Genes & Diseases (2023) 10, 671–674

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Haploinsufficiency of KCNAB3 causes sudden cardiac death through delayed inactivation of Kv1.5 channel upon depolarization and induced myocardial apoptosis

Sudden cardiac death (SCD) is the leading cause of death from out-of-hospital cardiovascular disease worldwide, with an annual incidence of 1.4 per 100,000 in females and 6.8 per 100,000 in males.[1] Most cases of SCD in older patients (>45 years) are caused by atherosclerotic coronary artery disease. SCD in younger patients (<45 years) is caused by inherited cardiovascular diseases, including premature atherosclerosis, primary electrical disease, cardiomyopathy, thoracic aortic aneurysm, and dissection.[1] Primary electrical disease, caused by molecular defects in the ion channels involved in the generation of cardiac action potentials, accounts for approximately 93% of SCDs from a hereditary disease in patients with negative autopsy findings.[1]

Primary electrical disease is caused by the disorder of one or more ionic currents, including sodium, potassium, and calcium currents, which alters the balance between depolarization and repolarization during the generation of ventricular action potentials.[1] The disturbance of potassium channels in cardiomyocytes is a significant cause of SCD. Mutations in genes encoding potassium channel-related proteins in cardiomyocytes cause different categories of primary electrical disease.[2] For example, mutations in Potassium Voltage-Gated Channel Subfamily A Member 5 (*KCNA5*) cause atrial fibrillation, while variants in Potassium Voltage-Gated Channel Subfamily H Member 2 (*KCNH2*) may result in long QT syndrome.[2]

The human Potassium Voltage-Gated Channel Subfamily A regulatory beta subunit 3 (*KCNAB3*) gene is located in chromosome 17p13.1, consists of 14 exons, and spans approximately 7.9 kilobases. *KCNAB3* encodes a beta subunit of the voltage-gated potassium channel, which forms a heterodimer with KCNA5 and regulates the activity of the Kv1.5 channel.[3] Mutations of *KCNAB3* in neurons may lead to epilepsy and febrile seizures.[4] *KCNAB3* is expressed in cardiomyocytes and interacts with *KCNA5*, an atrial fibrillation–causing gene; therefore, *KCNAB3* may be associated with primary electrical disease.[3] However, whether variants of *KCNAB3* affect cardiomyocyte function remains unclear. The aim of this study was to explore the function of *KCNAB3* in cardiomyocyte. The materials and methods were included in Supplemental materials.

In this study, we enrolled a patient with SCD from the south-central region of China (Fig. 1A). The proband (II-3), a 20-year-old man, underwent a general physical examination three times due to sudden syncope, but the examinations did not reveal any relevant symptoms. Seven months after the examination, the proband died suddenly in his sleep. According to a family history survey, neither his parents (I-1 and I-2) nor his sister (II-1) suffered syncope or arrhythmia-related disorders. Whole-exome sequencing was performed on the proband and his parents to identify any genetic lesions in the patient. After data filtering and Sanger sequencing validation, as previously described, a *de novo* mutation (c.697dupG: p.A233GfsX22) of *KCNAB3* (NM_004732) was identified in the proband (Fig. 1B). This novel mutation was absent in his parents and our 200 control cohorts, as well as from the dnSNP132 and 1000G databases. Bioinformatics analysis revealed that this mutation, located in an evolutionarily conserved domain, is predicted to be disease causing and may result in a truncated KCNAB3 protein (Fig. 1C, D).

To confirm the pathogenicity of this mutation, a wild-type (WT) KCNAB3 CDS with a C-terminal His-tag and a WT KCNA5

