European Review for Medical and Pharmacological Sciences 2017; 21 (4 Suppl): 79-84

Transient receptor potential melastatin 4 cation channel in pediatric heart block

J. TIAN, X.-J. AN, M.-Y. FU

Department of Cardiology, Xuzhou Children's Hospital, Xuzhou, Jiangsu, P.R. China

Abstract. – OBJECTIVE: Progressive cardiac conduction disease (PCCD) is a common pediatric heart conduction disorder. It is an autosomal inheritance of rare mutations, which leads to familial cases of PCCD. In these cases, the His-Purkinje system's conductive capacity is progressively deranged, involving either right or left bundle branch block. Also, QRS complexes display widening is an important characteristic that culminates in complete AV block, syncope, and sudden death. Mutations in TRPM4 gene that encodes for transient receptor potential melastatin 4 have recently been reported to cause familial cases of PCCD and heart block. TRPM4 conducts a Ca^{2+}-activated non-selective monovalent cationic current leading to a negative plasma membrane potential. TRPM4 channels let Na^+ ion influx, causing membrane depolarization, whereas, at positive membrane potentials, TRPM4 channels repolarize the membrane by facilitating K^+ ion efflux from the cell. TRPM4 protein contains many regulatory motifs that confer voltage dependence, ATP/ADP sensitivity, and Ca^{2+} responsiveness. Mutational studies revealed the significance of the two-calmodulin binding sites at the N-terminus of for Ca^{2+} dependent activation of this channel. Mutations that reduce deSUMOylation increase the steady-state levels of active TRPM4 channels on the membrane without alteration of its sensitivity to Ca^{2+} or ATP or its voltage dependence of activation. Increased TRPM4 function interferes with cardiac conduction and eventually contributes to heart block. Both gain and loss of function mutations of TRPM4 are implicated in the cardiac block. Currently, the major therapeutic management of cardiac block due to TRPM4 mutations is implantation of a pacemaker to reinstate normal current propagation through AV node.

Key Words:

Progressive cardiac conduction disease, TRPM4, His-Purkinje system, SUMOylation, AV block, Cardiac block.

Introduction

Heartbeat with its specific rhythm is dependent on electrical impulse. This impulse is transmitted through the heart by the presence of direct junctions between the cardiomyocytes and through the conduction system of the heart muscle. This system consists of the sinoatrial node, which is the physiological pacemaker, relatively ill-defined atrial tracks, and the atrioventricular (AV) node. Besides these, there is also a conduction system made up of a bundle of His, the left and right bundle branches, and Purkinje fibers called the ventricular conduction system[1]. Artificial pacemaker implantation is strongly suggested as a therapeutic measure under conditions of conduction block at the AV level or completes heart block. Progressive cardiac conduction disease (PCCD) is a common conduction disorder with a progressive disturbance in conduction through the His-Purkinje system, with either right or left bundle branch block. Moreover, characteristic widening of QRS complexes that culminates in complete AV block, syncope, and sudden death, are confirmed indicators[2]. Familial causes for the above pathological state involve an inheritance pattern that is autosomal dominant in nature, the presence of sporadic mutations in genes that code for components of cardiac impulse propagation systems and the intercellular communication. Some of these genes include SCN5A, coding for Nav1.5 sodium channel[3], SCN1B coding for sodium channel β-subunit[4] and TRPM4, coding for Transient Receptor Potential Melastatin 4 cation channel[1], and GJA5, coding for connexin[5]. TRPM4 is known to conduct a Ca^{2+}-activated non-selective cationic current. Therefore, at negative plasma membrane potential, TRPM4 channels let Na^+ ions to enter the cell, causing membrane depolarization. However, at positive plasma membrane potentials, TRPM4 channels help in repolarizing of the membrane by facilitating K^+ ion efflux from the cell[6,7]. Thus, the function of TRPM4 could either reduce or increase the driving force for Ca^{2+}, which has a significant impact on the frequency of intracellular Ca^{2+} oscillation in several cell types including

