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Mutations of KCNQ4 Channels Associated with Nonsyndromic Progressive Sensorineural Hearing Loss

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Abstract

Purpose of the review—This article provides an update on the current progress in identification of KCNQ4 mutations responsible for progressive hearing loss in DFNA2.

Recent findings—The *KCNQ4* gene has been identified at DFNA2 locus on the human chromosome 1p34. DFNA2 is a subtype of autosomal dominant nonsyndromic progressive hearing loss, characterized by hearing loss starting at high frequencies in the twenties and thirties, and then progressing to more than 60 dB with middle and low frequencies often affected as well, in less than 10 years. To date, eight missense mutations and two deletions of the *KCNQ4* gene have been identified in DFNA2 patients with various clinical phenotypes. In general, missense mutations are associated with younger-onset and all-frequency hearing loss, while deletion mutations are underlying later-onset and pure high-frequency hearing loss. The etiology of DFNA2 remains largely unknown at this point, even though the degeneration of cochlear outer hair cells, caused by dysfunction of KCNQ4 channels, might be one of the underlying mechanisms.

Summary—During the last decade, significant progress has been made in identifying KCNQ4 mutations in DFNA2 patients. Elucidation of the pathogenic effect of these mutations will help to gain insights to the molecular mechanisms of hearing and hearing loss, which, in turn, will facilitate informative genetic counseling, early diagnosis, and even treatment of hearing loss.

Keywords

Autosomal dominant; nonsyndromic; progressive hearing loss; voltage-gated potassium channels; mutations

Introduction

The past several years have witnessed a period of breathtaking discovery regarding mutations in a variety of ion channel and co-transporter genes, which result in dysfunctional K⁺ regulation in the inner ear and eventually hearing loss [1]. Channelopathies are associated with both syndromic and non-syndromic hearing loss, most of which progress from high to low frequencies [2]. For example, mutations of KCNQ channels results in deafness in humans, as seen in Jervell and Lange Nielsen syndrome (JLNS) and autosomal dominant nonsyndromic sensorineural hearing loss DFNA2 [3,4]. The latter has been ascribed to mutations in KCNQ4 channels [4]. KCNQ4 channel belongs to the family of voltage-gated K⁺ channels, which consists of six transmembrane domains (S1–S6) and a K⁺ selective pore [5]. Most of missense mutations associated with DFNA2 affect the pore structure of the channels, exerting strong

dominant negative effects on the channel function [4,6–13]. Deletions, on the other hand, cause a frame-shift, resulting in the truncated channels that are nonfunctional [6,14]. Clinically, patients with deletions have milder hearing loss than that observed in the patients with missense mutations [11]. Further study of KCNQ4 mutations will improve our understanding of the molecular mechanisms of progressive hearing Loss.

Gene mutation is a leading cause of hearing loss

Deafness is one of the most common communication disorders in humans. Approximately one out of every thousand infants is born with a significant hearing deficit. The prevalence of hearing loss increases dramatically with age. By age 65, one out of three of us will suffer from hearing impairment sufficient to interfere with the understanding of speech [15]. Hearing impairment is a very heterogeneous disorder with a wide range of causes. It has been estimated that more than half of the cases of childhood deafness are hereditary, while the rest are attributed to environmental factors. Hearing loss in the elderly is in most cases due to a combination of both [15]. Genetic factors interact with the environment even when there seems to be a clear environmental determinant, as individuals may be genetically predisposed to hearing loss induced by noise, drugs, or infection. Thus, genetic factors, a single gene mutation in most of the case, are the leading cause for hearing loss.

Genetic heterogeneity of hearing loss

Deafness may occur in isolation (nonsyndromic) or along with additional clinical abnormalities, such as blindness or cardiac or kidney disorders (syndromic). More than four hundred syndromes associated with hearing loss have been identified, which account for thirty percent of the cases of hereditary hearing loss [16]. The online Mendelian Inheritance in Man (OMIM) database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim>) provides comprehensive descriptions of the clinical features and molecular genetics of these syndromes. On the other hand, the vast majority (>70%) of inherited hearing disorders are non-syndromic [15]. Nonsyndromic deafness is classified according to its mode of inheritance: DFNA (autosomal dominant), DFNB (autosomal recessive), and DFN (X-linked). More than a hundred gene loci for nonsyndromic deafness have been mapped to the human genome and more than fifty genes have been identified. A current list of these genes can be found at [Hereditary Hearing Loss Homepage](#). Despite the extreme heterogeneity of genes involved in nonsyndromic hearing loss [17–19], mutations in a single gene (*GJB2*) are responsible for as many as 50% of autosomal recessive hearing loss [20–24]. By contrast, most genes responsible for autosomal dominant hearing loss have been detected in only a single or a few families so far [15,18], with the only exception of DFNA2 which has been found in families around world (table 1).

