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Impact of next-generation sequencing on molecular diagnosis of inherited non-syndromic hearing loss

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Abstract

Hearing loss is one of the most common birth defects, with inherited genetic defects play an important role, contributing to about 60% of deafness occurring in infants. However, hearing impairment is genetically heterogeneous, with both common and rare forms occurring due to mutations in estimated 500 genes. Due to the large number and presumably low mutation frequencies of those genes, it would be highly expensive and time-consuming to address this issue by conventional gene-by-gene Sanger sequencing. Next-generation sequencing is a revolutionary technology that allows the simultaneous screening of mutations in a large number of genes. It is cost effective compared to classical strategies of linkage analysis and direct sequencing when the number or size of genes is large, and thus has become a highly efficient strategy for identifying novel causative genes and mutations involved in heritable disease.

In this review, we describe major NGS methodologies currently used for genetic disorders and highlight applications of these technologies in studies of molecular diagnosis and the discovery of genes implicated in non-syndromic hearing loss.

Keywords: Next-generation sequencing; Molecular diagnosis; Inherited non-syndromic hearing loss; Whole genome sequencing; Whole exome sequencing

1. Introduction

Identifying the genetic basis of deafness provides important information for diagnosis, intervention and treatment of the disease. Non-syndromic hearing loss (NSHL) is extremely heterogeneous. To date, more than 100 genes and 100 genetic loci have been implicated in NSHL (http://hereditaryhearingloss.org/). The marked heterogeneity of genetic hearing loss can be explained by the complexity of the auditory system, which requires coordination of multiple processes involving the inner ear and nervous system. A defect in any part of this complex chain of events can lead to hearing impairment. For many decades, linkage analysis has been the most powerful and widely used strategy to identify the gene defects responsible for inherited disorders. However, this approach is time consuming and requires the availability of cohorts of homogeneous and informative large families, and a large proportion of NSHL remain genetically unexplained. These limitations, however, may be overcome by the next-generation sequencing (NGS) technologies.

NGS offers an unprecedented ability to identify rare variants and new causative genes. Several next generation sequencing platforms allow for a DNA-to-diagnosis protocol to identify the molecular basis of inherited non-syndromic hearing loss, including whole genome sequencing (WGS),

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whole exome sequencing (WES) and targeted deafness gene capture.

Updated guidelines from the American College of Medical Genetics and Genomics (ACMG) recommend that clinicians consider NGS when testing for genetic causes of hearing loss (Levenson, 2014). The guidelines, which are built on guidelines issued in 2002, include panel tests targeted at genes related to hearing loss, whole exome sequencing, and whole genome sequencing after negative results are returned on initial single-gene testing indicated by a patient's family medical history and presentation.

1.1. Whole genome sequencing

Whole genome sequencing (WGS) by next generation sequencing technologies has the potential for simultaneous, comprehensive, differential diagnostic testing of likely monogenic illnesses. In 2003, the cost of sequencing a single human genome was estimated to be 2.7 billion dollars, that price had dropped to 4000 dollars by 2012, and it is anticipated that this cost will soon be 1000 dollars. Clinical use of WGS by NGS has taken at least a month. It's now possible to complete sample collection, sequencing, and analysis in less than 50 h (Saunders et al., 2012). As this pace, WGS will be increasingly integrated into clinical care. Researchers already have been able to help clinicians aid some children born with rare birth defects by sequencing and analyzing their whole genomes to diagnose and treat their illness (Saunders et al., 2012). WGS data are also used to advance personalized medicine, including predicting an individual's risk of a hearing loss attack or determining the best dosage of medication for an individual patient. This is only the beginning of the whole genome sequencing era, which has the potential to revolutionize medicine.

However, there is no report about application of whole genome sequencing in inherited non-syndromic hearing loss. There are major obstacles to the clinical implementation of WGS, such as hidden costs, issues surrounding sequencing and analysis, quality assurance, standardization of protocols, ethical dilemmas, and difficulties with interpretation of the results. With the availability of human WGS data from many individuals, it is now clear that two unrelated individuals have at least two million differences in their genomic DNA sequences (Moore et al., 2011). WGS requires the analysis of ~3.2×10^9 bps of DNA sequences. The full potential of WGS can be realized only when we gain a much better understanding of functions in noncoding regions. WGS should be carefully implemented in the clinic to allow the realization of its potential to improve patient health in specific indications.