Corresponding Author: Xinjiang An, MD; e-mail: anxinjian001@163.com

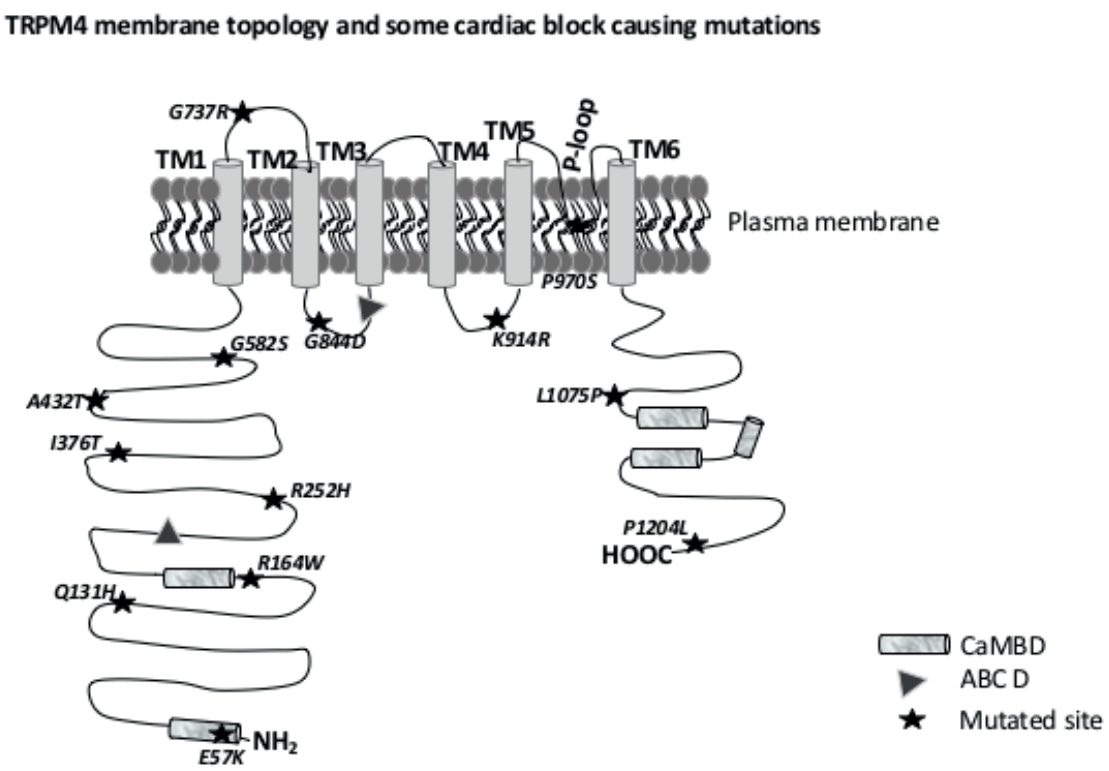

Figure 1. Plasma membrane topology of TRPM4 subunit and some cardiac block causing mutations. TM1-TM6, membrane-spanning segments. ABC D: ATP binding cassette domain; CaMBD: calmodulin binding site; Mutation sites are indicated in italics and "Stars". NH2, N-terminus of TRPM4; HOOC, C-terminus of TRPM4.

cardiomyocytes[8]. Also, TRPM4 is expressed in large quantities the heart[6] and the above channel represents the cardiac Ca^{2+}-activated transient inward current (Iti)[9,10]. So, it is quite probable that TRPM4 plays an important role in the cardiac conduction system[11].

Structure and Function of TRPM4

TRPM4 is a non-selective monovalent cation channel (NSCCa) that is activated by Ca^{2+} ions. However, it is not permeable to Ca^{2+} ions and has a single channel conductance in the range of 20-25pS. Moreover, it was initially described in rodent as well as in human cardiomyocytes[12,13]. Importance of TRPM4 in heart function has been confirmed by (I) the identification of TRPM4 specific inhibitor, 9-phenanthrol, which was instrumental in examining the TRPM4 mediated current modulation in the hearts of lab animals[14], (II) development of TRPM4-knockout mice for understanding the role of this cationic channel in heart[15] and (III) significance of different mutations of TRPM4 by positional cloning as well as their association with heart block and Brugada syndrome[16]. TRPM4 has been shown to play a role not only in heart function but also in the regulation of immune response, control of pancreatic β-cell insulin secretion apparatus, contraction of smooth muscle and apoptosis[17-19]. Expression-status of other membrane ion channels/transporters influences TRPM4 activation or inactivation and its cellular response[20]. TRPM4 belongs to the family of transient receptor potential channel proteins that includes TRPV1, TRPV4, TRPC, and others, which are encoded by nearly 28 genes in the human genome[19]. TRPM4 is a 1214-amino acid protein, coded by the TRPM4 gene and is present on chromosome 19[6]. Similar to other members of this transient receptor potential channels family, there are 6 transmembrane domains (TM1-TM6) in each TRPM4 monomer, and the actual channel pore is formed by a tetramer. Each of these four monomers possesses a membrane inserted loop structure, called P-loop, between TM5 and TM6 domains (Figure 1). Transmembrane domains TM5 and TM6 together with the P-loop constitute the central conduction pathway pore with the selectivity filter, which is selectively permeable to monovalent cations in the following preference: $Na^+ \geq K^+ >> Cs^+ > Li^+$. Voltage depen-

