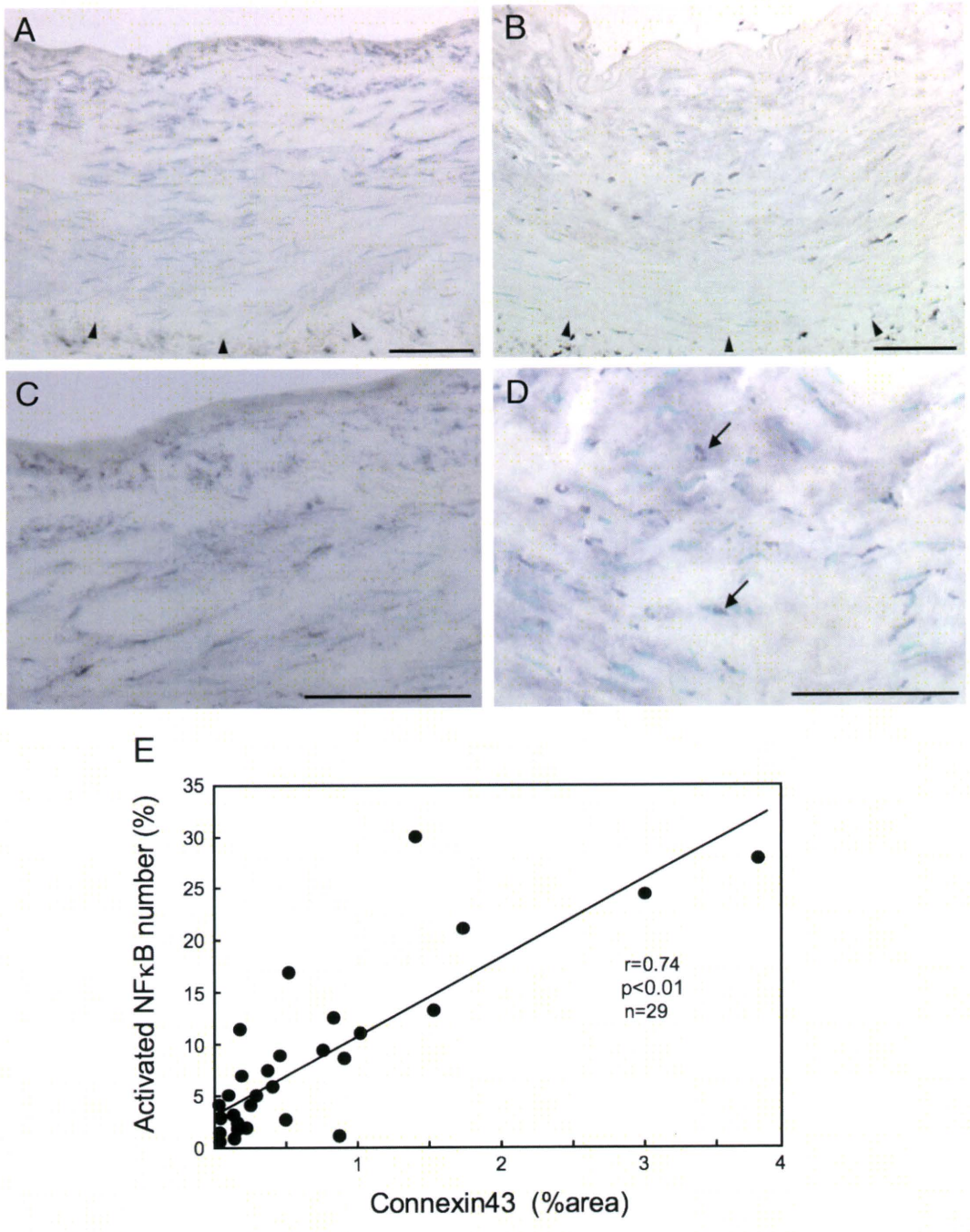

Fig. 1. Connexin 43 expression and NFκB activation in RA. Immunohistochemical staining for connexin 43 (A and C) and NFκB p65 subunit (B and D) in media of RA. C and D, positive staining at greater magnification. Arrowheads, external elastic laminae. Arrows, positive staining for NFκB p65 subunit. Bar, 100 μm. E, positive relationship between intensity of connexin 43 and NFκB p65 expression. RA, radial artery. NFκB, nuclear factor kappa B.

atherosclerosis [1,5]. Thus the regulation of Cx43 expression on medial SMC in ITA seems unique, whereas that in RA is universal. In addition, RA and coronary artery are similar in that they are both of the muscular type. Thus, Cx43 expression might be involved in the development of coronary atherosclerosis.

The fact that increased Cx43 expression correlated with NFκB activation in the distal portion of RA is of interest because bypasses might fail in patients with an RA bypass conduit in that location. In fact, bypasses became occluded at the RA-coronary anastomosis sites within 2 years of CABG in two of our patients who expressed high levels of Cx43 and activated NFκB in RA sections. Although we could not follow the remote patency of bypass conduits in all of the patients, Cx43 expression might be involved in its remote patency. Thus a strategy to decrease Cx43 expression in RA conduits might be useful in the clinical setting.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [6].

