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ORIGINAL

The Role of *HCN4* Variants in Human Systolic Heart Failure and Protein Interaction Network

El Papel de las Variantes del *HCN4* en la Insuficiencia Cardíaca Sistólica Humana y Red de Interacción de Proteína

Rasmaya Niruri[1,2], Zullies Ikawati[1], Agung Endro Nugroho[1], Habibie Arifianto[3]

[1]Faculty of Pharmacy, Universitas Gadjah Mada. Yogyakarta, Indonesia.
[2]Département of Pharmacy, Faculty Mathematics and Sciences. Universitas Sebelas Maret, Surakarta, Indonesia.
[3]Faculty of Medicine, Universitas Sebelas Maret. Surakarta, Indonesia.

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Corresponding author: Rasmaya Niruri

ABSTRACT

Introduction: an elevated heart rate (HR) results in adverse outcomes in human systolic heart failure with a sinus rhythm. Genetic variants may alter HCN4 interactions with regulatory proteins and increase HR. This study aimed to generate a protein interaction network (PIN) associated with elevated HR and to determine *HCN4* gene variants (718G>A, 1571G>A, and 2648C>G) in patients with systolic heart failure, sinus rhythm, and elevated heart.
Method: STRING protein database was used to generate a PIN. Laboratory exploration was performed to identify *HCN4* gene variants in patients with systolic heart failure using PCR and DNA sequencing.
Results: PIN revealed eight nodes and 13 edges. STRING functional enrichment showed the essential proteins (ADRB1 and HCN channels) involved in HR regulation. GNAS and ADCY1 contributed to the regulation process. *HCN4* gene variants (718G>A, 1571G>A, and 2648C>G) alter the properties of HCN4 channel. STRING scores of protein-protein interactions that involved HCN4, ADRB1, GNAS, and ADCY1 were high (in the range of 0,879-0,979). The three gene variants were evaluated in 49 study participants with HR≥70 bpm after 10 mg bisoprolol therapy. However, only 718G>A was identified in three of 49 patients.
Conclusion: PIN revealed that the three essential proteins associated with HCN4 channels in elevating HR were ADRB1, GNAS, and ADCY1. Only HCN4 718G>A was found in three out of 49 patients with systolic heart failure, sinus rhythm, and increased HR, according to a laboratory investigation on *HCN4* gene variants.

Keywords: Systolic Heart Failure; Heart Rate; HCN4.

RESUMEN

Introducción: una frecuencia cardíaca (FC) elevada produce resultados adversos en insuficiencia cardíaca sistólica humana con ritmo sinusal. Las variantes genéticas pueden alterar las interacciones del HCN4 con las proteínas reguladoras y aumentar la FC. Este estudio tuvo como objetivo crear una red de interacción de proteínas relacionadas con el aumento de la FC y determinar las variantes del gen HCN4 (718G>A, 1571G>A y 2648C>G) en pacientes con insuficiencia cardíaca sistólica, ritmo sinusal y corazón elevado.
Método: se utilizó la base de datos de STRING proteínas para describir la red de interacción de proteínas. Se aplicó exploración de laboratorio (con PCR y secuenciación de ADN) para identificar la variante del gen HCN4 en humanos.
Resultados: la PIN reveló ocho nodos y 13 bordes. STRING reveló proteínas importantes (ADRB1 y HCN) involucradas en la regulación de la frecuencia cardíaca. Las variantes del gen HCN4 (718G>A, 1571G>A y

2648C>G) alteran las propiedades del canal HCN4. Las puntuaciones STRING de interacción proteína-proteína para HCN4, ADRB1, GNAS y ADCY1 mostraron valores altos (que oscilan entre 0,879 y 0,979). Los tres genes fueron evaluados en 49 pacientes con FC ≥70 lpm después de un tratamiento con 10 mg de bisoprolol. Sin embargo, sólo se identificó 718G>A en tres de 49 pacientes.
Conclusiones: PIN reveló tres proteínas importantes (ADRB1, GNAS, and ADCY1) que interactúan con HCN4. Basado en investigación de laboratorio, sólo se encontró HCN4 718G>A en tres de 49 pacientes diagnosticados con insuficiencia cardíaca sistólica, ritmo sinusal y FC elevada.