dence, Ca^{2+} sensitivity (activation/inactivation) of TRPM4 are also dependent on several regulatory motifs present on this protein. These include the ABC transporter motifs on the cytoplasmic amino-termini, carboxy-termini sites for protein kinase C (PKC) phosphorylation, a TRP domain for calmodulin binding and a pleckstrin homology (PH) domain[21]. Mutational studies revealed that the two calmodulin binding sites at the N-terminus of TRPM4 are essential for Ca^{2+} dependent activation of this channel[19]. Besides, the Ca^{2+} sensitivity of TRPM4, it is found observed to be elevated by PIP2 (phosphatidylinositol biphosphate), which shifts the voltage-dependence of TRPM4 channel activation toward negative potentials[22,23]. Also, cytosolic ATP and ADP inhibit[24] whereas hydrogen peroxide activates TRPM4 channel activity[25]. Further, it has been shown that reduced deSUMOylation increases the steady-state levels of active TRPM4 channels on the membrane without alteration of its sensitivity to Ca^{2+} or ATP[1]. However, it has been speculated that SUMOylation likely interferes with the ubiquitination and proteosomal targeting of TRPM4 for degradation, thereby elevating its plasma membrane levels[1]. Furthermore, in neuronal cells, a functional association between sulfonylurea receptor (SUR1) and TRPM4 (analogous to K-ATP channel and SUR1), has been observed, but its significance in cardiac function is still not clear[21,26].

TRPM4 and Conduction Blocks

An E7K mutation on the TRPM4 was the first evidence for its direct involvement in human heart block diseases, and this mutation is related to the conduction block in cardiac bundle branch[1]. Later studies identified several other TRPM4 mutations that are inherited in the autosomal dominant manner in different families along with isolated heart conduction blocks[27]. Many of these TRPM4 mutations were observed in 25% of the right bundle branch blocks and also in 10% of the AV blocks[27]. Interestingly, none of the mutations directly affected TRPM4 with respect to its biophysical or regulatory functions. However, these mutations were associated with the elevated amplitude of the whole-cell current, which was attributed to increased TRPM4 channel protein molecules on the cell membrane due to decreased proteasomal targeting and degradation of TRPM4[1,11]. Initial evidence that linked TRPM4 with heart disease was noticed in two large pedigrees, a South African family[1] and a Lebanese family[11] by the identification of an association with progressive familial heart block type I (PFHBI). There is a progressive impairment of conduction through the His bundle branches in PFHBI. The right bundle branch block (RBBB) is the site where this loss of conduction typically starts followed by left anterior hemiblock (LAHB), which progresses to a complete AV block. In PFHBI, there is an increase in QRS duration with time even though no changes are seen in PR and QTc intervals[28]. Following these two initial reports on the involvement of TRPM4 in PFHBI, several gain-of-function and loss-of-function TRPM4 variants have been reported[21,27]. A recent study[13] on 95 unrelated PCCD patients identified variants of TRPM4 gene employing next generation sequencing technologies. The above report also observed a novel variant I376T in TRPM4, which is carried by the proband of a 4-generation French pedigree. TRPM4-p. I376T mutation leads to a gain of function of this ion channel, and also enhances current density in association with elevated expression of TRPM4 protein at the cell surface (Table I)[2].

Possible Mechanisms for TRPM4 Gain/Loss of Function Mediated Defect in Cardiac Conduction

Despite the convincing study of the E7K gain of function mutation of TRPM4 associated with heart block, other evidence associated with conduction disorders led to the speculation that TRPM4 variants likely act only as modifiers of cardiac conduction[27,29]. One possibility is that as TRPM4 generates a net inward depolarizing current, assuming a tonic activity under resting conditions. So, an increase in TRPM4 function would depolarize the resting membrane potential, while a reduction in its activity would be expected to lead to hyperpolarization. Because of this, the functionality of the excitable Na^{+} and Ca^{2+} channels become dependent on TRPM4 activity; thus, altered TRPM4 activity could cause defective conduction[20]. In a recent study, two losses of function variants of TRPM4 (A432T and A432T/G582S) (Figure 1; Table I) in two patients with childhood AV block were found to show decreased expression at the cell membrane, which is probably due to misfolding of the protein and reduced trafficking to the membrane. Even though G582S variant alone showed increased expression, this was not related to SUMOylation of this variant. Moreover, other rare variants (D198G, A432T, T677I, and V921I) observed in this work indicated alternate mechanisms that are possible for elevated TRPM4

Table I. Various TRPM4 mutations and their effect on cardiac conduction and pathology.

