced aberrations, such as segmental chromosome deletions and duplications, could only be detected with deep sequencing [8]. With the introduction of WISECONDOR, this can now be done using low sequence coverage. In fact, WISECONDOR allows detection of chromosomal copy number changes at a resolution comparable to the currently used microscopic chromosome analysis, but without the risks. This can be done at nearly the same cost as chromosome analysis but in an automated fashion and with less cytogenetic technicians. Together with growing evidence that NIPT has significantly lower false-positive rates and higher positive predictive values for detection of trisomy 21 and 18 [9], the introduction of WISECONDOR matures
NIPT with an important step. However, as with all NIPTs, WISECONDOR is done on placental DNA, and therefore it is recommended to confirm any abnormality detected by fetal chromosome analysis using an invasive test, preferably amniocentesis, as this test is the best representation of the fetal genome.

WISECONDOR calculates whether the number of sequencing reads for a given genomic region is higher (amplification) or lower (deletion) compared to normal (diploid). The breakthrough of the WISECONDOR method is the result of the use of a within-sample NGS read frequency correction algorithm. First, the genome is split into fixed-size regions, bins. The bins that behave alike in terms of NGS read frequencies over different samples are used to create a reference bin set for each bin. Then, for each bin, the read frequency can be corrected based on the statistics of its reference bin set in the same sample. Chromosomal gains and losses are identified by detecting statistically aberrant corrected read frequencies relative to the reference bin set in several adjacent bins. For reliable reference sets, WISECONDOR requires that the reference samples should not contain aberrations, while they should provide enough, and varied, information between samples to learn how read frequencies for the different regions co-vary. This thus implies that the set of reference samples should be large enough and show normal technical variation in read frequencies.

WISECONDOR assigns a score to each bin (z-score): large positive scores represent evidence for amplifications (duplications) and large negative scores for deletions. The advantage of this approach is that WISECONDOR not only detects and plots significantly aberrant chromosomal regions ($z < -3$, $z > 3$) but also plots the scores per region. This allows geneticists to closely examine the aberrant chromosomal region. This is extremely important for the detection of small aberrations (with little information from neighboring bins) as well as helpful for long subtle aberrations caused by mosaicism and for samples with a relatively small fetal DNA fraction.

Clearly, as read depth frequencies have strong natural fluctuations along the genome, WISECONDOR can only make reliable calls for relatively large regions. For a cost-effective read coverage of 0.5–1.0x, this implies that aberrations smaller than 20 Mb cannot be reliably detected. However, with the rapidly decreasing costs for NGS, it is anticipated that increased read coverage will soon be cost-effective, allowing the detection of smaller sized copy number aberrations using WISECONDOR.

A possible limitation of WISECONDOR is that it assumes that the sample contains sufficient fetal DNA to allow the identification of fetal chromosomal aberrations. There is currently no reliable method to determine the fraction of fetal DNA: for male fetuses, the fraction of the Y chromosome can be calculated based on the NGS data or using quantitative PCR for the Sex Determining Region Y (SRY) gene. For female fetuses, the ratio of fetal and maternal single-nucleotide polymorphism genotypes can be used to determine the fetal fraction, but this requires much higher sequence depth. In our experience, even qPCR for SRY is relatively imprecise. Consequently, additional methods should be evaluated to accurately determine the fraction of fetal DNA in a maternal plasma sample, such as using methylated markers [10]. An additional limitation is that
aberrations in the sex chromosomes were ignored during the development of the current version of WISECONDOR due to their low mappability.

An important consequence of WISECONDOR is that it also detects aberrations present in the maternal (germline) DNA, which will result in extreme \( z \)-scores as the main part of the DNA tested is maternal. Currently, WISECONDOR does not test the likelihood that aberrations are fetal or maternal in origin, which requires the contribution of Medical Geneticists’ to the analysis of WISECONDOR’s result. In our experience, WISECONDOR detects maternal aberrations in 1% of the samples requiring careful design of the reporting protocols and counseling regarding the possibility of identifying maternal disease including malignancies.

A more practical consequence of using WISECONDOR for prenatal screening, or NIPT in general, is that it generates a large amount of data, even with low read coverage. Since current practice requires long-term storage of diagnostic data, a flexible integration of information technology within the clinical genetics environment is required. Data storage in ‘the cloud’ could be a practical solution for this problem; however, the storage of medical data at a third-party data center is currently not acceptable and a long-term protection of privacy is required.

In conclusion, the introduction of WISECONDOR sets a new milestone in prenatal diagnosis as it allows the detection of fetal chromosomal aberrations using fetal DNA in maternal blood at a lower price and with a higher resolution than the NIPTs used currently. The declining costs of NGS will allow a further increase in detection resolution in the near future. The high resolution and low price will soon enable providing NIPT to all pregnant women. As putative maternal medical conditions may be detected; an ethical debate should be started on whether and how these should be reported is subject to ethical debate. Institutions that decide to apply this technique will also need to solve some practical issues including the acquisition and organization of the computational infrastructure needed to analyze large data sets and the safe storage of the large amounts of information obtained by this technique. Currently, many clinical genetics laboratories in the Netherlands are already using WISECONDOR in their daily practice, and since WISECONDOR is an open-source set of scripts, it is possible to integrate additional and dedicated in-house analysis tools into it.

In the next 5 years, we expect NGS to provide higher resolutions at lower prices, thus providing a replacement to prenatal chromosome analysis and become available as a screening test for all pregnant women. This will require the medical geneticists and the gynecologists to become familiar with this technique so they can provide accurate genetic counseling that will help patients to make informed decisions. The increase in resolution may identify both maternal and fetal aberrations that may not be always interpretable. This will require an informed consent including ‘the right not to know’.
References