Palabras clave: Insuficiencia Cardíaca Sistólica; Frecuencia Cardíaca; HCN4.

INTRODUCTION

Systolic heart failure had a high distribution and mortality rate compared with diastolic heart failure.[1] A resting heart rate (HR) of ≥70 beats per minute (bpm) in systolic heart failure subjects with sinus rhythm significantly enhanced the risk of death.[2] HR reduction demonstrated beneficial outcomes for decreasing mortality rates.[3] Bisoprolol, a beta-1 adrenergic receptor (ADRB1) blocker, was recommended for rate lowering in heart failure. Bisoprolol protects cardiac against excessive catecholamine stimulation, continuous beta-adrenergic signaling, and cardiotoxic neurohormonal systems.[4,5] In clinical practice, achieving a target HR using ADRB1 blockers was difficult.[6]

Genetic variations play a role in regulating resting HR and cardiovascular diseases.[7] Hyperpolarization-activated cyclic nucleotide-gated 4 (HCN4), the primary hyperpolarization-activated cyclic nucleotide-gated (HCN) channel, is found in the sinoatrial (SA) node in humans. HCN4 plays a crucial role in pacemaker current and autonomic regulation of HR. HCN4 prompts to initiate heart rhythm.[8,9] Mutations in the *HCN4* gene are reported to be associated with tachycardia.[10,11,12,13] The three identified *HCN4* variants associated with elevated HR/tachycardia were *HCN4* c.718G>A(p.Val240Met), c.1571G>A(p.Arg524Gln), and c.2648C>G (p.Pro883Arg).[10,11,12] However, the *effect of HCN4* gene variants on HR varies.[10,13,14,15] Regulatory proteins that are interacted/complexed with HCN channel define different functions of the channel.[8] Therefore, this study aimed to create a protein interaction network (PIN) associated with elevated HR and determine the gene variants in patients with systolic heart failure, sinus rhythm, and elevated HR. This research provided valuable insights into the genetic factors and proteins that increase HR and expanded the view of controlling the HR mechanism associated with HCN4. These findings will expand systolic heart failure's diagnostic and treatment options.

METHOD

Protein interaction network

The STRING database was used to generate a PIN and to analyze functional protein pathways. The PPI in STRING networks was performed according to the procedure provided by the STRING consortium 2023.[16] STRING scores of 0,700 and 0,900 indicated high confidence and the highest interaction, respectively.[17]

HCN4 gene variants identification in human systolic heart failure with sinus rhythm and elevated HR

This study was approved by the ethics committee of Universitas Sebelas Maret and conducted at Sebelas Maret Hospital, Surakarta, Indonesia. The inclusion criteria for this study were adult patients (≥18 years old) with systolic heart failure, sinus rhythm, and elevated HR (HR ≥70 bpm) who had received 10 mg once daily of bisoprolol. The exclusion criteria were unwillingness to sign an informed consent form. Laboratory exploration was applied to identify the *HCN4* gene variant in the patients using polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequencing. DNA isolation and amplification were performed according to the procedures provided with the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific Inc.) and GoTag Geen (Promega). *HCN4* variants (718G>A, 1571G>A, and 2648C>G) were amplified with PCR primers (table 1). The sequencing result aligned with the NCBI reference sequence (NG_009063,1) for determining the gene variants.[18]

Table 1. Primers for *HCN4* amplification

SNP (exon)	Primers
718G>A (1st) [10]	(F):5'AGCAGCCCTCGGTGGACA'3; (R):5'ACCCACAGGATCATCGCTGT'3; 277 bp. [19]
1571G>A (4th) [11]	(F): 5′ TTCCCTCTCATCCACTGTCCC3′; (R):5′ GACCAATGTGCGGGTGCTCC 3′; 295 bp.[11]
2648C>G (8th) [12]	(F): 5' GATCCCTTCTGCGCTGGGCT; (R):TGAGCAGGGGAGAGTCGGAG; 285 bp.[19]

Note: bp=base pair; F= forward primer; R=reverse primer; SNP = single nucleotide polymorphism.