Mutation	Location in protein	TRPM4 levels	Influence of mutation	ECG/phenotype	Reference
IL-1β (ng/mL)	Before treatment	156.4 ± 32.6	157.8 ± 35.5	0.069	0.924
	After treatment	133.4 ± 25.7	179.2 ± 43.5	5.326	0.032
TNF-α (ng/mL)	Before treatment	26.8 ± 7.2	25.4 ± 7.6	0.125	0.836
	After treatment	12.3 ± 5.2	44.7 ± 16.8	5.624	0.030
IL-6 (ng/mL)	Before treatment	45.6 ± 7.7	43.7 ± 8.2	0.326	0.724
	After treatment	37.6 ± 6.2	65.3 ± 9.6	5.127	0.035
IFN-γ (μmol/L)	Before treatment	15.5 ± 3.3	15.3 ± 3.5	0.215	0.766
	After treatment	14.5 ± 2.6	18.7 ± 3.4	4.968	0.037
IL-13 (ng/mL)	Before treatment	16.7 ± 5.2	16.55.3	0.426	0.695
	After treatment	21.8 ± 4.5	13.5 ± 3.4	5.102	0.035
IL-4 (ng/mL)	Before treatment	246.3 ± 64.9	234.7 ± 75.8	0.328	0.532

CaM: calmodulin; ND: not determined; RBBB: right bundle branch block; LBBB: left bundle branch block; LAHB: left anterior hemiblock; PFHBI: progressive familial heart block type I; AB block: atrioventricular block; BrS: Brugada syndrome; TM: transmembrane.

protein levels[20]. In line with what is said above, it has been noticed that the phenotype of different mutations of TRPM4 closely resembles the phenotypes of mutations in Nav1.5 Na^+ channel coding gene SCN5A. This reaffirms the idea that in cardiomyocyte excitability the main function of TRPM4 channel is to conduct Na^+ inward currents. The TRPM4 mRNA expression pattern in human heart revealed much higher levels of Purkinje fibers and weak expression in the left ventricle, with the relative order of TRPM4 expression being Purkinje fibers >> septum, right ventricle > right atrium, left ventricle, underlining the importance of this channel in cardiac conduction system[21]. The mutated TRPM4 and the increased current associated with ICCD and PFHBI probably lead to an elevated membrane leak conductance, which in turn disables action potential propagation down the Purkinje fibers. In agreement with this suggestion, there is often a broadened QRS complex in the electrocardiogram of patients with a right-bundle-branch block, bivascular block, and heart conduction block[1]. It is possible that TRPM4 channels are activated by an increase in intracellular Ca^{2+} during a late phase of cardiac action potentials, which results in slow down of action potential repolarization. Thus, both gain- and loss-of-function mutations in TRPM4 could cause conduction slowdown by decreasing the availability of Nav1.5 channels[16]. It has also been suggested that both gain and loss of function mutations of TRPM4 likely cause a rise or fall in extracellular K^+, thus, influencing the propagation velocity of cardiac impulse[29,30].

Pharmacological Agents Against TRPM4

TRPM4 channel was found[14] to be inhibited by the macrolide 9-phenanthrol, with an IC50 of about 2 μM, without affecting related channels like TRPM5, TRPC3, and TRPC6. However, at higher concentrations, 9-phenanthrol also affects other ion channels, like the voltage-gated Ca^{2+} and K^+ channels in cardiomyocytes, thus limiting its use as a probe and as a drug[31]. Since 9-phenanthrol acts as an anti-arrhythmic agent, it appears promising to develop better drugs to inhibit TRPM4, with high potency and specificity for use as anti-arrhythmic agents. The interaction between SUR1 and TRPM4 bestows sensitivity to drugs like glibenclamide in a manner and potency similar to that of K-ATP channels[32]. However, the significance of SUR1/TRPM4 interaction in heart and its role in cardiac blocks needs to be ascertained both experimentally as well as clinically so as to entertain the idea of exploiting SUR1/TRPM4 interaction for drug development. Other drugs such as clotrimazole, MPB-104, etc., that are also known to inhibit TRPM4 but due to less specificity they have limited application.

