TRANSLATIONAL PHYSIOLOGY

WTC deafness Kyoto (dfk): a rat model for extensive investigations of Kcnq1 functions

Hiroshi Gohma, Takashi Kuramoto, Mitsuru Kuwamura, Ryoko Okajima, Noriaki Tanimoto, Ken-ichi Yamasaki, Satoshi Nakanishi, Kazuhiro Kitada, Takeru Makiyama, Masaharu Akao, Toru Kita, Masashi Sasa, and Tadao Serikawa. WTC deafness Kyoto (dfk): a rat model for extensive investigations of Kcnq1 functions. Physiol Genomics 24: 198–206, 2006. First published December 20, 2005; doi:10.1152/physiolgenomics.00221.2005.—KCNQ1 forms K⁺ channels by assembly with regulatory subunit KCNE proteins and plays a key role in the K⁺ homeostasis in a variety of tissues. In the heart, KCNQ1 is coassembled with KCNE1 to produce a cardiac delayed rectifier K⁺ current. In the inner ear, the KCNQ1/KCNE1 complex maintains the high concentration of K⁺ in the endolymph. In the stomach, KCNQ1 is coassembled with KCNE2 to form the K⁺ exflux channel that is essential for gastric acid secretion. In the colon and small intestine, KCNQ1 is coassembled with KCNE3 to play an important role in transepithelial CAMP-stimulated Cl⁻ secretion. For further understanding of Kcnq1 function in vivo, an animal model has been required. Here we reported the identification of a coisogenic Kcnq1 mutant rat, named deafness Kyoto (dfk), and the characterization of its phenotypes. WTC-dfk rats carried intragenic deletion at the Kcnq1 gene and showed impaired gain of weight, deafness, and imbalance resulting from the marked reduction of endolymph, prolonged QT interval in the electrocardiogram (ECG), and gastric hyperplasia (16). The mechanism of deafness is that the lack of Iₖ, a voltage-dependent potassium channel; deafness; long-QT syndrome; achlorhydria; hypertension

KCNQ1 encodes a pore-forming (α) subunit of the voltage-gated K⁺ channel. It encodes six membrane-spanning domains (S1–S6), including the voltage sensor (S4) domain, and a K⁺-selective pore between S5 and S6 (2, 24). To form native channels, KCNQ1 coassembles with small β-subunits, so-called KCNE proteins. Although the stoichiometry of coassembly is not yet known, it is likely that four α-subunits assemble with four β-subunits to form the channels.

In the heart, KCNQ1 is coassembled with KCNE1. The KCNQ1/KCNE1 complex produces a slowly activating delayed rectifier K⁺ current (Iₖ₆) that contributes to the later phase of action potential repolarization, returning to the resting potential (2, 24). Mutations of the human KCNQ1 gene are associated with the congenital long-QT syndrome, an inherited disorder that is characterized by abnormal ventricular repolarization and increases the risk of sudden death from cardiac arrhythmias. There are at least two familial forms of long-QT syndrome. One is the Jervell and Lange-Nielsen syndrome, which is believed to be inherited as an autosomal recessive trait and associated with congenital deafness (12). A second, more common familial form is inherited as an autosomal dominant trait without other phenotypic abnormalities. This form, which is sometimes referred to as the Romano-Ward syndrome (34), is usually associated with a lower arrhythmia risk than the autosomal recessive form.

In the inner ear, both KCNQ1 and KCNE1 are expressed. In this tissue, the KCNQ1/KCNE1 complex produces a K⁺-rich fluid known as endolymph that bathes the organ of Corti, the cochlear organ responsible for hearing, and the utricle, saccule, and semicircular canal, which are responsible for balance and equilibrium. Functional loss of KCNQ1 provokes congenital deafness in the individuals with Jervell and Lange-Nielsen syndrome. The mechanism of deafness is that the lack of Iₖ₆ leads to inadequate endolymph production and deterioration of the organ of Corti (31).

