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Non-syndromic hearing loss gene identification: A brief history and glimpse into the future

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ABSTRACT

From the first identified non-syndromic hearing loss gene in 1995, to those discovered in present day, the field of human genetics has witnessed an unparalleled revolution that includes the completion of the Human Genome Project in 2003 to the $1000 genome in 2014. This review highlights the classical and cutting-edge strategies for non-syndromic hearing loss gene identification that have been used throughout the twenty year history with a special emphasis on how the innovative breakthroughs in next generation sequencing technology have forever changed candidate gene approaches. The simplified approach afforded by next generation sequencing technology provides a second chance for the many linked loci in large and well characterized families that have been identified by linkage analysis but have presently failed to identify a causative gene. It also discusses some complexities that may restrict eventual candidate gene discovery and calls for novel approaches to answer some of the questions that make this simple Mendelian disorder so intriguing.

1. Introduction to non-syndromic hearing loss

Hearing loss (HL) is the most prevalent sensory deficit primarily originating from genetic etiologies and clinically occurring without any additional phenotypes [1]. Non-syndromic hearing loss (NSHL) generally follows simple Mendelian inheritance and is predominantly transmitted as an autosomal recessive trait (75–80%), although autosomal dominant (20%), X-linked (2–5%) and mitochondrial mutations (1%) can also cause HL [1]. With certain exceptions, the onset and severity of these inherited types of HL follow a similar clinical pattern. For example, autosomal recessive NSHL is generally prelingual, non-progressive (stable) and severe-to-profound [2], whereas autosomal dominant NSHL is primarily characterized as post-lingual (with an onset often spanning the second through fifth decades of life) and progressive [3]. The prevalence of NSHL, beginning in prelingual children, doubles from approximately 1.33 per every 1000 newborns to 2.7 per every 1000 children at five years of age [4]. With the steadily aging global population, the number of individuals with various types of HL is expected to reach approximately 1 billion by the year 2020 [5], with age-related hearing loss (ARHL) accounting for a large proportion of this estimate. ARHL is thought to be multifactorial and the result of unclarified genetic susceptibility and environmental factors such as excessive noise exposure, exposure to ototoxic chemicals, and medical conditions that exacerbate HL in advanced age, specifically diabetes and cardiovascular disease [5]. Furthermore, ARHL shows a high degree of heritability within nuclear families and twin studies [6,7], and males are generally more affected than females [5]. The apparently complex pathological processes of ARHL can often mimic those of late-onset autosomal dominant NSHL, clouding their strict distinctions [8].

Genotype-phenotype correlations have intrigued physicians and scientists for as long as these principles have known to exist. The wealth of information that has emerged from connecting genetic mutations to impact on health has dramatically expanded the general understanding of inherited diseases. However, in the case of NSHL, full comprehension of these correlations is a great challenge due to extreme clinical and genetic heterogeneity [9]. Mutations in broad subsets of genes confer HL and the initial development and progression due to diverse genetic underlying

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causes are often indistinguishable. To resolve the molecular basis of a process as complex and delicate as hearing necessitates a fundamental understanding of participating genes.

The systematic elucidation of NSHL genes has translational consequence to improved diagnostic, prognostic, and therapeutic options. The identification of new NSHL genes has been met with astonishing success, uncovering a total of 85 genes (Fig. 1). Many of the recently identified genes have been established using massively parallel sequencing (MPS) or next generation sequencing (NGS) (Table 1); a process in which targeted enrichment and MPS with the so-called "next generation" of sequencing technologies post-−Sanger sequencing era, permit the scaling up of sequencing data by orders of magnitude [10]. Before expanding on how this relatively new method has forever transformed the face of molecular genetics by alleviating a significant data generation bottleneck, it is necessary to review the classical methods of NSHL gene discovery.

2. A brief history of the identification of non-syndromic hearing loss genes

Whole-genome linkage analysis within a large, clinically well-characterized family provides a powerful approach for mapping critical chromosomal intervals. This process integrates informative marker co-segregation in a large family for localizing a genetic region encompassing a disease-causing mutation to a defined chromosomal position [11] for a variety of subsequent positional cloning methods. Similarly, homozygosity mapping is an alternative approach for delineating autosomal recessive loci by mapping long stretches of homozygous genotypes that has proven successful with consanguineous families [12] and homogeneous populations sharing similar clinical features [13]. Due to limiting meiotic crossing-over events, these locus mapping approaches typically identify large chromosomal regions spanning several mega bases that include hundreds of genes for positional cloning strategies, namely bacterial artificial chromosome (BAC)-mediated cloning [14,15], cDNA library preparation from tissues of interest for expression analysis [16,17], as well as selection and sequencing of candidate genes based on *a priori* hypotheses linking a candidate gene to a certain phenotype [18,19] or candidate gene selection with the aid of pre-existing deafness mouse models for selection of human orthologous genes to sequence [20,21]. Candidate genes in the locus are ranked and sequentially sequenced to pinpoint mutations.