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.05.023

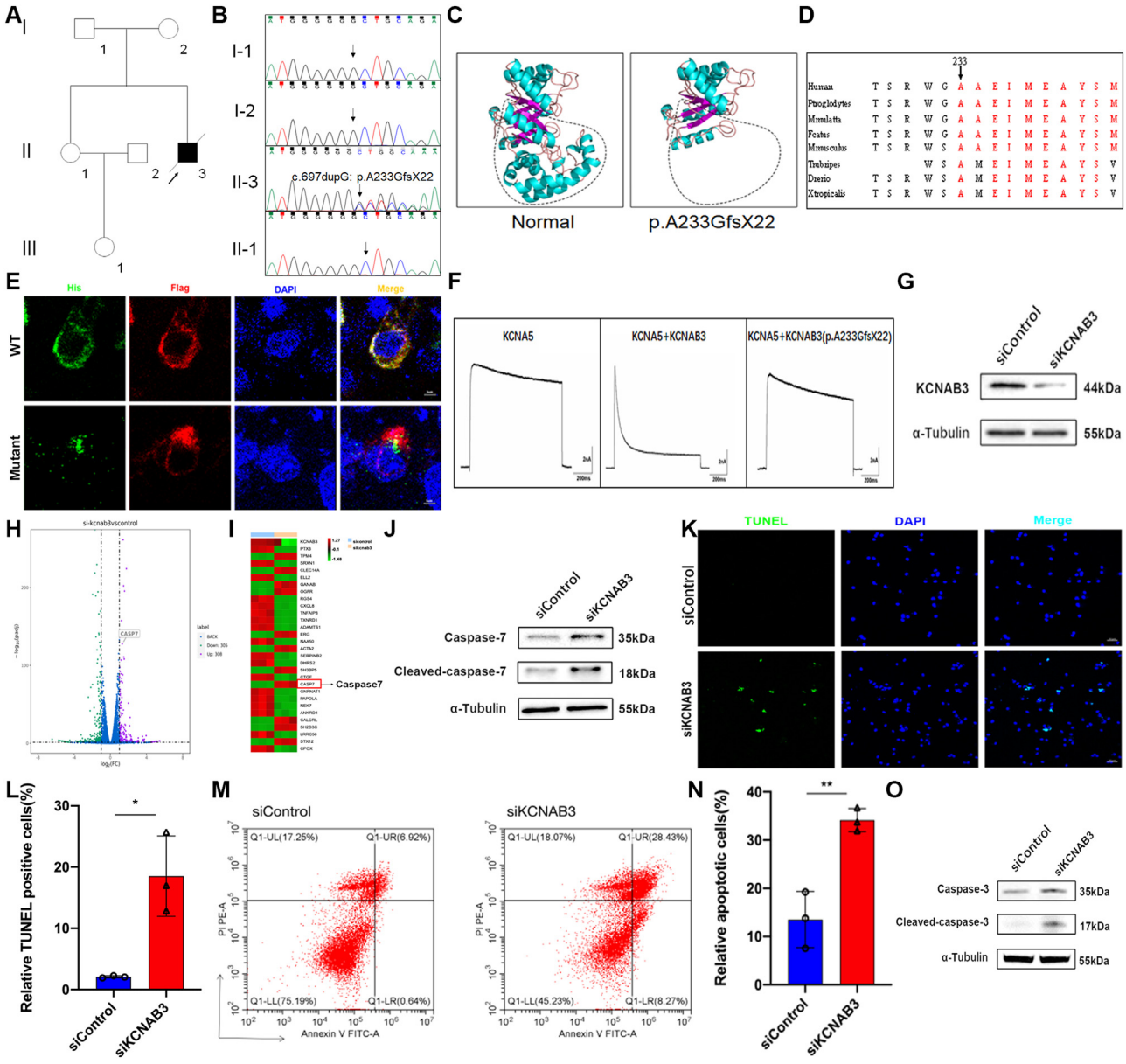

Figure 1 The genetic analysis and functional studies of KCNAB3 gene in SCD. **(A)** Pedigree of the SCD family. Circles refer to female subjects. Squares refer to male subjects. Solid symbols refer to affected subjects. The arrow indicates the proband. **(B)** Sanger sequencing analysis of the variant (c.697dupG: p.A233GfsX22) of *KCNAB3* (NM_004732) in the proband's parents and sister. **(C)** Swiss-model program predicts the structure of the KCNAB3 (WT and mutated) **(D)** Conservation analysis of the alanine residue at position A233 of KCNAB3. **(E)** The subcellular localization analysis of the WT and mutated KCNAB3 in HEK293 cells by laser confocal. Green shows KCNAB3, red represents KCNA5, blue exhibits cell nucleus. **(F)** Patch clamp experiments reveal the effect of the mutated KCNAB3 on Kv1.5. **(G)** Western blot analysis the expression of KCNAB3 in AC16 cells transfected with si-control and si-KCNAB3 si-RNA. **(H)** The volcano figure of RNA-seq in AC16 cells transfected with si-control and si-KCNAB3 si-RNA. **(I)** The heatmap analysis of the differentially expressed genes in AC16 cells transfected with si-control and si-KCNAB3 si-RNA. **(J)** Western blot analysis the expression of caspase7 and cleaved caspase7 in AC16 cells transfected with si-control and si-KCNAB3 si-RNA. **(K)** The TUNEL staining analysis of AC16 cells transfected with si-control and si-KCNAB3 si-RNA. Green represents apoptotic cells, blue exhibits cell nucleus. **(L)** Quantification of TUNEL staining. * means $P < 0.05$. **(M)** Flow cytometry analysis using Annexin V-FITC/PI staining detects the apoptosis of AC16 cells transfected with si-control and si-KCNAB3 si-RNA. **(N)** Quantification of flow cytometry analysis using Annexin V-FITC/PI staining. ** represents $P < 0.01$. **(O)** Western blot analysis the expression of caspase7 and cleaved caspase3 in AC16 cells transfected with si-control and si-KCNAB3 si-RNA. Results represent the mean ± SEM of at least 3 independent experiments as indicated in the figure legends.

