JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2017 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER

VOL. 70, NO. 3, 2017
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2017.05.039

Progressive Atrial Conduction Defects Associated With Bone Malformation Caused by a Connexin-45 Mutation

Akiko Seki, MD, PhD,[a,b] Taisuke Ishikawa, DVM, PhD,[c] Xavier Daumy, PhD,[d] Hiroyuki Mishima, DDS, PhD,[e] Julien Barc, PhD,[d] Ryo Sasaki, DDS, PhD,[f] Kiyomasa Nishii, MD, PhD,[g] Kayoko Saito, MD, PhD,[h] Mari Urano, MA,[h] Seiko Ohno, MD, PhD,[i] Saki Otsuki, BS,[c] Hiroki Kimoto, MPharm,[c] Alban-Elouen Baruteau, MD, PhD,[d,j] Aurelie Thollet, PharmD,[k] Swanny Fouchard, PhD,[k] Stéphanie Bonnaud, PhD,[d] Philippe Parent, MD, PhD,[l] Yosaburo Shibata, MD, PhD,[m] Jean-Philippe Perrin, MD,[n] Hervé Le Marec, MD, PhD,[d,k] Nobuhisa Hagiwara, MD, PhD,[a] Sandra Mercier, MD, PhD,[o] Minoru Horie, MD, PhD,[i] Vincent Probst, MD, PhD,[d,k] Koh-Ichiro Yoshiura, MD, PhD,[e] Richard Redon, PhD,[d,k] Jean-Jacques Schott, PhD,[d,k] Naomasa Makita, MD, PhD[c]

ABSTRACT

BACKGROUND Inherited cardiac conduction disease is a rare bradyarrhythmia associated with mutations in various genes that affect action potential propagation. It is often characterized by isolated conduction disturbance of the His-Purkinje system, but it is rarely described as a syndromic form.

OBJECTIVES The authors sought to identify the genetic defect in families with a novel bradyarrhythmia syndrome associated with bone malformation.

METHODS The authors genetically screened 15 European cases with genotype-negative de novo atrioventricular (AV) block and their parents by trio whole-exome sequencing, plus 31 Japanese cases with genotype-negative familial AV block or sick sinus syndrome by targeted exon sequencing of 457 susceptibility genes. Functional consequences of the mutation were evaluated using an in vitro cell expression system and in vivo knockout mice.

RESULTS The authors identified a connexin-45 (Cx45) mutation (p.R75H) in 2 unrelated families (a de novo French case and a 3-generation Japanese family) who presented with progressive AV block, which resulted in atrial standstill without ventricular conduction abnormalities. Affected individuals shared a common extracardiac phenotype: a brachyfacial pattern, finger deformity, and dental dysplasia. Mutant Cx45 expressed in Neuro-2a cells showed normal hemichannel assembly and plaque formation. However, Lucifer yellow dye transfer and gap junction conductance between cell pairs were severely impaired, which suggested that mutant Cx45 impedes gap junction communication in a dominant-negative manner. Tamoxifen-induced, cardiac-specific Cx45 knockout mice showed sinus node dysfunction and atrial arrhythmia, recapitulating the intra-atrial disturbance.

CONCLUSIONS Altogether, the authors showed that Cx45 mutant p.R75H is responsible for a novel disease entity of progressive atrial conduction system defects associated with craniofacial and dentodigital malformation.
(J Am Coll Cardiol 2017;70:358–70) © 2017 by the American College of Cardiology Foundation.

Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

From the [a]Department of Cardiology, Tokyo Women's Medical University, Tokyo, Japan; [b]Support Center for Women Health Care Professionals and Researchers, Tokyo Women's Medical University, Tokyo, Japan; [c]Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [d]INSERM, CNRS, UNIV Nantes, L'Institut du Thorax, Nantes, France; [e]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [f]Department of Oral and Maxillofacial Surgery, Tokyo Women's Medical University, Tokyo, Japan; [g]Department of Anatomy and Neurobiology, National Defense Medical College, Tokorozawa, Japan; [h]Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan; [i]Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan; [j]Department of Congenital Cardiology, Evelina London Children's Hospital, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom; [k]CHU Nantes, L'institut du thorax, Service de Cardiologie, Nantes, France; [l]CHRU Brest, Service de Génétique, Brest, France; [m]Fukuoka Prefectural University, Tagawa, Japan; [n]CHU Nantes, Service de Chirurgie Maxillo-Faciale et Stomatologie, Nantes, France; and [o]CHU Nantes, Service de Génétique Médicale, Nantes, France.