KCNQ4 mutations identified in the DFNA2 families

The DFNA2 locus was originally assigned to chromosome 1p by linkage analysis in a large seven-generation Indonesian family with autosomal dominant nonsyndromic hearing loss [25]. The hearing loss first affected the high frequencies in the second or third decade of life and progressed in less than 10 years to more than 60 dB [25]. Additional genetic studies have restricted the DFNA2 locus to a 1.25-Mb region on chromosome 1p34 [26]. Currently, two deafness genes have been identified and a third gene was speculated at the DFNA2 locus [4, 27–30]. The *GJB3* gene encodes connexin31, a gap junction protein, whereas the *KCNQ4* gene encodes a voltage-gated potassium channel. To date, eight missense mutations and two small deletions of KCNQ4 channels have been identified [4,6–14]. The first KCNQ4 mutation, G285S, was found in all three affected members of a small French family with DFNA2 [4]. Another mutation affecting the same amino acid of the KCNQ4 channel, G285C, was detected in an American family [6]. Both mutations alter the first glycine of the K⁺ channel signature

sequence (GYG), which forms the K⁺ selectivity filter [5]. Mutations of these highly conserved amino acids disrupt the ability of the channel to discriminate between K⁺ and Na⁺ [31]. Not surprisingly, KCNQ4_{G285S} did not yield any detectable K⁺ currents when expressed in *Xenopus* oocytes [4]. Most importantly, the mutated channels exert a strong dominant-negative effect on wild type KCNQ4 currents, which may explain the autosomal dominant inheritance in DFNA2 families with *KCNQ4* mutations [4].

Apart from G285S and G285C, four more missense mutations were identified in the pore region of KCNQ4 channels. The W276S mutation was originally identified in a Dutch family [6] and four additional DFNA2 families from Japan and Europe [9–11]. This mutation alters Trp276, which is located in the pore loop of KCNQ4 channels, next to another tryptophan residue. These two adjacent tryptophan residues are highly conserved across different potassium channel families and play a crucial role in K⁺ channel function, presumably holding the pore open at a correct diameter [32]. Mutations in these conserved tryptophan residues result in a complete loss of function of K⁺ channels [33]. So far, W276S is the only mutation of KCNQ4 channel that has been detected in multiple DFNA2 families from different racial origins, suggesting that Trp276 might be a mutational hot spot in the *KCNQ4* gene [10]. Two leucines substitutions at the pore region of KCNQ4 channels, L281S and L274H, have been identified in the affected members of DFNA2 families [7,8]. Leucines are hydrophobic amino acids, which are usually buried within the interior of the protein structure. Leu 281 and 274 are highly conserved among KCNQ channels [7,8,13]. The replacement of these residues by a hydrophilic amino acid such as serine or histidine might significantly interrupt the structure of the KCNQ4 channel, therefore, leading to a loss of channel function. G296S is the sixth mutation found in the pore-forming region and was identified recently in a small Spanish family via a phenotype-guided mutation screening [13]. The glycine is located in a stretch of five amino acids that connects the P-loop domain and the S6 transmembrane domain of KCNQ4 channels and is perfectly conserved among voltage-gating K⁺ channels in all species. The G296S mutation results in not only a significant reduction of the surface expression of KCNQ4 channels but also impaired channel function [13]. Moreover, the G296S mutation exerts a strong dominant negative effect on wild type KCNQ4 channels, by reducing the channel expression at the cell surface. Thus, trafficking abnormality of heteromeric KCNQ4 channels has been proposed as the cause for DFNA2 pathology [13].