CDS with a C-terminal Flag-tag were designed in pENTR and pcDNA3.1+ vectors, respectively. The p.A233GfsX22-KCNAB3 missense mutation was engineered into the vector using the MutanBEST Kit. HEK293 cells were transiently transfected with KCNAB3-pENTR (WT or Mutated) and/or KCNA5-pcDNA3.1+ using the Lipofectamine™ 2000 CD Transfection Reagent. Immunofluorescence analysis revealed that the subcellular localization of Mutated-KCNAB3 differed from that of WT-KCNAB3 (Fig. 1E). Analysis of electrophysiological currents showed that the transfection of *KCNA5* alone delayed the inactivation of Kv1.5-mediated currents and caused incomplete decay, whereas the co-expression of WT-KCNA5 and WT-KCNAB3 resulted in the rapid inactivation of Kv1.5-mediated currents and virtually complete decay. These findings are consistent with those of previous studies in Chinese hamster ovary cells.[3] However, the result of the co-expression of WT-KCNA5 and Mutated-KCNAB3 was similar to that obtained after transfection of *KCNA5* alone. This indicated that p.A233GfsX22 may be a loss-of-function mutation that causes *KCNAB3* to lose its ability to regulate Kv1.5 (Fig. 1F). Because mutations in *KCNA5* may cause atrial fibrillation,[2] we identified this *de novo* mutation as a likely genetic lesion in the patient.

To further reveal the function of *KCNAB3*, we knocked down *KCNAB3* expression in AC16 cell lines using siRNA (Fig. 1G). RNA-seq data showed that in *KCANB3* knockdown cells, the expression of 305 genes, including *KCNBA3*, was reduced, and the expression of 308 genes was increased, compared to that in cells transfected with control siRNA (Fig. 1H). Interestingly, among the top 30 genes with the most significant differences in expression, caspase7 was highly expressed in the *KCNAB3* knockdown group (Fig. 1I). Real-time PCR and western blotting further confirmed that the expression of caspase7 and cleaved caspase7 increased when *KCNAB3* was knocked down in AC16 cells (Fig. 1J). Because the activation of caspase7 may induce cell apoptosis, we analyzed cell death in AC16 cells transfected with si-KCNAB3. TUNEL staining suggested that *KCNAB3* knockdown promoted cell apoptosis (Fig. 1K, L). Furthermore, as shown in Figure 1M, flow cytometry analysis using Annexin V-FITC/PI staining showed that the apoptotic rate was significantly increased in cells transfected with si-KCNAB3, compared to that in the control (Fig. 1N). Finally, western blotting revealed that the expression of another apoptosis marker, cleaved caspase3, also increased in *KCNAB3* knockdown cells (Fig. 1O).