In the stomach, KCNQ1 and KCNE2 are both expressed in the luminal membrane of the acid-secreting parietal cells (6, 11), where H⁺/K⁺-ATPase, a pump responsible for the transfer of H⁺ ions into the stomach, is coexpressed (9). The KCNQ1/KCNE2 complex yields K⁺ currents that are activated by acidic pH stimulation at resting membrane potential. Thus KCNQ1/KCNE2 forms acid-activated luminal K⁺ channels whose function is to supply K⁺ to the luminal surface to allow H⁺-for-K⁺ exchange by the pump (11). Disruption of Kcnq1 in mice causes a large increase in stomach pH that is accompanied by gastric hyperplasia (16).
In the small intestine and colon, KCNQ1 colocalizes with KCNE3 in the basolateral membranes of crypt cells (6). The KCNQ1/KCNE3 complex is constitutively opened at the more negative membrane potential of intestinal epithelial cells and can be further activated by the action of cAMP (25). This complex is thought to be important for maintenance of transepithelial transport in the colon and the small intestine, by recycling K\(^+\) that is transported into the cell by basolateral Na\(^+\)/K\(^+\)-2Cl\(^-\) (NaK2Cl) cotransporters and Na\(^+\)/K\(^+\)-ATPases. Thus the KCNQ1/KCNE3 channel is required for transepithelial cAMP-stimulated Cl\(^-\) secretion.

In the airway epithelia, KCNQ1 mediates a basolateral K\(^+\) conductance that plays an important role in maintaining cAMP-dependent Cl\(^-\) secretion (10, 17). The \(\beta\)-subunit coassembled with KCNQ1 in the airway epithelia has been a matter of debate. In the mouse, KCN3 is thought to be coupled with KCNQ1 (10). In the pancreas, KCNQ1 is expressed in the insulin-secreting cells. Inhibitors for the KCNQ1 channels increase the insulin secretion, which suggested that KCNQ1 would be involved in insulin secretion by the regulation of membrane potentials in the insulin-secreting cells (29).

Kcnq1 expression is not only confined to the tissues described above but also occurs in many epithelial tissues, such as placenta, kidney, liver, and thymus (7, 33, 38). However, no clear functional role of Kcnq1 in these tissues has been found as yet. For a further understanding of Kcnq1 functions in these tissues as well as the establishment of novel therapeutic procedures for diseases associated with Kcnq1 dysfunction, such as the long-QT syndrome, deafness, and achondroplasia, an animal model that is easy to investigate and manipulate is required. To address this issue, a rat model would be suitable, because of its easier clinical examination and sampling required. To address this issue, a rat model would be suitable, because of its easier clinical examination and sampling.

In this report, we describe the characteristics of a novel Kcnq1 mutation, deafness Kyoto (dfk), in the rat. Positional candidate cloning revealed that the dfk allele is an intragenic deletion including exon 7 of the Kcnq1 gene. WTC-dfk rats suffer from deafness and imbalance resulting from profound morphological abnormalities of the inner ear. These rats exhibit prolonged QT intervals and T-wave abnormalities on electrocardiogram (ECG) measurements and elevation of pH to almost neutral in the gastric secretion. Additionally, WTC-dfk rats display hypertension.

**MATERIALS AND METHODS**

**Animals.** Rats showing abnormal behaviors characterized by head tossing, drawing back, stepping back, and circling were found in the N\(_2\)F\(_1\) generation of a WTC.ZI-Arrn\(^{\text{a-}}\) congenic strain (15) at the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, in 1999. Even after elimination of the Arrn\(^{\text{a-}}\) mutation on chromosome (Chr) 3 (14), these phenotypes were observed and were inherited in an autosomal recessive manner. These mutant phenotypes resembled behavioral features of some types of deafness mutants. Therefore, we called the causative gene “deafness Kyoto” (gene symbol, “dfk”). Because the backcross generation in which the dfk mutation was found was sufficient to replace the genetic background with WTC, dfk was thought to be synonymous with a mutation arising on the genetic background of an inbred WTC strain. Therefore, WTC-dfk and WTC are isogenic; their genetic backgrounds were identical except for the dfk mutation. ACI/NKyo and WTC rat strains were maintained at the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University. Animal care was conducted according to the Guideline for Animal Experiments of Kyoto University. All experimental procedures were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