Conclusions

PCCD is a common pediatric heart conduction disorder responsible for complete AV block, syncope, and sudden death. Mutations in TRPM4 are prime causes of PCCD and heart block. The major therapeutic management of cardiac block due to TRPM4 mutations is implantation of a pacemaker to reinstate normal current propagation through

AV node. Pharmacological treatment options are still in the research stage and are not yet available.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Kruse M, Schulze-Bahr E, Corfield V, Beckmann A, Stallmeyer B, Kurtbay G, Ohmert I, Schulze-Bahr E, Brink P, Pongs O. Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. J Clin Invest 2009; 119: 2737-2744.
2) Daumy X, Amarouch MY, Lindenbaum P, Bonnaud S, Charpentier E, Bianchi B, Nafzger S, Baron E, Fouchard S, Thollet A, Kyndt F, Barc J, Le Scouarnec S, Makita N, Le Marec H, Dina C, Gourraud JB, Probst V, Abriel H, Redon R, Schott JJ. Targeted resequencing identifies TRPM4 as a major gene predisposing to progressive familial heart block type I. Int J Cardiol 2016; 207: 349-358.
3) Probst V, Kyndt F, Potet F, Trochu JN, Mialet G, Demolombe S, Schott JJ, Baro I, Escande D, Le Marec H. Haploinsufficiency in combination with aging causes SCN5A-linked hereditary lenegre disease. J Am Coll Cardiol 2003; 41: 643-652.
4) Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, Demolombe S, Probst V, Anselme F, Escande D, Wiesfeld AC, Pfeufer A, Kaab S, Wichmann HE, Hasdemir C, Aizawa Y, Wilde AA, Roden DM, Bezzina CR. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest 2008; 118: 2260-2268.
5) Makita N, Seki A, Sumitomo N, Chkourko H, Fukuhara S, Watanabe H, Shimizu W, Bezzina CR, Hasdemir C, Mugishima H, Makiyama T, Baruteau A, Baron E, Horie M, Hagiwara N, Wilde AA, Probst V, Le Marec H, Roden DM, Mochizuki N, Schott JJ, Delmar M. A connexin 40 mutation associated with a malignant variant of progressive familial heart block type I. Circ Arrhythm Electrophysiol 2012; 5: 163-172.
6) Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca^{2+}-activated nonselective cation channel mediating cell membrane depolarization. Cell 2002; 109: 397-407.
7) Xu XZ, Moebius F, Gill DL, Montell C. Regulation of melastatin, a trp-related protein, through interaction with a cytoplasmic isoform. Proc Natl Acad Sci U S A 2001; 98: 10692-10697.
8) Burt R, Graves BM, Gao M, Li C, Williams DL, Fregoso SP, Hoover DB, Li Y, Wright GL, Wondergem R. 9-phenanthrol and flufenamic acid inhibit calcium oscillations in hl-1 mouse cardiomyocytes. Cell Calcium 2013; 54: 193-201.
9) Guinamard R, Demion M, Chatelier A, Bois P. Calcium-activated nonselective cation channels in mammalian cardiomyocytes. Trends Cardiovasc Med 2006; 16: 245-250.
10) Nilius B, Vennekens R. From cardiac cation channels to the molecular dissection of the transient receptor potential channel TRPM4. Pflugers Arch 2006; 453: 313-321.
11) Liu H, El Zein L, Kruse M, Guinamard R, Beckmann A, Bozio A, Kurtbay G, Megarbane A, Ohmert I, Blaysat G, Villain E, Pongs O, Bouvagnet P. Gain-of-function mutations in TRPM4 cause autosomal dominant isolated cardiac conduction disease. Circ Cardiovasc Genet 2010; 3: 374-385.
12) Guinamard R, Rahmati M, Lenfant J, Bois P. Characterization of a Ca^{2+}-activated nonselective cation channel during dedifferentiation of cultured rat ventricular cardiomyocytes. J Membr Biol 2002; 188: 127-135.
13) Guinamard R, Chatelier A, Demion M, Potreau D, Patri S, Rahmati M, Bois P. Functional characterization of a Ca(2+)-activated non-selective cation channel in human atrial cardiomyocytes. J Physiol 2004; 558: 75-83.
14) Grand T, Demion M, Norez C, Mettey Y, Launay P, Becq F, Bois P, Guinamard R. 9-phenanthrol inhibits human TRPM4 but not TRPM5 cationic channels. Br J Pharmacol 2008; 153: 1697-1705.
15) Barbet G, Demion M, Moura IC, Serafini N, Leger T, Vrtovsnik F, Monteiro RC, Guinamard R, Kinet JP, Launay P. The calcium-activated nonselective cation channel TRPM4 is essential for the migration but not the maturation of dendritic cells. Nat Immunol 2008; 9: 1148-1156.
16) Liu H, Chatel S, Simard C, Syam N, Salle L, Probst V, Morel J, Millat G, Lopez M, Abriel H, Schott JJ, Guinamard R, Bouvagnet P. Molecular genetics and functional anomalies in a series of 248 Brugada cases with 11 mutations in the TRPM4 channel. PLoS One 2013; 8: e54131.
17) Guinamard R, Demion M, Launay P. Physiological roles of the TRPM4 channel extracted from background currents. Physiology (Bethesda) 2010; 25: 155-164.
18) Simard JM, Woo SK, Gerzanich V. Transient receptor potential melastatin 4 and cell death. Pflugers Arch 2012; 464: 573-582.
19) Mathar I, Jacobs G, Kecskes M, Menigoz A, Philippaert K, Vennekens R. TRPM4. Handb Exp Pharmacol 2014; 222: 461-487.
20) Syam N, Chatel S, Ozhathil LC, Sottas V, Rougier JS, Baruteau A, Baron E, Amarouch MY, Daumy X, Probst V, Schott JJ, Abriel H. Variants of transient receptor potential melastatin member 4 in childhood atrioventricular block. J Am Heart Assoc 2016; 5: pii: e001625.
21) Kruse M, Pongs O. TRPM4 channels in the cardiovascular system. Curr Opin Pharmacol 2014; 15: 68-73.
22) Nilius B, Mahieu F, Prenen J, Janssens A, Owsianik G, Vennekens R, Voets T. The Ca^{2+}-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-biphosphate. EMBO J 2006; 25: 467-478.