The year 1988 marked the first reported NSHL locus mapping to chromosome Xq using a linkage approach [22] that served as the first step in a seven year journey toward identifying the gene *POU3F4* (MIM: *300039) [23]. Similarly, the first autosomal locus was linked to chromosome 5q31 in 1992 [24] leading to the identification of *DIAPH1* (MIM: *602121) five years later [25]. To grasp the full appreciation of this work, these loci were mapped at a time when human molecular genetics was still in its infancy and newly developed physical maps still unknowingly harboured serious errors [26], increasing the risk of incorrect map distances and reduced linkage analysis power [27]. This emerging work served as an example from which many classical NSHL candidate gene studies materialized.

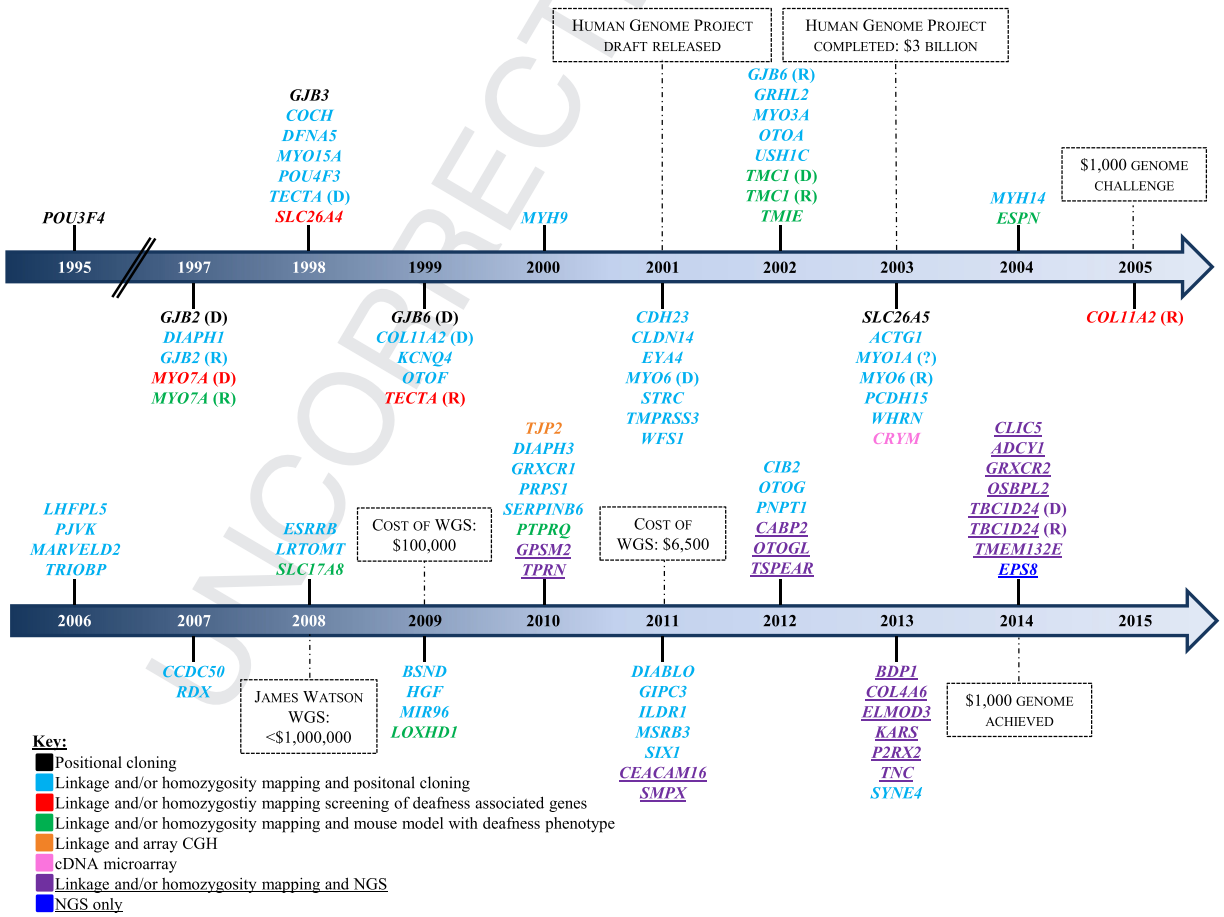

Fig. 1. Timeline of NSHL genes identified according to method of discovery and major achievements in the development of NGS technologies per year [97,98]. Abbreviations: D, dominant form of HL; R, recessive form of HL; WES, whole exome sequencing; WGS, whole genome sequencing.

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