The ACI/NKyo (NBRP no. 0001), WTC (NBRP no. 0020), and WTC-dfk (NBRP no. 0289) strains have been deposited in the National BioResource Project for the Rat in Japan and can be obtained from the Project (http://www.anim.med.kyoto-u.ac.jp/nbrf/).

**Auditory brainstem response measurement.** Auditory brainstem response (ABR) measurements were performed in three individuals for each WTC and WTC-dfk rats at 26 wk of age. The following experiments were done on animals that were anesthetized with ketamine hydrochloride (60 mg/kg, im) and pentobarbital sodium (21 mg/kg, ip) and kept at 38°C. Stainless steel needle electrodes were inserted subcutaneously into the vertex (indifferent) and one side (active) and the other side (ground) of the retroauricular region, respectively. The ABR was obtained by averaging 1,000 evoked responses to clicks stimulus intensities of 60, 65, 70, 75, 80, 85, 90, 100, 120, and 135 dB peak equivalent sound pressure level (peSPL), with 50-ms intervals generated by an acoustic stimulator (MEB-5504; Nihon Koden, Tokyo, Japan). Clicks were delivered through an inner ear type earphone facing the meatus acusticus externus.

**Genetic mapping.** (WTC-dfk × ACI)\(_F_2\) rats were intercrossed to obtain \(F_2\) rats. Homozygous WTC-dfk animals were identified on the basis of appearance of head-tossing and/or circling behaviors and inability to swim at 3–4 wk of age. Two hundred and forty WTC-dfk animals were obtained from 1,000 \(F_2\) progeny and used for genetic mapping. For the initial mapping of dfk, we employed pooled simple sequence-length polymorphism (SSLP) analysis (27). DNAs from 45 randomly selected rats were standardized to 20 ng/\(\mu\)L and equal amounts of individual DNAs were pooled. The dfk DNA pool was genotyped for 75 microsatellite markers distributed among all autosomes. For the fine mapping of dfk, all dfk/dfk animals were genotyped.

**Sequencing.** PCR products were treated with ExoSAP-IT (Amer sham Biosciences, Piscataway, NJ) to digest single-strand DNAs and excess primers. Cycle sequencing was performed with the BigDye Terminator Ready Reaction Mix according to the manufacturer’s instructions (Applied Biosystems, Fater City, CA). PCR samples were purified with CENTRI-SEP spin columns and were then loaded into an ABI PRISM 3100 genetic analyzer (Applied Biosystems).

**Northern blotting.** Poly(A) RNA was purified from total RNA by using Oligotex-dT30 Super (Roche Diagnostics). Two micrograms of poly-A RNA were electrophoresed on formaldehyde-denaturing agarose gels and blotted onto Hybond N+ membrane (Amer sham Biosciences). Bands on the autoradiograph were detected by using an imaging plate and a computerized image display system (BAS2000; Fuji Film, Tokyo, Japan). A DNA fragment containing exons 9–14 of rat Kcnq1 was used as a probe.

**Electrocardiograms.** ECG recordings were obtained from adult (12–18 wk) wild-type (\(n = 6\)) and WTC-dfk (\(n = 6\)) rats using implantable Physiotel TA10EA-F20 radio frequency transmitters and receivers (Data Sciences International, Arden Hills, MN). After an animal was anesthetized with chloral hydrate (600 mg/kg, ip), the transmitter was placed within the peritoneal cavity. The electrodes were placed at the right axilla and at the left side of the xiphoid. Each electrode was sutured subcutaneously. After implantation of the transmitters, the animals were allowed to recover for at least 72 h. Twenty consecutive beats were recorded from individual animals under resting conditions. Unfiltered data were analyzed offline, and QRS, QT, PQ, and RR intervals were measured. Rate-corrected QT values (QTc) were derived using the formula QTc = QT/SQRT(RR/100) (19).