Cardiac action potentials are generated and propagated through the cardiac conduction system, which consists of the atrial conduction system (including the sinoatrial [SA] and atrioventricular [AV] nodes) and the His-Purkinje system. Proper action potential propagation is ensured by gap junctions (GJs) responsible for cell-to-cell communication. GJs consist of 3 major connexin (Cx) isoforms—Cx43, Cx40, and Cx45—each of which are characterized by chamber-specific regional distribution and permeation properties. Cx45 is strongly expressed in the early embryonic myocardium and is required for cardiac development (1). Homozygous Cx45-deficient mice are embryonic lethal; however, in the adult heart, Cx45 is expressed mainly at the SA and AV nodes and is not essential for survival of adult mice. Disease-causing mutations in GJ gamma-1 protein (*GJC1*), which encodes Cx45, have not yet been identified.

SEE PAGE 371

Dysfunction of the cardiac conduction system is primarily due to acquired conditions, such as age-related degeneration, pathological conditions, post-operative complications, and drug toxicity (2). Inherited cardiac conduction diseases (CCDs) were first described by Lenègre (3) and Lev (4) as a progressive fibrotic process in the His-Purkinje system, characterized by bundle branch blocks with wide QRS complexes, leading to complete AV block, syncope, and sudden death. CCDs are rare arrhythmia disorders that involve mutations in genes that encode cardiac ion channels (*HCN4, SCN5A, TRPM4, SCN1B*), membrane adaptor proteins (*ANK2*), transcription factors (*NKX2-5, TBX5, GATA4*), components of the inner nuclear membrane (*LMNA, EMD*), and Cx40 (*GJA5*), which all regulate action potential generation and propagation (5,6). Most CCDs are isolated cardiac conditions, but a few syndromic forms have been described, such as Andersen-Tawil syndrome (periodic paralysis) (7), Holt-Oram syndrome (upper limb skeletal abnormalities) (8,9), and Emery-Dreifuss muscular dystrophy (muscular dystrophy) (10).

Congenital AV block is a rare arrhythmic disorder with an estimated prevalence of 1 in 14,000 to 20,000 live births (11). Transplacental passage of maternal anti-Ro-SSA and/or anti-La-SSB autoantibodies accounts for 90% to 99% of cases of congenital AV block diagnosed before 6 months of age (12). The remaining cases of congenital and childhood nonimmune AV block, without underlying structural heart disease, exhibit strong heritability (13), but the genetic basis of such idiopathic AV block is largely unknown.

To identify genetic determinants for nonimmune familial AV block, we performed trio whole-exome sequencing in 15 European cases with genotype-negative de novo AV block, as well as targeted exon sequencing of 457 CCD-susceptibility genes in 31 Japanese cases with genotype-negative familial AV block or sick sinus syndrome (SSS). We identified a Cx45 mutation, p.R75H, in 2 unrelated families: a de novo French case and a 3-generation Japanese family. Affected individuals commonly presented with progressive AV block, which resulted in atrial standstill, and was associated with an extracardiac phenotype of a brachyfacial cranial profile, finger deformity, and dental dysplasia, which suggests this is a novel disease entity of syndromic familial bradyarrhythmia.

ABBREVIATIONS AND ACRONYMS

AV = atrioventricular
CCD = cardiac conduction disease
Cx = connexin
ECG = electrocardiogram/electrocardiographic
GJ = gap junction
N2A = Neuro-2a
ODDD = oculodentodigital dysplasia
PM = pacemaker
SA = sinoatrial
SSS = sick sinus syndrome
WT = wild type

METHODS

All individuals who were enrolled in the study gave written informed consent before genetic and clinical investigations in accordance with the standards of the Declaration of Helsinki and the local ethics committees. AV block and SSS were defined as previously described (14). Subjects with underlying structural heart diseases, acquired autoimmune diseases,

Drs. Makita and Schott were supported by JSPS/CNRS Bilateral Joint Research Projects; Drs. Makita and Yoshiura were supported by the Japan Agency for Medical Research and Development (16km0405109h0004 and 15ek0109034h0002); Drs. Makita and Mishima were supported by JSPS KAKENHI (JP15H04823 and JP15K15311; JP25430183); Dr. Seki was supported by a research grant from Miwa Mikami Foundation; Dr. Ishikawa was supported by a research grant from the Takeda Science Foundation; Dr. Schott was supported by the Fondation pour la Recherche Médicale (DEQ20140329545); Dr. Redon was supported by the National Agency for Research (ANR-GENSUD-14-CE10-0001) and the Regional Council of Pays-de-la-Loire; Dr. Barc was supported by the H2020-MSCA-IF-2014 Program of the European Commission (RISTRAD-661617); and Dr. Probst was supported by the French Ministry of Health (PHRC-I PROG11/33), the Coeur et Recherche Foundation (R15074NN), and the Fédération Française de Cardiologie (RC13_0012). The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Seki, Ishikawa, and Daumy contributed equally to this work. Drs. Yoshiura, Redon, Schott, and Makita jointly directed this work and are joint senior authors. Drs. Schott and Makita equally contributed as corresponding authors.

Manuscript received February 10, 2017; revised manuscript received April 29, 2017, accepted May 9, 2017.