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Original Research

An intronic mutation leading to incomplete skipping of exon-2 in *KCNQ1* rescues hearing in Jervell and Lange-Nielsen syndrome

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ABSTRACT

Romano-Ward syndrome (RWs) and Jervell and Lange-Nielsen Syndrome (JLNs) are two inherited arrhythmia disorders caused by monoallelic or bi-allelic mutations, respectively, in the *KCNQ1* or *KCNE1* genes. Both disorders could cause Long QT syndrome either without deafness (RWs), or with deafness (JLNs). We have performed clinical, molecular and functional investigation in two consanguineous Arabian families with history of sudden death of several children. Importantly, none of the affected individuals had (or have) any hearing impairment. Homozygosity mapping followed by molecular analysis identified a novel splice acceptor site mutation (homozygously) in intron-1 of the *KCNQ1* gene (c.387 −5 T > A), in these two apparently unlinked families. RNA analysis revealed that this splice site mutation causes incomplete transcriptional aberration of the *KCNQ1* gene, leaving 10% of the normal allele transcript intact, which restores the hearing function. Our molecular and functional data provide the first evidence that small amount (as low as 10%) of normal KCNQ1 current can effectively maintain the hearing function but fails to maintain cardiac repolarization characteristics within normal limits. Additionally, we have revealed four extra low frequency aberrant isoforms emphasizing the importance of intronic and other non-coding sequences in maintaining cellular homeostasis as pathologic changes in a single nucleotide can affect splicing events at distant sites. The novel *KCNQ1* mutation found in this study is very likely a founder mutation in the southern province of Saudi Arabia emphasizing its screening in the LQT population in this region.

Contents

Abbreviations: SCN5A, sodium channel, voltage-gated, type V, alpha subunit; KCNH2, potassium voltage-gated channel, subfamily H (eag-related), member 2; KCNQ1, potassium voltage-gated channel, KQT-like subfamily, member 1; KCNE1, potassium voltage-gated channel, Isk-related family, member 1; KCNE2, potassium voltage-gated channel, Isk-related family, member 2; ANK-B, ankyrin 2; KCNJ4, potassium inwardly rectifying channel, subfamily J, member 4; KCNJ11, potassium inwardly rectifying channel, subfamily J, member 11; KCNJ12, potassium inwardly rectifying channel, subfamily J, member 12; CALNA2, calcineurin A beta subunit; CLCN2, chloride channel 2; CACNA1C, calcium channel, voltage-dependent, L type, alpha 1C subunit.

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1. Introduction

Jervell and Lange-Nielsen syndrome (JLNs) is an autosomal recessive disorder clinically manifested by severe cardiac arrhythmias and congenital bilateral sensory neural deafness (Jervell and Lange-Nielsen, 1957). On ECG, the cardiac phenotype is characterized by a prolonged QT interval and polymorphic ventricular arrhythmias (torsade de pointes). These cardiac arrhythmias may result in recurrent syncopes, seizure, or sudden death. Homozygous or compound heterozygous mutations in the *KCNQ1* or *KCNE1* genes could cause JLNs (Neyroud et al., 1997; Schulze-Bahr et al., 1997; Duggal et al., 1998; Chen et al., 1999; Wang et al., 2002). In contrast to JLNs, heterozygous mutations in either of the genes (*KCNQ1*, *KCNE1*) are responsible for autosomal dominant Romano-Ward LQT1 syndrome (RWs) (Duggal et al., 1998; Wang et al., 1996; Donger et al., 1997; Splawski et al., 1997). Heterozygous carriers for JLNs causing mutations usually show a milder cardiac phenotype including only moderate prolongation of the QT interval (Wilde and Escande, 2001). Another important distinguishing clinical feature between the homozygous and heterozygous mutation carriers (*KCNQ1/KCNE1* genes) is that the heterozygous carriers don't show any congenital hearing impairment (Neyroud et al., 1997; Schulze-Bahr et al., 1997; Duggal et al., 1998; Wang et al., 1996; Donger et al., 1997; Splawski et al., 1997).