**Histopathological analyses.** To evaluate the histological phenotype of dfk rats, WTC-dfk rats were killed under anesthesia at 34 wk of age (\(n = 3\)). Age-matched WTC rats (\(n = 3\)) were examined as controls.
Perfusion fixation through the left ventricle was performed with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains, spinal cords, inner ears, stomach, and representative visceral organs were removed and fixed with the same fixative solution. Before processing for paraffin embedding, the ear tissues were decalcified with 5% EDTA-dipotassium salt. The samples were embedded in paraffin, and sections (4μm) were stained with hematoxylin and eosin stain (HE). The cochlea was also embedded in epoxy resin, and then semithin sections (1μm) were stained with toluidine blue.

Stomach pH and acid output measurements. The WTC-dfk rats (11 wk old, n = 5) and the age-matched, wild-type, control WTC rats (n = 5) were fasted overnight before the experiment with free access to water. Each rat was anesthetized, and the abdominal cavity was opened and the pylorus ligated. Four hours after treatment, the rat was killed and the stomach was removed. The intraluminal contents were drained into a centrifuge tube after a small nick was made in the stomach wall along the greater curvature adjacent to the pyloric ligature. The solutions with the stomach contents were centrifuged to pellet the insoluble material. The pH of the supernatants was measured, and then acid was titrated using an automatic titrator COMTITE-500 (Hiranuma Sangyo, Ibaraki, Japan). The results were expressed as microequivalents of acid per liter of gastric volume.

Blood pressure measurement. Systolic blood pressure was measured by the tail-cuff method using a nonpreheated, noninvasive blood pressure monometer (MK-2000; Muromachi Kikai, Tokyo, Japan). The average values of three measurements were determined for individual animals. All measurements were performed on 11 male rats at 9 or 10 wk of age (18).

RESULTS

Clinical features of WTC-dfk rats. The dfk rats were recognized at 10 days after birth by their twisting of their necks toward the back when lifted by the tail. After weaning, they exhibited hyperactivity and usually showed rapid head bobbing and occasionally a head tilt. Mature WTC-dfk rats displayed bidirectional circling behavior. In addition, they were unable to swim. When WTC-dfk rats were placed into a deep tank filled with warm water, WTC-dfk rats immediately began rotating along their long axis and sank underwater. While underwater, the rats were still rotating along their body length. The rats seldom resurfaced until they were rescued. These findings suggested that WTC-dfk rats might have lost their balance and have defects in the inner ear, which is responsible for linear and angular acceleration.

The adult WTC-dfk rats seemed to be smaller than the normal littermates, so we measured the body weights of the WTC-dfk rats (male, n = 6) at 5, 6, and 10 wk of age. At 5 or 6 wk of age, body weights were not significantly different between WTC and WTC-dfk rats. At 10 wk of age, WTC-dfk rats showed significantly lower body weight than WTC rats (246.3 ± 3.2 vs. 190.6 ± 8.8 g, P < 0.01) (Fig. 1A).