References

[1] Yeh HI, Lupu F, Dupont E, Severs NJ. Upregulation of connexin43 gap junctions between smooth muscle cells after balloon catheter injury in the rat carotid artery. Arterioscler Thromb Vasc Biol 1997;17:3174–84.

[2] Blackburn JP, Peters NS, Yeh HI, Rothery S, Green CR, Severs NJ. Upregulation of connexin43 gap junctions during early stages of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol 1995;15:1219–28.
[3] Ko YS, Yeh HI, Haw M, et al. Differential expression of connexin43 and desmin defines two subpopulations of medial smooth muscle cells in the human internal mammary artery. Arterioscler Thromb Vasc Biol 1999;19:1669–80.
[4] van Son JA, Smedts F, de Wilde PC, et al. Histological study of the internal mammary artery with emphasis on its suitability as a coronary artery bypass graft. Ann Thorac Surg 1993;55:106–13.
[5] Chadjichristos CE, Matter CM, Roth I, et al. Reduced connexin43 expression limits neointima formation after balloon distension injury in hypercholesterolemic mice. Circulation 2006;113:2835–43.
[6] Coats AJ. Ethical authorship and publishing. Int J Cardiol 2009;131:149–50.

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The stoichiometric relationship between KCNH-2 and KCNE-2 in I_{Kr} channel formation☆

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Ventricular arrhythmias are caused by reentrant circuits that occur due to the blockade of a conduction circuit in the muscle of the heart [1]. Torsade des pointes (TdP), a type of ventricular tachycardia characterized by twisting of the wave on an electrocardiogram, may be caused by the malfunctioning of inwardly rectifying potassium channels (I_{Kr} channels), which play significant roles in phase 3 of the cardiac action potential [2].

The pores of I_{Kr} channels are encoded by two genes, *KCNH-2* and *KCNE-2*, which serve as alpha- and beta-subunits, respectively. Mutations in either of these genes can cause long QT-like syndromes [3–6].

However, contrary to the assumption that the expression of KCNE-2 exceeds that of KCNH-2, a number of articles have shown that the ratio of *KCNH-2* mRNA to *KCNE-2* mRNA is inadequate to support the formation of a channel complex (i.e., the expression of KCNH-2 is much higher than that of KCNE-2) and this has resultantly lead to the weakness of KCNH-2 and KCNE-2 complex theory [7–9].

In this study, we examined the stoichiometric relationship between *KCNE-2* and *KCNH-2* to confirm whether the proteins they encode comprise a functional channel in cardiac muscle. To do so, we evaluated the expression of the two genes at the mRNA level by real-time polymerase chain reaction (PCR) and microarray analysis and at the protein level by Western blotting. The stoichiometry between two proteins was obtained using densitometer.

mRNA was extracted from the ventricles of 6-week-old SD rats ($n=3$; Orient Bio, Seongnam, Korea) using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and purified using an RNeasy Total RNA Isolation Kit (Qiagen, Hilden, Germany). Total RNA from the hearts of three individuals was quantified using a NanoDrop ND-1000 spectrophotometer (Wilmington, DE) and its integrity was assessed using a 2100 Bioanalyzer (Agilent, Böblingen, Germany). *KCNE-2* and *KCNH-2* mRNA were detected and quantified using SYBR Green (QunatiTect SYBR Green PCR Master Mix; Qiagen, Valencia, CA) according to the manufacturer's instructions using a Rotor Gene 6000 real-time rotary analyzer (Corbett Research, Sydney, Australia). Primers were designed using Primer 3 software (http://frodo.wi.mit.edu/; Table 1 in Appendix A). To ensure the specificity and integrity of the amplified products, melting curve analyses were performed on all amplified products. *GAPDH* was used as an internal control, and the fold-changes in expression were calculated according to the $2^{-\Delta\Delta CT}$ method. For the microarray analysis, an Affymetrix Rat Genome 230 2.0 array was used; all processing was performed according to the manufacturer's instructions (Affymetrix, Santa Clara, CA). Data processing of the cell intensity files (CEL) and microarray analysis were performed using GenPlex software (Istech Inc., Goyang, Korea). The data were normalized by global-scale normalization. The accession numbers of each probe used in the detection of *KCNH-2* and *KCNE-2* are given in Table 2 in Appendix A.

Astemizole (60 mg/kg), a selective I_{Kr} blocker, was administered to 6-week-old SD rats ($n=3$; Orient Bio) by oral gavage; rats given an equal volume of 0.5% methylcellulose ($n=3$) were included as a control. Four hours later, the rats' hearts were collected under isoflurane and RNA was extracted for microarray analysis as described above. Changes in the mRNA expression of *KCNH-2* and *KCNE-2* in response to astemizole were subsequently determined.

For deglycosylation study of KCNE-2, approximately 50 mg ventricular muscle was isolated from the 6-week-old SD rats ($n=3$). The samples were lysed with lysis buffer and automated sonicator. 60 μg of lysed samples was incubated with N-glycosidase at 37 °C for

☆ The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, see: http://www.textcheck.com/certificate/xRKStB.

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