common genetic diseases, such as cardiomyopathy and congenital deafness. In cancer testing, gene panels are being widely adopted to perform detailed tumor profiling. Each tumor has a unique set of somatic mutations, and these assays aim to detect as many treatable or prognostic mutations as possible to offer individually tailored patient care. Currently available panels vary widely in size, from a few dozen genes up to nearly a thousand. For cancer, targeted testing allows for high-depth sequencing at low cost, helpful for detecting clinically relevant mutations present at low allelic percentage due to tumor or sample heterogeneity.

- *Whole exome sequencing (WES).* Exome sequencing is really just a type of targeted sequencing. It uses hundreds of thousands of custom probes to pull out the roughly 1.5% of the genome that consists of protein-encoding exons. At a time when whole genome sequencing is still costly, WES enables a broad survey for protein coding mutations (which are responsible for as much as 80% of Mendelian disease) at significantly reduced cost. This has led to some wonderful success stories, allowing physicians to deliver answers and even therapies for children with orphan diseases who had suffered through prolonged and unsuccessful diagnostic odysseys. WES is also used in oncology to perform a very broad analysis, mostly in the research setting but also in some clinical laboratories.
- *Whole genome sequencing (WGS).* Whole genome sequencing is the most comprehensive type of DNA analysis that can be performed on an individual. However, current costs and informatic challenges still preclude its routine use in clinical practice. Indications for use in medical genetics are mostly limited to cases where exome sequencing has failed to provide an answer but the clinical suspicion of genetic disease remains high. For cancer applications, WGS is the only NGS application that can detect novel structural rearrangements (e.g., insertions, deletions, translocations) that may be clinically relevant. Because of associated costs, WGS is generally performed to lower sequencing depth than either targeted panels or exomes, and may suffer from a lack of statistical power to detect low percentage mutations in heterogeneous tumor samples.

The choice of approach is mainly a function of sequencing cost and interpretive workload. Interpreting NGS clinical assays can be laborious, with considerable effort required to research the potential relevance of novel, suspicious variants. These interpretive challenges should lessen over time with improvements to variant databases.

Future Applications

Because NGS can be used to detect genetic anomalies of essentially any size scale, from SNPs to very large rearrangements and even aneuploidy, almost all of today's genetic diagnostic tests could in principle be supplanted by NGS. This includes RNA analysis, because NGS-based analysis of the transcriptome (RNA-seq) is straightforward. As costs continue to drop, it is reasonable to expect NGS to occupy an increasingly prominent place in the diagnostics lab. Additionally, NGS holds promise for application into novel areas, including microbiome analysis and blood screening for early markers of diseases, including cancer. Continuing technologic advances may even extend the applications further. Already third generation (or "single molecule" or "next next generation") technologies are emerging that can rapidly sequence single molecules in parallel without the need for focal amplification, and these could soon have an impact in the clinical laboratory.

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SUGGESTED READING

Molecular Basis of Single Gene Disorders—General

Dietz HC: New Therapeutic Approaches to Mendelian Disorders. *New Engl J Med* 363:852, 2010. *[An excellent discussion of treatment of genetic disorders based on molecular pathogenesis.]*

Disorders Associated with Defects in Structural Proteins

De Paepe A, Malfait F: The Ehlers-Danlos syndrome, a disorder with many faces. *Clin Genet* 82:1, 2012. *[A modern appraisal of the molecular defects in this disorder of collagen.]*

Jondeau G, Michel JB, Boileau C: The translational science of Marfan syndrome. *Heart* 97:1206, 2011. *[An excellent review of the pathogenesis of Marfan syndrome.]*

Disorders Associated with Defects in Receptor Proteins

Faiz F, Hooper AJ, van Bockxmeer FM: Molecular pathology of familial hypercholesterolemia, related dyslipidemias and therapies beyond the statins. *Crit Rev Clin Lab Sci* 49:1, 2012. *[An update on molecular pathology of familial hypercholesterolemia.]*

Disorders Associated with Defects in Enzymes

Lieberman AP, Puertollano R, Raben N, et al: Autophagy in lysosomal storage disorders. *Autophagy* 8:719, 2012. *[Discussion of the emerging role of autophagy in lysosomal storage disorders.]*

Platt FM, Boland B, van der Spoel AC: Lysosomal storage disorders: The cellular impact of lysosomal dysfunction. *JCell Biol* 199:723, 2012. *[A review of the multiple mechanisms that underline lysosomal disorders.]*

Cytogenetic Disorders Affecting Autosomes

Karayiorgou M, Simon TJ, Gogos JA: 2q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci* 11:402, 2010. *[Discussion of molecular basis of neurologic deficits in 22q11.2.]*

Lana-Elola E, Watson-Scales SD, Fisher E M, et al: Down syndrome: searching for the genetic culprits. *Dis Model Mech* 4:586, 2011. *[Review of candidate genes involved in pathogenesis of Trisomy 21.]*

Simpson JL: Cell-free fetal DNA and maternal serum analytes for monitoring embryonic and fetal status. *Fertil Steril Inc* 99:1124, 2013. *[Recent advances in the diagnosis of cytogenetic disorders by analysis of fetal DNA in maternal serum.]*

Cytogenetic Disorders Affecting Sex Chromosomes

Groth KA, Skakkebaek A, Host C, et al: Klinefelter syndrome—A Clinical Update. *J Clin Endocrinol Metab* 98:20, 2013. *[A comprehensive discussion of clinical and genetic features of Klinefelter syndrome.]*

Heard E, Turner J: Function of the Sex Chromosomes in Mammalian Fertility. *CSH Perspectives Biol* 3:a002675, 2011. *[A review of the basic biology of sex chromosomes and their abnormalities.]*

Oliveira CS, Alves C: The role of the *SHOX* gene in the pathophysiology of Turner syndrome. *Endocrinol Nutr* 58:433, 2011. *[Discussion of molecular basis of Turner syndrome.]*

Pinsker JE: Turner Syndrome: Updating the Paradigm of Clinical Care. *J Clin Endocrinol Metab* 97:E994, 2012. *[A clinical review of Turner syndrome]*