ABR. In addition to the imbalance, WTC-dfk rats showed no response to sounds such as rapping and clapping. To test the...
Fig. 2. Positional cloning of the dfk mutation. A: distribution of haplotypes observed among 22 dfk-homozygous F2 progeny carrying a recombinant chromosome between D1Rat133 and D1Rat295. White boxes, homozygote for the WTC allele. Grey boxes, heterozygote. Black boxes, homozygote for the ACI allele. B: dfk was genetically mapped to 0.8-cM region between D1Kyo9 and D1Mgh10. The dfk showed no recombination with D1Wox9 in 480 informative meioses. The dfk locus was physically localized to the 1-Mb region defined with D1Kyo9 and D1Mgh10. Within the dfk locus, 4 genes have been mapped. In the WTC-dfk rats, a genomic sequence containing Kcnq1 exon 7 was deleted. The 5'- and 3'-breakpoints of the deleted sequence are indicated by arrowheads. C: expression of Kcnq1 assessed by Northern blot analysis. Poly(A) RNAs from the hearts of WTC and WTC-dfk rats are hybridized with a probe containing exons 9–14 of rat Kcnq1. A smaller Kcnq1 transcript due to the loss of exon 7 was observed in WTC-dfk. Hybridization signals of β-actin on the same blot are shown at bottom. Molecular weight markers are indicated to the left in kilobases. D: genomic sequences around the breakpoints of the dfk deletion. A 2,040-bp sequence containing the Kcnq1 exon 7 was deleted in the WTC-dfk genome. E: molecular diagnosis of the dfk deletion. With use of the primers rKcnq1–51 and rKcnq1–42, a 2,735-bp PCR product was obtained from the WTC genome, while a 695-bp fragment was obtained from the WTC-dfk genome.
auditory organ function, we measured ABR in WTC-dfk and WTC rats. In WTC rats, ABR composed of I, II, III, IV, and V peaks was observed with intensity of over 100 dB (Fig. 1B). The WTC rats showed an average hearing threshold of 68.3 ± 2.58 dB, whereas all WTC-dfk rats exhibited no ABR up to the maximum level (>135 dB) of acoustic stimulation (Fig. 1B), indicating that WTC-dfk rats were completely deaf.

Identification of the dfk mutation. The pooled SSLP analysis showed a linkage relationship between D1Rat429 and the dfk locus. A genetic linkage study of 240 WTC-dfk rats using 19 additional markers on Chr 1 narrowed down dfk to a 0.8-cM interval between D1Kyo9 and D1Mgh10 (Fig. 2, A and B). The dfk gene showed no recombination with D1Wox9 in 480 informative meioses. Within the dfk locus, four genes, Cd81 (CD 81 antigen), Kcnq1 (potassium voltage-gated channel, subfamily Q, member 1), Mrgrg (Mas-related G-protein coupled receptor member G), and Dhcr7 (7-dehydrocholesterol reductase), have been mapped, and these genes were thought to be candidates for dfk.

Because human KCNQ1 mutation is associated with deafness (22), and Kcnq1-deficient mice showed similar behavioral phenotypes to those of WTC-dfk rats (5), Kcnq1 was considered to be the strongest candidate. Northern blot analyses showed that Kcnq1 transcript of the WTC-dfk was smaller than that of WTC (Fig. 2C). Sequencing analyses of the entire coding region and all of the exon-intron boundaries of Kcnq1 revealed that the entire exon 7 and its flanking sequences were lacking in the WTC-dfk rats. The deletion was 2,040 bp in length and flanked with TG dinucleotide tandem repeats (Fig. 2D). No nucleotide alternations were observed in the coding sequences of either the Cd81, Mrgrg, or Dhcr7 genes between WTC and WTC-dfk rats.

To verify and diagnose the deletion at the molecular level, we designed the PCR primers rKcnq1–51 (5'-ACCTGTCATGGCTCCCTAGA-3') and rKcnq1–42 (5'-AGGCTGTCCTCAGCAAGAAG-3'), which are located outside of the 5'- and 3'-breakpoints, respectively (Fig. 2B). These primers yielded a 695-bp PCR product from WTC-dfk and a 2,735-bp PCR product from the wild-type WTC (Fig. 2E).