RESULT

The PIN revealed a STRING average local clustering coefficient of 0,779 (figure 1). Protein-protein interaction (PPI) data was presented on node-node interaction. Most of the PPI scores were around the highest confidence interaction. However, it was not applied to the protein interaction with guanine nucleotide-binding protein G(t) subunit alpha-3 (GNAT3), in which all the scores were lower than 0,700. Functional enrichment of the proteins (HCN, ADRB1, Guanine nucleotide subunit alpha (GNAS) and adenylyl cyclase type 1 (ADCY1)) network showed clusters of biological processes and molecular functions with low false discovery rates. Proteins involved in HR regulation were ADRB1 and four types of HCN channels. GNAS and ADCY1 contributed to the regulation process.[16] *HCN4* variants (c.718G>A (p.Val240Met), c.1571G>A (p.Arg524Gln), and c.2648C>G (p.Pro883Arg)) alter the properties of HCN4 channel[10,11,12] we identified a mutation (p.V240M)

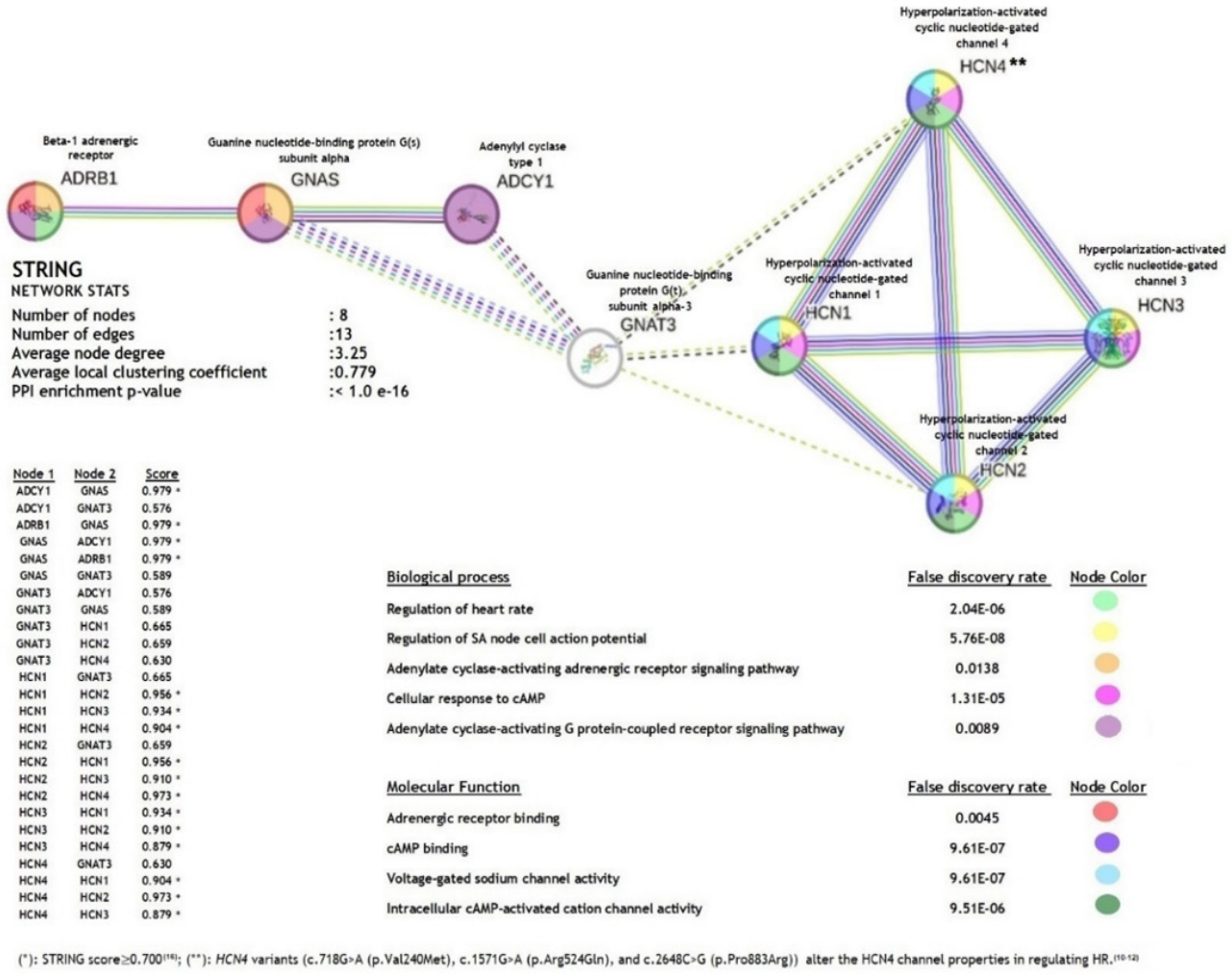

Figure 1. HCN4 channel and protein-protein interaction network

HCN4 gene at 718, 1571, and 2648 positions were evaluated in 49 patients (table 2, figure 2). Three subjects carried 718GA, and the rest had a wild type (WT) variant (718GG), which revealed double peaks and a single peak on the sequencing chromatogram, respectively (figure 2(A)). The WT gene variants (*HCN4* 1571GG and 2648CC) were identified in all patients (figure 2(B and C)).

Table 2. *HCN4* gene variant in human systolic heart failure with sinus rhythm and elevated HR after receiving 10 mg bisoprolol

Subjects *	***HCN4* 718**			***HCN4* 1571**			***HCN4* 2648**		
(N_{total}=49)	G	GA	AA	GG	GA	AA	CC	CG	GG
N	46	3	0	49	0	0	49	0	0
%	94 %	6 %	0 %	100 %	0 %	0 %	100 %	0 %	0 %

Note: (*) = All 49 patients were not diagnosed with anemia, fever, infectious disease, thyroid disorder, or decompensated heart failure; elevated HR=HR ≥70bpm