The Kv1.5 channel is closely associated with cell apoptosis and SCD. Several mutations of KCNA5 have been identified in patients with atrial fibrillation, which is one category of SCD.[2] Our study suggested that loss-of-function mutations in *KCNAB3* may delay the inactivation of the Kv1.5 channel upon depolarization and induce apoptosis in cardiomyocytes, potentially leading to SCD. Mutations in *KCNAB3* have been detected in patients with epilepsy,[4] and we hypothesized that the potassium ion channels play a crucial role in both brain and heart diseases; *KCNAB3* may exhibit genetic heterogeneity. Similarly, mutations in dipeptidyl aminopeptidase-like protein-6 (*DPP6*), an essential b-subunit of the human transient outward current encoded by Kv4.3, can cause idiopathic ventricular fibrillation or neurodevelopmental disorder.[5]

In summary, we identified a *de novo* mutation (c.697dupG: p.A233GfsX22) of *KCNAB3* (NM_004732) in a patient with SCD. Functional studies in HEK293 cells revealed that the novel mutation affects the subcellular localization of KCNAB3 and causes loss of function, which can delay the inactivation of the Kv1.5 channel upon depolarization. Further experiments in AC16 cells indicated that knocking down the expression of *KCNAB3* activates cleaved caspase7 and cleaved caspase3, ultimately inducing myocardial apoptosis. Our study is the first to establish a link between *KCNAB3* mutations and SCD. Mutations in *KCNAB3* may cause SCD by delaying the inactivation of the Kv1.5 channel upon depolarization and inducing myocardial apoptosis.

Ethics declaration

The studies involving human subjects were reviewed and approved by Ethics Committee of The Second Xiangya Hospital of Central South University (Approval No. 20190422) and conformed to the principles outlined in the Declaration of Helsinki. All the participants in the study have signed the written informed consent.

Author contributions

Ji-He Yu and Jun-Mei Xu enrolled the family. Zhao-Jing Lin performed the DNA extraction and Sanger sequencing. Chan Chen performed bioinformatic analysis. Ji-He Yu and Lv Liu performed the cell culture and functional studies. Ji-He Yu and Lv Liu wrote the draft of the manuscript. Rong Yu and Lv Liu designed and supported the project.

Conflict of interests

The authors declare no conflict of interests.

Funding

This study was supported by the National Natural Science Foundation of China (No. 81800220 and 82000079), Hunan Province Natural Science Foundation (China) (No. 2019JJ50890).

Acknowledgements

We thank all subjects for participating in this study. We thank Dr. Liang-Liang Fan from the department of cell biology, school of life science, central south university provided the assistance in patch clamp experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.023.

References

1. Waldmann V, Jouven X, Narayanan K, et al. Association between atrial fibrillation and sudden cardiac death: pathophysiological and epidemiological insights. *Circ Res*. 2020;127(2):301—309.
2. Christophersen IE, Olesen MS, Liang B, et al. Genetic variation in KCNA5: impact on the atrial-specific potassium current IKur in patients with lone atrial fibrillation. *Eur Heart J*. 2013;34(20):1517—1525.
3. Leicher T, Bähring R, Isbrandt D, et al. Coexpression of the *KCNA3B* gene product with Kv1.5 leads to a novel A-type potassium channel. *J Biol Chem*. 1998;273(52):35095—35101.
4. Ding J, Miao QF, Zhang JW, et al. H258R mutation in *KCNAB3* gene in a family with genetic epilepsy and febrile seizures plus. *Brain Behav*. 2020;10(12):e01859.
5. Ding DB, Fan LL, Xiao Z, et al. A novel mutation of dipeptidyl aminopeptidase-like protein-6 in a family with suspicious idiopathic ventricular fibrillation. *QJM*. 2018;111(6):373—377.

He-Yu Ji [a,c], Zhao-Jing Lin [a], Chan Chen [a], Jun-Mei Xu [a], Rong Yu [a,**], Lv Liu [b,*]

[a] *Department of Anesthesiology, The Second Xiangya Hospital of Central South University, Changsha, Hunan 410011, China*

[b] *Department of Respiratory Medicine, Diagnosis and Treatment Center of Respiratory Disease, The Second Xiangya Hospital of Central South University, Changsha, Hunan 410011, China*

[c] *Department of Anesthesiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100005, China*

*Corresponding author.

**Corresponding author.

E-mail addresses: yurong@csu.edu.cn (R. Yu), docliulv@csu.edu.cn (L. Liu)

11 April 2022
Available online 4 June 2022