Mutations were also found in the positions other than the P-loop of KCNQ4 channels in DFNA2 patients. A mutation in the sixth transmembrane domain (G321S) was identified in a Dutch family [24], while another mutation at the third transmembrane domain (F182L) was recently reported in a Chinese family [14]. It is known that the S6 transmembrane domain contributes to the overall pore structure of the K⁺ channel. The replacement of glycine by serine at position 321 is expected to have strong impact on the channel function. However, S3 transmembrane domain is not directly involved with the pore formation, it would be plausible to predict that the F182L mutation causes hearing loss through a mechanism unrelated to altering the pore structure.

Two deletion mutations of KCNQ4 channels have been identified in DFNA2 families. The first deletion (211del13) was found in a Belgian family, lacking 13 nucleotides between positions 211 and 224 of the *KCNQ4* gene [6]. This deletion results in a frame-shift after Gly70 (FS71), followed by 63 novel amino acids and a premature stop codon. Consequently, the channel is truncated before the first transmembrane domain and is rendered nonfunctional. Most recently, a one-base deletion (211delC) was found in a Japanese family [12]. This deletion generates truncated KCNQ4 proteins similar to that result from the deletion 211del13 [12].

Phenotypes between DFNA2 patients with missense mutations and those with deletions are distinct [11]. A genotype–phenotype correlation has been suggested, based on the present data,

in which younger-onset and all-frequency hearing loss is associated with missense mutations, and later-onset and pure high-frequency hearing loss with deletion mutations. The phenotypic difference among DFNA2 patients may be caused by the difference in pathogenic mechanisms. Haploinsufficiency might be the pathogenesis for hearing loss in DFNA2 patients with deletions [6,11]. Due to the fact both deletions found so far generate proteins that are truncated before the first transmembrane domain, it is unlikely that the resulting proteins form tetramers with the wild type KCNQ4 subunits [11,12]. In contrast, the missense mutations, at least G285S and G296S, exert a strong dominant-negative effect on the wild type subunits and render heteromeric channels nonfunctional [4,13]. Since the presence of a heterozygous missense mutation leads to half of the channel subunits being defective and 15 out of 16 of channel tetramers dysfunction, it is not surprising that missense mutations cause more profound hearing loss than that which would result from deletions.

Mechanisms of progressive hearing loss in DFNA2

Although a strong correlation between KCNQ4 mutations and DFNA2 has been established, the precise pathology of progressive hearing loss remains largely unknown. Recently, a growing amount of evidence supports the hypothesis that $G_{K,n}$ in cochlear outer hair cells is composed of KCNQ4 subunits [34,35]. Loss of KCNQ4 currents may lead to a chronic K^+ overload in these cells and their degeneration, resulting in progressive hearing loss [36]. First, there is a strong correlation between the spatiotemporal expression of KCNQ4 channels [37, 38] and $G_{K,n}$ [35,39]. Second, $G_{K,n}$ in cochlear hair cells can be blocked by linopirdine and XE991, the KCNQ channel blockers [39–41]. Additionally, the deletion of KCNQ4 in the mouse model resulted in progressive hearing loss, which was paralleled by the loss of cochlear outer hair cells [36]. Furthermore, the G285S mutation of KCNQ4 abolished $G_{K,n}$ in both of the knock-in mouse line [36] and the transfected hair cell culture in vitro [35]. However, as a complete loss of outer hair cells results in only a hearing loss of about 30–50 dB [42], other processes must contribute to the final, severe deafness in DFNA2. There are several issues regarding the properties of KCNQ4 channels and the etiology of progressive hearing loss in DFNA2 that remain unresolved at this point. For example, why are the properties of the native $G_{K,n}$ distinct from the KCNQ4 current observed in heterologous systems? What is the functional significance of differential expression of multiple KCNQ4 splicing variants in the cochlea? What are the distinct roles of KCNQ4 channels in the inner ear that cannot be compensated for by other K^+ channels in diseased conditions? Answers to these questions will greatly improve our understanding of the cellular and molecular mechanisms of hearing in both normal and disease states.

Conclusion

DFNA2 is characterized by clinical and genetic heterogeneity. Two genes have been assigned and the third is proposed to DFNA2 locus. A series of mutations in both genes have been identified and more will be added to the list. At this moment, little is known about the molecular mechanisms of progressive hearing loss in DFNA2. Further study is required to elucidate the pathogenic effect for each of the mutations. With this information, there will be increased opportunities for early diagnosis and intervention, as well as the improvement of therapeutics.

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