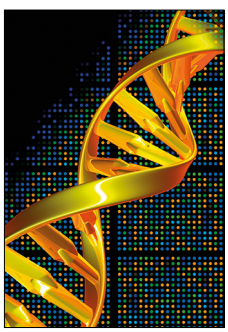
Introduction of genomics into prenatal diagnostics



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See Articles pages 747 and 758

The introduction of new technologies presents unique challenges for invasive prenatal genetic testing. Clinical phenotyping is indirect, many traits cannot be assessed in utero, and rapid turnaround times are crucial. In their Articles in *The Lancet*, Jenny Lord and colleagues[1] and Slavé Petrovski and colleagues[2] explore the relative value of whole-exome sequencing (WES) in the prenatal assessment of fetal structural anomalies. These studies will have immediate translational implications; they provide roadmaps for clinical interpretation of WES and crucial benchmarks of diagnostic yields for health-care consumers, providers, and payers alike.

Karyotyping to visualise megabase-scale copy number variants (CNVs) and balanced chromosomal abnormalities was the mainstay of cytogenetic testing for several decades. In the early 2000s, microarray technology enabled access to submicroscopic CNVs,[3-5] and discoveries over the subsequent decade demonstrated the clinical usefulness of this technology.[6] A seminal study[7] in 2012 marked a transition point for prenatal testing, in which microarray of 4406 pregnancies revealed a 6% yield of clinically significant CNVs in fetal structural anomalies; however, the improved resolution came at a cost since microarray cannot detect balanced chromosomal abnormalities.[8-10] At about the same time, WES transformed gene discovery research by providing a method to capture and sequence most of the protein-coding genome.[11,12] These two independent studies by Lord and colleagues[1] and Petrovski and colleagues[2] now apply WES in the prenatal setting, and they arrive at almost identical conclusions: routine WES will enable more molecular diagnoses of causal genetic variation in fetuses with structural anomalies relative to conventional cytogenetic methods.

Early studies[13] of WES in fetuses with structural anomalies reported diagnostic variants (ie, genetic alterations likely to be associated with the observed ultrasound anomaly) in 6·2–80% of these fetuses, dependent on the selection of fetal anomalies being studied and interpretation criteria applied. In the presence of such variability, the studies by Lord and colleagues[1] and Petrovski and colleagues[2] are notable for their systematic designs and transparent analyses. The larger study from the PAGE Consortium[1] evaluated WES in a prospective cohort of 610 fetuses with structural anomalies that were detected by ultrasound (596 fetus–parent trios, 14 fetus–parent dyads). The second study from Petrovski and colleagues[2] screened consecutive fetuses with structural anomalies at Columbia University from 2015 to 2017 and performed WES on 234 fetus–parent trios. Both studies pre-screened fetuses for pathogenic chromosomal abnormalities or CNVs, and plausible pathogenic variants from WES were reviewed by multidisciplinary teams to determine final molecular diagnoses. These carefully designed studies found remarkably consistent results: the PAGE study[1] reported a clinically significant genetic variant in 8·5% of fetuses, with an additional 3·9% of fetuses harbouring variants of possible clinical significance (12·4% total), and the study by Petrovski and colleagues[2] found a diagnostic genetic variant in 10·3% of fetuses.

In the PAGE study,[1] the proportion of fetuses with phenotype-specific diagnostic genetic variants were found to range from 3·2% (in fetuses with increased nuchal translucency) to 15·4% (in those with multisystem fetal structural anomalies). Petrovski and colleagues[2] found that these proportions ranged from 6·3% in fetuses with a single affected anatomical system to 18·9% in fetuses with multisystem fetal structural anomalies. The greatest source of pathogenic variation was dominant de-novo mutations in both studies (representing 32 [61·5%] of 52 diagnostic variants in the PAGE study and 15 [63%] of 24 diagnostic variants in the Petrovski and colleagues' study).

Both studies also discuss topics related to parental decisions, clinical outcomes, and incidental findings. The PAGE study[1] provided pregnancy outcome data for 474 (78%) of 610 fetuses, and they revealed that parents opted for termination in 142 (30%) pregnancies, 14 (3%) ended in miscarriage, and there were 22 (5%) stillbirths, 14 (3%) neonatal deaths, and 282 (59%) liveborn babies. The proportion of fetuses with pathogenic or likely pathogenic genetic variants that did not survive beyond birth was significantly higher than those that were liveborn (14·1% *vs* 7·1%; p=0·0181). The study by Petrovski and colleagues[2] reported outcomes for 220 (94%) of

234 fetuses: 57% of pregnancies resulted in livebirths, 33% were terminations, and 3% were stillborn or died in utero. Incidental findings were also reported for four (2%) fetuses, in which the pathogenic variant was presumed to be unrelated to the structural anomaly. Both studies also considered the sensitive issue of return of results. In the PAGE study, no results were provided during the current pregnancy, but results related to the ultrasound findings were subsequently reported to parents, whereas the study by Petrovski and colleagues reported diagnoses during the pregnancy.

