IN THIS ISSUE

Longer-term impacts of early exome sequencing in infants with suspected monogenic disorders

https://doi.org/10.1038/s41436-018-0006-8

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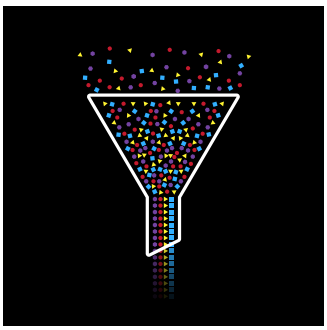
For children suspected of having monogenic disorders, diagnostic yield and short-term cost comparisons support utilization of exome sequencing (ES) early in the pursuit of a diagnosis. Little is known, however, about the longer-term impacts of ES on patients and families or the cost-effectiveness of data reanalysis for patients with uninformative initial results. In this issue, Stark et al. discuss these gaps in knowledge and report the findings of their follow-up study of the impacts of singleton ES in 80 infants suspected of having a monogenic disorder. Patients were recruited from the Royal Children's Hospital in Melbourne, Australia, and followed for 12 or more months after receiving ES results. The research team explored patient survival, further diagnostic evaluations, changes in clinical management as a result of ES results, uptake of genetic testing by first-degree relatives, and reproductive outcomes for parents. Among undiagnosed infants, further diagnostic evaluations were conducted. For nine infants, these evaluations cost AU$15,584 but resulted in no additional diagnoses. In contrast, reanalysis of ES data resulted in four additional diagnoses: two from reanalysis at 6 months, one from reanalysis at 12 months, and one from reanalysis at 18 months. Compared with standard of care, reanalysis once at 18 months was cost-effective with a savings of AU$1058 per additional diagnosis, while reanalysis of data every 6 months resulted in a cost increase of AU$3578 per additional diagnosis. Among diagnosed infants, the authors estimated that changes in clinical management resulted in a cost savings of AU$1578 per quality-adjusted life year (QALY) gained compared with standard of care, while increased utilization of hospital services was not observed. Parents of diagnosed infants were substantially more likely than parents of undiagnosed children to use reproductive genetic services, including preimplantation and prenatal genetic diagnosis, and to become pregnant again. When genetic testing of relatives and reproductive services were included in cost assessments, the authors estimated a cost increase of AU $8118 per QALY gained. The authors conclude that these data provide further support for the early utilization of ES in the diagnosis of children suspected of having monogenic diseases and that reanalysis of ES data 18 months after initial testing is more cost-effective than more frequent reanalysis. The authors suggest that these data offer evidence for payers and laboratories to support policies related to utilization and reimbursement of ES and data reanalysis in clinical and laboratory practice. — *Raye Alford, News Editor*

Optimizing the analytical sensitivity and specificity of exome sequencing

https://doi.org/10.1038/s41436-018-0016-6



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Confirmatory Sanger sequencing of variants identified by massively parallel sequencing (MPS) adds time, labor, and cost to testing and is impractical on a large scale. Filtering of variants based on quality, coverage, and frequency criteria can reduce the number of variants requiring confirmation, but overly stringent thresholds for variant calling adversely affect the sensitivity of MPS-based tests. Analytical algorithms capable of faithfully predicting true and false positives are needed to reduce the number of variants requiring Sanger-based confirmation without unfavorably impacting sensitivity and specificity. In this issue, Bauer et al. discuss these and other concerns related to MPS-based tests and report their construction of an algorithm for filtering variants identified by exome sequencing (ES). DNA samples from 773 unrelated patients suspected of having genetic disease were subjected to ES by the research team. Nonstringent variant calling criteria for Phred-based quality score, total number of reads, and frequency were applied to the sequence data, and 1048 variants were identified in genes known to be associated with the presumptive clinical diagnoses of patients. Sanger sequencing by the team confirmed 858 of the variants (81.9%). To increase precision, the authors applied more stringent criteria for variant calling based on commonly used cutoffs for panel-based or ES-based MPS tests. The team found that more stringent variant calling criteria increased precision but also decreased sensitivity. The authors then sought to customize an algorithm for filtering variants that did not compromise sensitivity or specificity. The team delineated 11 features of variants—6 analogous features and 5 digital features—and assessed each feature for its capability to predict confirmation status from Sanger sequencing. Iterative analyses using quality score followed by number of reads for the variant allele and fraction of reads for the variant allele allowed the team to establish evidence-based cutoffs that correctly predicted 813 true and 146 false positives among the original 1048 variants, leaving only 87 variants requiring Sanger sequencing; two true positives were, however, filtered out in the process. The authors present a decision tree that exhibited 99.8% sensitivity and 100% specificity and reduced the percentage of identified variants requiring Sanger confirmation to 8.3%. The authors caution, however, that their cutoff values might not hold for other platforms and operators, and attempts to adapt the algorithm should be tested prior to implementation. — *Raye Alford, News Editor*

Genetics in Medicine (2019) https://doi.org/10.1038/s41436-018-0394-9