1.2. Whole exome sequencing

Most Mendelian disorders are caused by exonic or splice-site mutations that alter the amino acid sequence from the affected gene. An effective compromise between the competing goals of genome-wide comprehensiveness and cost-control is realized in the concept of Whole exome sequencing (WES) (Ng et al., 2009). Approximately 85% of disease-related mutations in Mendelian disorders have been found in the protein-coding region, although this portion constitutes only approximately 1% of the human genome (Teer and Mullikin, 2010). WES has become a highly efficient strategy for identifying novel causative genes and mutations involved in heritable disease.

Over 1778 publications since 2009, whose abstracts contain the term “whole exome sequencing”, confirm the success of exome sequencing as a new and effective technological paradigm within human genetics. Exome sequencing has proven useful for identifying molecular defects underlying single gene disorders (Mendelian inheritance), as well as some genetically heterogeneous disorders, such as inherited non-syndromic hearing loss.

Inherited non-syndromic hearing loss can be resolved efficiently using WES, especially in small families with distinct and interesting phenotypes that were once too small to map using linkage analysis. Recently, there have been many successful applications of WES in identifying the causative

Table 1
List of genes and mutations related with non-syndromic hearing loss identified by WES.

Gene name	Mutation (protein)	Reference
OSBPL2	p.Gln53Argfs*100	Xing et al. (2014)
	p.Leu195Met	
TBC1D24	p.Ser178Leu	Azaiez et al. (2014)
TNC	p. Thr 1796Ser	Zhao et al. (2013)
	p.V1773M	
ELMOD3	p.Leu265Ser	Jaworek et al. (2013)
KARS	p.Asp377Asn	Santos-Cortez et al. (2013)
	p.Tyr173His	
GRXCR2	c.714dupT	Imtiaz et al. (2014)
ATP1A2	p.Val191Met	Oh et al. (2014)
ADCY1	p.Arg1038X	Santos-Cortez et al. (2014)
BDP1	p.*2625Gluext*11	Girotto et al. (2013)
EPS8	p.Gln30*	Behlouli et al. (2014)
PNKP	p.Gly292Arg	Nakashima et al. (2014)
PCDH15	p.Met65Ile	Nakashima et al. (2014)
	p.Ser404Arg	
CDH23	p.Pro240Leu	Woo et al. (2014)
	p.Glu1595Lys	
	p.Asn342Ser	
POU4F3	p.Arg326Lys	Kim et al. (2013)
MYO15A	p.Ser1481 Pro	Diaz-Horta et al. (2012);
	p.Gln1425X	Gao et al. (2013a);
	p.Ala1551Asp	Woo et al. (2013)
	IVS11 + 1	
	p.Arg2146Q	
TMC1	p.Ser530X	Diaz-Horta et al. (2012);
	p.Gly197Arg	Gao et al. (2013b)
	p.Gln391X	
ACTG1	p.Met305Thr	Park et al. (2013)
LOXHD1	p. Arg1494X	Diaz-Horta et al. (2012)
	p. Glu955X	
GIPC3	p.His170Asn	Diaz-Horta et al. (2012)
ILDR1	p. Gln 274X	Diaz-Horta et al. (2012)
MYO7A	p.Gly2163 Ser	Diaz-Horta et al. (2012)
TECTA	p. Tyr 1737Cys	Diaz-Horta et al. (2012)
TMPRSS3	p.F13Lfs*10	Diaz-Horta et al. (2012)
TRIOBP	p. Arg785 Ser fs*50	Diaz-Horta et al. (2012)

genes and mutations of inherited non-syndromic hearing loss (Table 1).

To date, 10 non-syndromic deafness genes and more than 30 novel causative mutations have been identified by WES (Table 1). These studies show that WES, followed by verification and functional and immunolabeling examinations, can reveal critical disease-causing genes from small pedigrees.

Even with the rapid maturation of this field, there are a number of areas that require additional work: (1) WES fails to solve a substantial proportion of presumably Mendelian phenotypes (Fairfield et al., 2011). (2) There is tremendous interests in understanding the contribution of rare variations to the genetic basis of common diseases. Many such studies have been initiated using WES, but are still ongoing as they require additional samples to testify the results. (3) The discrete prioritization of protein-altering variations over all other variations has clearly proven to be useful, but is undeniably crude.