Histopathology of the inner ear. To identify the histopathological alterations responsible for the deafness and imbalance observed in WTC-dfk rats, we examined the inner ear struc-

Fig. 3. Histological features of the inner ear of WTC and WTC-dfk rats. A and B: epon-embedded sections of the cochlea stained with toluidine blue. Note that collapsed Reissner’s membrane, atrophied stria vascularis, loss of hair cells, and marked reduction of neurons in the spiral ganglion are observed in the 34-wk-old WTC-dfk rat. C and D: details of the saccule macula. Note that the membranous labyrinth is collapsed onto the macula and compresses the statoconia, statoconial membrane, and hair cells in the 34-wk-old WTC-dfk rat. E and F: details of the ampullary crest. Note that the membranous labyrinth is collapsed onto the culupa. A small cavity is present between the membrane and the culupa (indicated by an asterisk), and vacuoles are seen in the marginal region of the ampullary crest (indicated by arrows) in the 34-wk-old WTC-dfk rats. DC, dark cell; HC, hair cell, LM, membranous labyrinth, RM, Reissner’s membrane, SG, spiral ganglion, SV, stria vascularis.
tures. In the cochlea of the 34-wk-old WTC-dfk rats, the Reissner’s membrane was collapsed and the volume of the cochlear duct was markedly reduced. The stria vascularis was severely atrophied. The inner and outer hair cells in the Corti were degenerated, and swelling of supporting cells was observed. The number of neurons in the spiral ganglion was markedly reduced (Fig. 3). Such lesions were scattered in the mucosa, and their production was impaired in the WTC-dfk rats.

Gastric abnormalities in WTC-dfk rats. To evaluate Kcnq1 function in the rat stomach, we examined gastric functions of the WTC-dfk rats. The volume of the secretion products in the pylorus-ligated stomach collected during the 4-h experimental period was not different between WTC-dfk and WTC rats. However, the pH of the stomach fluids was elevated to almost neutral (pH = 7.24 ± 0.2) in the WTC-dfk rats, whereas the WTC stomach retained strong acidity (pH = 1.47 ± 0.1). The acidity of the stomach contents could not be detected in the WTC-dfk rats, while the acidity of the WTC stomach was 84.2 ± 18.6 meq/l (Table 2). These findings indicated that acid production was impaired in the WTC-dfk rats.

The most prominent pathological feature in the stomach of 34-wk-old WTC-dfk rats was the appearance of hypertrophic gastric glands in the mucosa of the stomach body (Fig. 5, B and C). Such lesions were scattered in the mucosa, and their cytoplasm was deeply stained with eosin. Additionally, dilatation of the fundic glands and fibrosis in the lamina propria were observed in the stomach body of WTC-dfk rats. Fibrosis was

### Table 1. Comparison of ECG parameters in WTC and WTC-dfk rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WTC</th>
<th>WTC-dfk</th>
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<tbody>
<tr>
<td>HR, bpm</td>
<td>319.4 ± 30.9</td>
<td>350.4 ± 31.1</td>
</tr>
<tr>
<td>RR, ms</td>
<td>189.0 ± 16.9</td>
<td>174.2 ± 17.7</td>
</tr>
<tr>
<td>PQ, ms</td>
<td>42.3 ± 2.8</td>
<td>40.8 ± 3.6</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>15.8 ± 1.9</td>
<td>15.9 ± 2.5</td>
</tr>
<tr>
<td>QT, ms</td>
<td>63.7 ± 13.7</td>
<td>86.8 ± 13.7†</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>46.4 ± 10.1</td>
<td>65.8 ± 9.2†</td>
</tr>
</tbody>
</table>

All data are presented as means ± SD. Data sets were obtained from WTC (n = 6) and WTC-dfk (n = 6) rats and were compiled using Student’s t-test. HR, heart rate; bpm, beats/min; QTc, rate-corrected QT values. *P < 0.05 vs. WTC. †P < 0.01 vs. WTC.

