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Current mutation discovery approaches in Retinitis Pigmentosa

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ABSTRACT

With a worldwide prevalence of about 1 in 3500–5000 individuals, Retinitis Pigmentosa (RP) is the most common form of hereditary retinal degeneration. It is an extremely heterogeneous group of genetically determined retinal diseases leading to progressive loss of vision due to impairment of rod and cone photoreceptors. RP can be inherited as an autosomal-recessive, autosomal-dominant, or X-linked trait. Non-Mendelian inheritance patterns such as digenic, maternal (mitochondrial) or compound heterozygosity have also been reported. To date, more than 65 genes have been implicated in syndromic and non-syndromic forms of RP, which account for only about 60% of all RP cases. Due to this high heterogeneity and diversity of inheritance patterns, the molecular diagnosis of syndromic and non-syndromic RP is very challenging, and the heritability of 40% of total RP cases worldwide remains unknown. However new sequencing methodologies, boosted by the human genome project, have contributed to exponential plummeting in sequencing costs, thereby making it feasible to include molecular testing for RP patients in routine clinical practice within the coming years. Here, we summarize the most widely used state-of-the-art technologies currently applied for the molecular diagnosis of RP, and address their strengths and weaknesses for the molecular diagnosis of such a complex genetic disease.

1. Introduction

The term Retinitis Pigmentosa (RP) encompasses a broad group of genetically determined retinal diseases caused by a large number of mutations that result in rod photoreceptor cell death followed by gradual death of cone cells, eventually leading to blindness. Thus, typical RP is also described as a rod-cone dystrophy, in which loss of rod function exceeds the reduction in cone sensitivity (Hamel, 2006; Hartong, Berson, & Dryja, 2006). RP is the most common form of hereditary retinal degeneration with a worldwide prevalence of about 1 in 3500–5000, with a total of more than 1 million affected individuals (Chang et al., 2011; Chizzolini et al., 2011; Collin et al., 2010). Affected individuals first experience defective dark adaptation (night blindness), followed by reduction of the peripheral visual field (known as tunnel vision) and sometimes, loss of central vision late in the course of the disease. In the progression of RP symptoms, night blindness generally precedes tunnel vision by years or even decades. At the cellular level, the retinal pigment epithelium is altered in most cases, presenting clusters of pigment within the retina of RP patients; hence the name to the disease. The onset, progression and severity of the disease is genetically determined in most cases and also influenced by the mode of inheritance. In extreme cases, patients may present a rapid evolution over two decades, but in contrast, other patients exhibit slow progression, which may never lead to blindness (Hamel, 2006). Nevertheless, symptoms typically start in the early teenage years and severe visual impairment occurs by ages 40–50 years (Sahni et al., 2011).

RP can be divided into two groups: non-syndromic RP in which RP is restricted to the eyes, without other systemic manifestations and syndromic RP in which patients present associated non-ocular diseases, the latter representing 20–30% of total cases (Chang et al., 2011; Ferrari et al., 2011). The most common forms of syndromic RP are: Usher's syndrome, which is characterized by RP and sensory-neural hearing impairment, with or without vestibular dysfunction (Williams, 2008) and the Bardet–Biedl syndrome, which is characterized by RP with obesity, polydactyly, mental retardation, hypogonadism and renal failure in some cases (Beales et al., 1999). The Bardet–Biedl syndrome is due to mutations in at least 11 genes, with cases of triallelic and digenic inheritance (Hamel, 2006). On the basis of its inheritance pattern and prevalence, RP can be divided into three main groups: autosomal-dominant (30–40% of cases), autosomal-recessive (50–60%) and X-linked (5–15%). Patients with no other affected relatives are typically autosomal

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recessive, although a few might represent new dominant mutations, instances of uniparental isodisomy or, for males, X-linked mutations, or even non-Mendelian inheritance patterns, such as digenic inheritance, compound heterozygosity or maternal (mitochondrial) inheritance (Audo, Bujakowska, et al., 2011; Audo, Lancelot, et al., 2011; den Hollander et al., 2007; Hartong, Berson, & Dryja, 2006).

Most cases of RP are monogenic. More than 65 associated genes have been identified, 43 of which correspond to non-syndromic RP as of May 2012 (http://www.sph.uth.tmc.edu/retnet/). Most genes for RP cause only a small proportion of cases, with the exception of the rhodopsin (RHO), the USH2A and the RPGR genes which together cause ~30% of all cases of RP (Daiger, Bowne, & Sullivan, 2007; Hamel, 2006; Hartong, Berson, & Dryja, 2006; Musarella & Macdonald, 2011; Pomares et al., 2010; Sergouniotis et al., 2011; Waseem et al., 2007). However, these genes account for only about 60% of all RP patients, while heritability in about 40% of RP patients remains unknown (Daiger, Bowne, & Sullivan, 2007; Ferrari et al., 2011; Sahni et al., 2011). For an update on genetic and genomic information regarding complex genetic retinal disorders, several databases are available, including: http://www.ensembl.org; http://www.ncbi.nlm.nih.gov; http://www.ncbi.nlm.nih.gov/gene; http://www.ncbi.nlm.nih.gov/homologene; http://www.sph.uth.tmc.edu/retnet/; http://genome.ucsc.edu.