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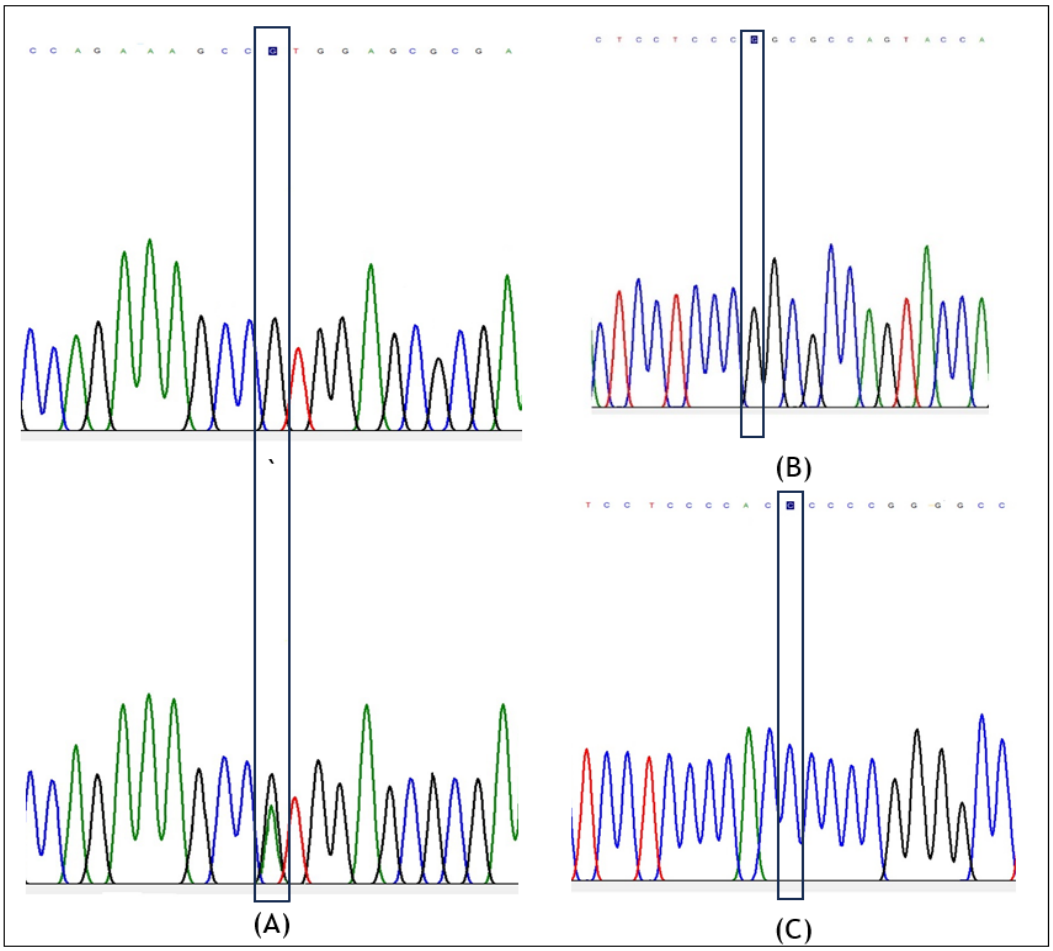

Figure 2. Sequencing chromatogram of *HCN4* variants: (A) 718GG (top) and 718GA (bottom); (B) 1571GG; (C) 2648CC

DISCUSSION

The PIN revealed the association of HCN channels, ADRB1, GNAS, and ADCY1 (figure 2). HCN channels are crucial in generating cardiac automaticity.[8] HCN4 is predominantly expressed in the SA node region.[20,21,22] Adenylyl cyclase (ADCY) is an enzyme that catalyzes cAMP production in the beta-adrenergic signaling pathway. ADCY1 is the predominant isoform in the SA node.[21,22] The synchronous work between HCN4 and ADCY1 is essential in the SA node after beta-adrenergic stimulation. ADCY1 contributes to the higher level of cAMP in the SA node. The role of cAMP is crucial in regulating cardsiac contractility and chronotherapy. The gene variants affect HCN4 channel properties and protein interactions.[11,21,22] *HCN4* 1571 variant alters the cAMP binding to the HCN4 channel.[11] The nucleotide change on *HCN4* 1571G>A (p.Arg524Gln) enhances cAMP sensitivity. The location of Arg524Gln is in the C-linkers (a part connecting cyclic nucleotide-binding domain (CNBD) to the S6 transmembrane domain) of the HCN4 channel, which is the binding site of cAMP for channel activation. Based on HEK293 cell and an animal study, as compared to the wildtype (WT) channel, the mutation (p.Arg524Gln)) was more sensitive to cAMP, which led to more sensitivity to adrenergic stimulation and a faster pacemaker rate. Ivabradine blocked both the mutant and WT HCN4 channel carriers similarly.[11,21,22]

HCN4 is crucial in regulating SA node potential (figure 1).[8] HCN4 expression affects pacemaker current (If) and activity. *HCN4* 2648C>G (p.Pro883Arg) and 718G>A (p.Val240Met) alter the HCN4 current.[11] The location of p.Pro883Arg is in the distal C-terminus of HCN4 comprised C-linker, which is linked to the transmembrane region with the CNBD. Based on functional analyses, the replacement of proline by arginine (p.Pro883Arg) altered the channel's current to a more positive potential, and the channel property to a more depolarized potential.[12,23]Meanwhile, the location of p.Val240Met is in HCN-domain (HCND) at the N terminus.[10] HCND interacts with the chanel voltage-sensing domain (VSDs) and cyclic nucleotide-binding domain (CNBD) of HCN channel gating.[22] The couple of HCND and VSD are crucial for HCN4 channel expression. The mutant channel increased the channel current and beating rate.[10]