### Table 2. Acidity of stomach contents in WTC and WTC-dfk rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WTC</th>
<th>WTC-dfk</th>
</tr>
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<tbody>
<tr>
<td>Gastric volume, ml</td>
<td>2.8 ± 0.9</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>1.47 ± 0.1</td>
<td>7.24 ± 0.2†</td>
</tr>
<tr>
<td>Acidity, meq/l</td>
<td>84.2 ± 18.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

All data are presented as means ± SD. Data sets were obtained from WTC (n = 5) and WTC-dfk (n = 5) rats at 11 wk of age and were compiled using Student’s t-test. ND, not detected. †P < 0.01 vs. WTC.
prominent at the bottom part of the mucosa (Fig. 5B). No pathological alteration was observed in the pylorus of the WTC-dfk rats.

**Hypertension in WTC-dfk rats.** To examine the physiological phenotypic characteristics of the WTC-dfk rats, we collected phenotypic data about blood pressure, biochemical blood test values, hematology, and urology. The most striking finding was hypertension in the WTC-dfk rats. They showed significant increases in the systolic blood pressure (SBP) compared with WTC (165.8 ± 22 vs. 125.8 ± 14.4 mmHg, *P* = 0.0001). The blood pressure level of WTC-dfk rats was highly placed on the ranking list of the SBP of 98 rat inbred strains of the Rat Phenome Project of the NBRP in Japan (http://www.anim.med.kyoto-u.ac.jp/nbr) (18). The SBP of WTC-dfk rats was greater than the mean value plus one standard deviation of the SBP of the 98 rat strains (142.7 ± 19.4 mmHg), while that of WTC rats was within the mean value plus one standard deviation.

DISCUSSION

We have described here the molecular basis and phenotypes of a novel mutation of the rat Kcnq1 gene. WTC-dfk rats had an intragenic deletion that included whole exon 7 of the Kcnq1 gene. The rat Kcnq1 cDNA encodes a 669-amino acid polypeptide with six membrane-spanning segments and a pore-associated domain (13). Kcnq1 exon 7 is 111 bp in length and encodes a 37-amino acid peptide that forms a pore-associated domain and part of the sixth transmembrane domain. Therefore, the dfk deletion allele was thought to result in a smaller protein that lacked the pore, which plays important roles in generating K+ current.

We found several intriguing phenotypes of the WTC-dfk rat. First, these rats showed circular movements, imbalance, and complete deafness. Histological analyses demonstrated the marked reduction of the endolymph of the inner ear and the collapse of sensory hair cells. These findings implied that endolymph reduction followed by hair cell collapses resulted in inner ear defects in the WTC-dfk rats. Kcnq1-deficient mice also show circular movements, imbalance, deafness, and inner ear pathologies that are very similar to those observed in the WTC-dfk rats (5, 16). The phenotypic similarities and deduced functions of the mutated KCNQ1 of the WTC-dfk rat suggested that WTC-dfk rats would be deficient for KCNQ1 protein.

Second, the WTC-dfk rats manifested prolonged QT interval and abnormal T-wave form in the ECG, indicating that the ventricular repolarization was prolonged in the WTC-dfk rats. The ventricular repolarization derives from transient outward K+ currents (*I*o) and delayed, outwardly rectifying K+ currents (*I*K) (21). In the rat myocytes, two transient outward K+ currents, *I*o,t and *I*o,s (1, 36), and five distinct delayed rectifier currents, *I*Ks, *I*Kur, *I*K, *I*K,slow, and *I*ss, have been identified (1, 3, 20). However, *I*Ks current generated from KCNQ1/KCNE1
complex is not a prominent repolarizing current in adult rats or in mice. Therefore, the cardiac phenotype seen in the WTC-\textit{dkf} rats would not be a direct effect of \textit{Kcnq1} deficiency but would be induced by extracardiac stimuli such as autonomic nerve and hormonal factors (32, 35). This idea is supported by the data on the cardiac phenotypes of the \textit{Kcnq1}-deficient mice. They displayed abnormal T-wave form and prolongation of the QT interval when measured in vivo, but not in isolated hearts (5). In addition, nicotine challenge of the isolated heart and acute stress due to saline injection in vivo revealed that sympathetic stimulation induced a long-QT phenotype in \textit{Kcnq1}-deficient mice (28).