KCNQ1 and KCNE1 proteins co-assemble to form the cardiac K^+ channel, responsible for the slowly activating delayed rectifier outward K^+ current (I_{Ks}) (Sanguinetti et al., 1996). However, I_{Ks} channel has also been detected in the inner ear as a functional channel (Wangemann, 2002a,b). In the inner ear, I_{Ks} channel functions as K^+ charge carrier for sensory transduction and the generation of the endocochlear potential in the endolymph (Wangemann, 2002a,b) required to maintain normal hearing. In a mouse model Knipper et al. (2006) elucidated decline of functional I_{Ks} channel as the primary cause of deafness.

Though, JLNs causing homozygous or compound heterozygous *KCNQ1* mutation carriers in general suffer from congenital deafness and cardiac arrhythmias, yet, there are several reports where homozygous mutation carriers suffered only from cardiac arrhythmias and no deafness (Priori et al., 1998; Larsen et al., 1999; Wei et al., 2000; Novotny et al., 2006). Mechanism of hearing function preservation in severe arrhythmia patients (with homozygous/compound heterozygous *KCNQ1/KCNE1* mutations) remained unclear. It was suggested that these recessive mutations are probably mild mutations and thus are unable to abolish the I_{Ks} channel completely like in the JLNs patients (Wollnik et al., 1997), and the residual I_{Ks} could effectively maintain hearing, but not the normal cardiac electrical potential properties (Priori et al., 1998; Larsen et al., 1999). Proper and quantitative experimental data to support this hypothesis is lacking.

To date only one splice site mutation in *KCNQ1* has been described in connection to JLNs patients (Zehelein et al., 2006). A homozygous mutation ($c.477+1\,G>A$) at the splice donor site in exon-2–intron-2 junction (mentioned as exon-2 in the study of Zehelein et al., 2006) in the *KCNQ1* gene found as causal mutations in JLNs patients. At the mRNA level this donor site mutation exclusively produced *KCNQ1* transcripts lacking exon-2 leading to a frameshift at the 129th amino acid (p.129fs205X) (Zehelein et al., 2006), homozygous mutation carrier siblings are profoundly deaf in addition to the severe LQT cardiac phenotype (Zehelein et al., 2006).

In the present study, we have performed clinical, molecular and functional studies in two consanguineous Arabian families with history of sudden cardiac death of several children. Importantly, none of the affected individuals had any hearing impairment. Our study has elucidated a novel mutation, which is highly likely to be a founder mutation, in the *KCNQ1* gene pathogenic to severe recurrent and familial arrhythmias in a Yemeni tribe from southern part of Saudi Arabia. In depth molecular, functional and clinical analysis elucidated several new findings: a) intronic mutation in our patients did affect transcriptional aberration, but 10% of *KCNQ1* gene still could escape aberrant transcription, which could effectively rescue the hearing in our patients, but not the cardiac repolarization function; b) intronic mutation detected in this study could jeopardise the homoeostasis in the exon–intron splicing leading to aberrant splicing which includes exon skipping, inclusion of introns in the mRNA and also activation of cryptic splicing. Further, this aberration affects not only the following exon/intron immediately downstream to the mutation-spot, exons/introns further downstream/in a distant location could also be affected.

This is the first real evidence of dose effect of KCNQ1 protein on functional and clinical consequences into the heart and ear function.

2. Experimental procedures

2.1. Clinical analysis

Clinical consultation of the patients was performed at the Prince Sultan Cardiac Centre, Riyadh, Saudi Arabia. We have done our investigation in two probands from two unlinked families originating from the southern part of Saudi Arabia. Both probands are offsprings of asymptomatic, consanguineous, Arabian parents (Fig. 1A, families A and B). We have done our study on these two symptomatic and alive individuals who were affected by repeated syncope. The subjects underwent detailed cardiovascular examination and audiological examination.