Despite the fact that many technically diverse approaches are being investigated for the treatment of RP, there is currently no standardized and efficient treatment for this disease. The discovery of the molecular basis of the disease has led to the development of several assays in animal models and more recently in human trials, with promising results. More advanced lines of research in RP therapy include: the use of neurotrophic factors (Zhang et al., 2012); gene therapy (Allocca et al., 2011; Jacobson et al., 2012; Pang et al., 2011); retinal transplant (MacLaren et al., 2006; West et al., 2012) and electronic prosthesis (Barry & Dagnelie, 2012; Zrenner et al., 2011).

Several factors have made the molecular characterization of RP a real challenge. These include the high number of genes and variants involved, as well as non-Mendelian inheritance patterns, such as incomplete penetrance, digenic or triallelic inheritance. To further complicate things, two different mutations in the same gene can generate different diseases and the same mutation in different individuals may cause distinctly different symptoms. For instance, although mutations in the rhodopsin gene are usually linked to dominant RP, a few rare rhodopsin mutations can cause recessive RP. Finally, the fraction of disease-causing mutations varies with ethnicity and geography (Daiger, Bowne, & Sullivan, 2007; Ferrari et al., 2011; Hamel, 2006; Stone, 2003).

Methods to determine DNA sequences were developed in the late 1970s by Frederick Sanger and Walter Gilbert, and have revolutionized the science of molecular genetics. Development of the Chain-Termination method in 1977, and especially publication of the first draft of the human genome in 2001 (Lander et al., 2001; Venter et al., 2001), have boosted the blossoming of novel technologies that deliver fast, inexpensive and accurate genome information with unprecedented cost-effectiveness, leading to the generation of what is known as Next Generation Sequencing (NGS) technologies. Thanks to these new methods, the technical challenges, time and cost involved in full or partial sequencing of the human genome have exponentially plummeted (Bowne, Humphries, et al., 2011; Bowne, Sullivan, et al., 2011; Ferrari et al., 2011; Lander, 2011). For instance, the per-base cost of DNA sequencing has dropped by 100,000-fold over the last decade and the current generation of sequencing systems can read up to 250 billion bases in a week compared with 25,000 in 1990 and 5 million in 2000 (Lander, 2011; Service, 2006). High cost involved in conventional methods of genome sequencing, are responsible of the current lack of large-scale genome sequencing studies aimed at disease gene discovery. However, this may soon change, considering that the cost of sequencing a genome at $1000 per individual is near to becoming a fact (Duggal, Ibay, & Klein, 2011).

Since the discovery of the rhodopsin gene, the first one directly linked to RP (Dryja et al., 1990), almost 65 genes have been found to be associated with this disease. Considering recent advances in genomic sequencing, which are changing mutation discovery paradigms, it seems reasonable to assume that the genetic cause of RP will be identified in 90–95% of cases in the near future. Identification and classification of all RP causing mutations will contribute to a better understanding of disease variants and will be central in order to provide improved diagnosis and prognosis for each patient. This is particularly important when children, young adults or affected women planning to have family are involved (Daiger, Bowne, & Sullivan, 2007; Ferrari et al., 2011; Hamel, 2006; Shintani, Shechtman, & Gurwood, 2009).

In this review, we summarize the most widely used, state-of-the-art technologies currently employed for the molecular diagnosis of RP. We present their main features, advantages and disadvantages and assess their capabilities and limitations to accomplish an accurate molecular diagnosis of such a complex genetic disease. These techniques are presented in two main groups: sequencing technologies and technologies specialized in detecting genetic variants.

2. Sequencing methods

2.1. Chain-Termination or Sanger sequencing

The Chain-Termination sequencing method or Sanger method (Sanger, Nicklen, & Coulson, 1977) has revolutionized molecular biology, providing the backbone technology for DNA sequencing for almost three decades and has led to a number of monumental accomplishments, including the analysis of the whole human genome sequence (Lander, 2011). This method is considered to be the first-generation technology, with latest technologies being denominated as Next-Generation Sequencing systems (NGSs) (Metzker, 2010).

The Sanger method is performed in four separate reactions, in each of which are included a DNA polymerase, specific DNA primers flanking the target sequence and the four different types of deoxynucleotides (dNTPs). The key principle of this technique is the use of chain-terminating dideoxynucleotide triphosphates (ddNTPs). One of the four different types of radioactively labeled ddNTPs is added to each reaction. Instead of having an –OH group, like dNTPs, ddNTPs have a hydrogen atom attached to the 3′ carbon, which causes the termination of the elongation reaction due to their inability to form a phosphodiester bond with the next deoxynucleotide. Thus, DNA chain length in each reaction will depend on how long the chain was when a ddNTP is randomly incorporated. Once the four reactions have been completed, high-resolution electrophoretic separation in a polyacrylamide gel is applied for separating these chains by length with a resolution of one nucleotide (Pettersson, Lundeberg, & Ahmadian, 2009; Shendure & Ji, 2008). Finally, the gel is dried onto chromatography paper and exposed to X-ray film allowing direct reading of the DNA sequence, where a dark band in a lane indicates a DNA fragment that is the result of chain termination after incorporation of a ddNTP (Fig. 1A).

In the early 1990s, a methodological improvement of the Sanger method, called Dye Termination sequencing, was introduced and has become the mainstay in automated sequencing. The main improvement of this technique lies in the fact that each ddNTP is labeled with a different fluorescent dye, allowing sequencing in a single reaction, rather than in four as in the Sanger method, with