All 49 patients carried the wild type of *HCN4* 1571 and 2648 gene. *HCN4* 1571G>A and 2648C>G were not identified in this study participants (table 2, figure 2). Heterozygous 1571GA was detected in five of ten Italian patients with IST or sinus tachycardia.[11] Heterozygous 2648CG is a genetic factor associated with tachycardia-induced cardiomyopathy in German patients with atrial fibrillation.[12] Both of these variants occur infrequently

in a population. Based on NCBI data (NM_005477.3(*HCN4*):c.1571G>A(p.Arg524Gln)), the frequencies are 0,0040 %, 0,000 %, and 0,000 % in 26588 European, 2918 African, and 112 Asian populations.[24,25] Meanwhile, frequencies of the variant (NM_005477.3(*HCN4*):c.2648C>G(p.Pro883Arg))are 1,0840 %, 0,3140 %, and 0,000 % in 35526 European, 3574 African, and 168 Asian populations.[23,26]

Based on the gene variant exploration in this study, only HCN4 718G>A was identified in three patients (table 2, figure 2). The mutant allele frequency was rare (0,0004 %) in South Asian population. Meanwhile, the variant was identified in nine of 16 Spanish patients. HCN4 718G>A was responsible for inappropriate sinus tachycardia (IST) in Spanish patients. [10]Anemia, fever, hyperthyroidism, and decompensated heart failure increase HR.[4] Increased body temperature is associated with HR modulation.[27] Anemia and hyperthyroidism enhance sympathetic activity, increasing HR.[28,29] None of these disorders were identified in this study participants (table 2). Heterozygous HCN4 718GA enhanced the pacemaker beating rate[10], thus explaining the elevated HR of the carriers (table 2). Ivabradine (HCN channel inhibitor) treatment with or without bisoprolol was reported to reverse the IST of the HCN4 718G>A carriers with or without a low ejection fraction.[10]Therefore, a combination with ivabradine was recommended for 718G>A carriers not controlled with bisoprolol alone.

CONCLUSION

PIN revealed that the three important proteins associated with HCN4 channels in elevating HR were ADRB1, GNAS, and ADCY1. According to the laboratory investigation of *HCN4* gene variants, only HCN4 718G>A was found in three of 49 patients with systolic heart failure, sinus rhythm, and increased HR.

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CONFLICT OF INTEREST

None.

AUTHORSHIP CONTRIBUTION

Conceptualization: Rasmaya Niruri, Zullies Ikawati, Agung Endro Nugroho, Habibie Arifianto.
Data curation: Rasmaya Niruri, Zullies Ikawati.
Formal analysis: Rasmaya Niruri, Zullies Ikawati.
Research: Rasmaya Niruri, Habibie Arifianto.
Methodology: Rasmaya Niruri, Zullies Ikawati, Agung Endro Nugroho, Habibie Arifianto.
Project management: Rasmaya Niruri, Zullies Ikawati.
Resources: Rasmaya Niruri, Zullies Ikawati, Habibie Arifianto.
Software: Rasmaya Niruri, Zullies Ikawati.
Supervision: Zullies Ikawati, Agung Endro Nugroho, Habibie Arifianto.
Validation: Rasmaya Niruri, Zullies Ikawati, Agung Endro Nugroho, Habibie Arifianto.
Display: Rasmaya Niruri, Zullies Ikawati.
Drafting-original draft: Rasmaya Niruri, Zullies Ikawati.
Writing- proofreading-editing: Rasmaya Niruri, Zullies Ikawati, Agung Endro Nugroho, Habibie Arifianto.