Interpretation from WES remains a source of variability, and the underlying data between studies highlight instructive differences in their approaches. The PAGE study restricted interpretation to a virtual panel of 1628 genes that are implicated in developmental disorders and prenatal findings. In this narrowed search space, 255 potential diagnoses required manual review, which was a mean of 0·42 potential diagnoses per fetus. Otherwise stated, 33·6% of all fetuses had a variant that warranted consideration and 8·5% received a diagnosis. The design of the study by Petrovski and colleagues was more expansive; this study included all genes and used methods that are often applied to annotate variants in association studies of neurodevelopmental disorders.[14-18] This tiered interpretation incorporated so-called bioinformatic signatures, meaning that it allowed variants in genes not yet linked to disease to be considered if they had similar properties to variants that occur at higher frequency in developmental disorders relative to their occurrence in the general population. This approach should increase sensitivity, as well as interpretation workload, which bears out in their identification of 1182 qualifying genotypes that warranted consideration, which was a mean of 4·8 qualifying genotypes per fetus (ie, more than ten times as many as those identified in the PAGE study). After review by the multidisciplinary panel, this approach yielded molecular diagnoses in 24 (2%) of 1182 variants considered. There was justification for this increased interpretation burden since some variants in novel genes were re-interpreted as pathogenic with support from emerging literature. In one fetus, a bioinformatic signature was not reported to the parents because of insufficient evidence at the time of analysis, and a subsequent pregnancy revealed a recurrent fetal anomaly.

These data underscore the potential clinical value of computational prediction to prioritise variants when genes without a known phenotypic match are included in analyses, and they highlight the delicate balance between conservative interpretation and under-reporting of uncertain results. Nonetheless, it remains important to look in detail at phenotype-matching genetic variants, regardless of signatures, since two molecular diagnoses derived from de-novo variants in the study by Petrovski and colleagues were not captured by the bioinformatic signatures. Both studies emphasise the crucial need for manual review and re-interpretation over time. As more sophisticated methods are developed to leverage population-scale references and more complete functional annotations, this process can become standardised for routine clinical practice.

We suggest that these studies serve as the introduction of WES into prenatal testing and document a compelling justification for its adoption. The technology is mature, the data are reproducible, and the processes are established in many clinical laboratories. There remain sobering limitations to the technology since adoption of WES as a single test could create voids in the screening of CNVs, balanced chromosomal abnormalities, and complex structural variation. It is of note that both studies pre-screened fetuses for these variants with conventional technologies. Although CNV detection from WES is feasible, and was applied in the PAGE study, it remains a bioinformatic challenge and has not been widely adopted by clinical diagnostic labs. Implementation of WES as a first-tier screen will thus leave clinicians and patients with some blind spots, and the time-compressed prenatal setting likely precludes serial reflexive testing for structural variations. Whole-genome sequencing holds promise to displace these methods because it can capture coding and non-coding point mutations as well as CNVs and balanced chromosomal abnormalities in a single test.[19,20] However, until its arrival for routine genetic testing, it is likely that a combination of WES and molecular cytogenetic methods will represent the most comprehensive approach. The data from these studies[1,2] indicate that such a combination will offer substantial improvements in diagnostic precision and clinical management of fetal anomalies.

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Network analyses to rank pharmacological treatments for generalised anxiety disorder

Many treatments exist for generalised anxiety disorder. Guidelines, such as the National Institute for Health and Care Excellence guidelines,[1] usually provide a list of possible treatment options, but they do not systematically list the treatments in preferential order with regard to efficacy and tolerability because there are few head-to-head comparisons of these interventions. However, it makes a difference for patients when one treatment reduces the Hamilton Anxiety Scale (HAM-A), a common self-reported measure of feelings of anxiety, from 26 points to 10 points, for example, compared with another that has been shown to achieve a reduction to 16 points. The network meta-analysis is a newer method for putting all available treatments in a ranking order: when treatment B was better than treatment C and A was better than B, the network meta-analysis can indirectly conclude that A is better than C (even if A and C have never been compared directly). This can conceivably be extended to all available treatments by creating a network of direct and indirect head-to-head and placebo comparisons.

In *the Lancet,* April Slee and colleagues[2] report their comprehensive network meta-analysis on medications for generalised anxiety disorder and found that some drugs—eg, duloxetine, pregabalin, venlafaxine, or escitalopram—were efficacious with relatively good acceptability. Quetiapine showed the largest reduction of HAM-A scores but was poorly tolerated, which is probably the reason why it is not licensed for


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See Articles page 768