1.3. Targeted deafness gene capture and NGS

The popular application of targeted gene capture can be implemented for all of the genes involved in causing hearing loss, including all exons, exon/intron boundaries and promoter sequences that can be fully sequenced on a diagnostic platform to produce a specific genetic test for hearing loss. Targeted deafness gene capture combined with NGS is suited to identify the causative mutations of non-syndromic hereditary hearing loss owing to the following advantages: 1) comprehensive coverage of large numbers of genes and large genes associated with the disease; 2) significant cost saving; 3) higher sequencing accuracy because of deeper achievable coverage; 4) a significantly shorter turnaround time and 5) more convincing dataset by excluding other deafness genes (Lin et al., 2012).

To date, more than 100 human genes implicated in non-syndromic hearing loss are confirmed (Lin et al., 2012). Approximately 100 Gbp of sequencing is needed to obtain NGS results for one human genome (~3.2 Gbp) at about 30× average coverage. Targeted gene enrichment typically increases this proportion by at least 1000 fold (Brownstein et al., 2011; Shearer et al., 2010; Tang et al., 2012). Therefore, the same sequencing capacity can theoretically be used to sequence more than 1000 samples for a panel of genes associated with deafness.

Recent studies have demonstrated the feasibility of conducting diagnostic tests for all deafness genes by targeted gene capture and NGS (Brownstein et al., 2011; Shearer et al., 2010). Its success in research has already resulted in its translational uses in clinical care, and many of them are for diagnostic mutation detection of focused panels of disease genes. OtoSCOPE (Otological Sequence Capture Of Pathogenic Exons) is the first massively parallel sequencing platform that utilizes targeted sequence capture and NGS for genetic testing of hearing loss (Shearer et al., 2010). It has been developed by the University of Iowa and is being used in a research setting to fully sequence all exons of 57 deafness genes (http://www.healthcare.uiowa.edu/labs/morl/index_CDS.htm). At this stage in its development various methods of targeted sequence capture and NGS are being compared to determine which combination has the greatest level of sequence coverage. Otogenetics Corporation in the USA is currently offering a genetic mutation testing service using targeted sequence capture and NGS for the detection of variants in 131 known deafness genes for approximately $500 per sample (www.otogenetics.com).

Richard J.H. Smith, MD, Director of the Molecular Otolaryngology and Renal Research Laboratories at the Iowa Institute of Human Genetics at University of Iowa in Iowa City, is a proponent of panel testing for hearing loss. His lab offers a test that covers 90 genes known to cause hearing loss, which he suggests over any initial single-gene test. Because panel tests offer more depth of coverage in genes associated with hearing loss, they are superior to WES at some extent, which looks at 20,000 genes and may miss parts of them.

If single-gene tests yield no diagnosis, panel tests is recommended. The drawbacks of panel tests are these tests, which use disease-targeted exon capture focused on specific genes, may only sequence a subset of the genes known to cause hearing loss, and there is limited knowledge of which genes are involved in hearing loss.

1.4. Challenge and future

It is anticipated that the next five to ten years will see yet more improvements in the speed and cost of DNA sequencing. There is already discussion about “third generation” DNA sequencing, which aims to increase the speed of sequencing and reduce cost even further.

Applications of NGS technologies are now beginning to enter clinical practice. Interpreting the data and translating the research results into applications that improve healthcare is still challenging. Filtering through the millions of variants in an individual's genome for the pathogenic mutation seems to be the most urgent task at hand. Another important aspect is the concurrent development of genetic counseling capabilities to interpret the large amount of data revealed by NGS for clinical use. In the near future, physicians may combine a past medical history and family history with NGS diagnostic data to identify disease predisposition variants and variants that affect drug metabolism in individuals.

Although NGS-based molecular diagnostic tests are still in their infancy, they have demonstrated excellent clinical utility for single-gene disorders. With further developments in NGS technologies for data generation and with more effective bioinformatics tools for data analysis and clinical extraction, the full potential of WES/WGS that we expect to be revealed in the coming years will greatly enrich and empower the practice of genomic medicine beyond the rare single-gene disorders. The improvements in patient care demonstrated in recent studies justify all effort and cost for moving these new and exciting approaches into molecular diagnostics practice.

In our opinion, WES, WGS and targeted deafness gene capture should remain as options to be considered for inherited non-syndromic hearing loss and be used according to a

patient's specific conditions. Alternatively, a combined approach can be used to capture all variety of genomic variations.

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