23) Zhang Z, Okawa H, Wang Y, Liman ER. Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. J Biol Chem 2005; 280: 39185-39192.

24) Nilius B, Prenen J, Voets T, Droogmans G. Intracellular nucleotides and polyamines inhibit the Ca^{2+}-activated cation channel TRPM4B. Pflugers Arch 2004; 448: 70-75.

25) Simon F, Leiva-Salcedo E, Armisen R, Riveros A, Cerda O, Varela D, Eguiguren AL, Olivero P, Stutzin A. Hydrogen peroxide removes TRPM4 current desensitization conferring increased vulnerability to necrotic cell death. J Biol Chem 2010; 285: 37150-37158.

26) Guinamard R, Bouvagnet P, Hof T, Liu H, Simard C, Salle L. TRPM4 in cardiac electrical activity. Cardiovasc Res 2015; 108: 21-30.

27) Stallmeyer B, Zumhagen S, Denjoy I, Duthoit G, Hebert JL, Ferrer X, Maugenre S, Schmitz W, Kirchhefer U, Schulze-Bahr E, Guicheney P, Schulze-Bahr E. Mutational spectrum in the Ca(2+)--activated cation channel gene TRPM4 in patients with cardiac conductance disturbances. Hum Mutat 2012; 33: 109-117.

28) Stephan E. Hereditary bundle branch system defect: survey of a family with four affected generations. Am Heart J 1978; 95: 89-95.

29) Abriel H, Syam N, Sottas V, Amarouch MY, Rougier JS. TRPM4 channels in the cardiovascular system: physiology, pathophysiology, and pharmacology. Biochem Pharmacol 2012; 84: 873-881.

30) Aksoy H, Okutucu S, Sayin BY, Ercan EA, Kaya EB, Ozdemir O, Inanici F, Aytemir K, Oto A. Assessment of cardiac arrhythmias in patients with ankylosing spondylitis by signal-averaged P wave duration and P wave dispersion. Eur Rev Med Pharmacol Sci 2016; 20: 1123-1129.

31) Simard C, Salle L, Rouet R, Guinamard R. Transient receptor potential melastatin 4 inhibitor 9-phenanthrol abolishes arrhythmias induced by hypoxia and re-oxygenation in mouse ventricle. Br J Pharmacol 2012; 165: 2354-2364.

32) Woo SK, Kwon MS, Ivanov A, Gerzanich V, Simard JM. The sulfonylurea receptor 1 (Sur1)-transient receptor potential melastatin 4 (TRPM4) channel. J Biol Chem 2013; 288: 3655-3667.