Third, we found achlorhydria in the WTC-\textit{dkf} rats. Because gastric H\textsuperscript{+} is secreted by the H\textsuperscript{+}/K\textsuperscript{+}-ATPase with coupling to the uptake of the luminal K\textsuperscript{+} (23, 37), it is possible that the mutated \textit{Kcnq1} polypeptide fails to transport K\textsuperscript{+} into the gastric lumen. Histological findings of the WTC-\textit{dkf} stomach were hypertrophy of the gastric glands and fibrosis in the lamina propria. Because the administration of proton pump inhibitor sometimes induces hypertrophic gastric glands in the rat, these pathological changes are thought to have been produced as a direct effect of the elevation of pH in the stomach (4). \textit{Kcnq1}-deficient mice also show achlorhydria and increased weight of the stomach resulting from mucous neck cell hyperplasia (8, 16).

It is likely that the lower body weight found in the WTC-\textit{dkf} rats is related to their hyperactivity associated with the inner ear defects. Additionally, the loss of \textit{Kcnq1} functions in the stomach, the intestine, or the pancreas might give another possible explanation. Gastric H\textsuperscript{+} is required to converse pepsinogen to pepsin, the protease largely responsible for initiating the digestion of proteins in the stomach. \textit{Kcnq1} expressed in the epithelium of the small intestine and colon is believed to regulate K\textsuperscript{+} transport into the lumen (25). Luminal K\textsuperscript{+} is required for transepithelial Cl\textsuperscript{−} secretion, which regulates the osmolarity of the intestinal or colonic mucus. In the insulin-secreting cells, the \textit{Kcnq1} channels might play a role in regulation of the insulin secretion (29). Thus investigating the digestive capability and insulin secretion levels in the WTC-\textit{dkf} rats would be helpful for determining the cause of the lower body weight.

Lastly, the WTC-\textit{dkf} rats displayed hypertension. This is the first evidence that \textit{Kcnq1} might be involved in the regulation of blood pressure in the rat. Hypertension is provoked by a variety of etiologies. We have not yet determined which factor(s) induces hypertension in the WTC-\textit{dkf} rats, but it seems likely that it is due to a defect in reabsorption in the proximal tubule of the kidney. In the mouse kidney, \textit{Kcnq1} colocalizes with \textit{Kcnqe1} in the brush border of the mid to late proximal convoluted tubule as well as in the proximal straight tubule (30). It is thought that the \textit{Kcnq1}/\textit{Kcnqe1} complex would make K\textsuperscript{+} flux to the lumen, which is essential to counteract membrane depolarization due to electrolytic Na\textsuperscript{+}-coupled transport (30). Considering the important role of reabsorption in the regulation of blood pressure, it is possible that there might be some defect(s) in the kidney proximal tubule of the WTC-\textit{dkf} rats.

WTC-\textit{dkf} rats offer a sophisticated genetic system for studies of the physiological functions of \textit{Kcnq1}, because this strain has a strict control strain, WTC. The two strains are cosogenic and have an identical genetic background except for the \textit{dkf} deletion. Thus phenotypic differences found between them would only result from the \textit{dkf} mutation, which would imply the involvement of the \textit{Kcnq1} in such phenotypes. This genetic system was obtained simply by a spontaneous mutation arising in an inbred strain. In the gene knockout mouse, one cannot exclude effects of genes closely linked to the targeted gene on their phenotypes, even after producing congenic strains (26). \textit{Kcnq1} has been shown to be associated with several diseases such as long QT, deafness, achlorhydria, and hypertension. To develop more effective treatments for these disorders, WTC-\textit{dkf} could offer a powerful new tool as a \textit{Kcnq1}-related disease model. Furthermore, because \textit{Kcnq1} is expressed in various epithelial tissues, including lung, colon, small intestine, and thymus (7, 33), the functions of \textit{Kcnq1} in these tissues could be clarified using the cosogenic system of WTC-\textit{dkf} and WTC.


