Long QT syndrome KCNH2 mutation with sequential fetal and maternal sudden death

Jon M. Tuveng[1], Britt-Marie Berling[1], Gabor Bunford[1], Carlos G. Vanoye[2],

Richard C. Welch[2], Trond P. Leren[3], Alfred L. George Jr[2], Torleiv Ole Rognum[4]

[1]Ringerike Hospital, Hønefoss, Norway

[2]Department of Medicine, Vanderbilt University, Nashville, TN 37232-0275 USA

[3]Section of Laboratory Diagnostics, Oslo University Hospital, Oslo, Norway

[4]Section of forensic Pediatric Forensic Medicine, Clinic of Laboratory Medicine, Oslo University Hospital, and Institute of Laboratory Medicine, University of Oslo, Oslo, Norway

Corresponding Author:
Torleiv Ole Rognum
Oslo Universitetssykehus HF
Avdeling for rettsmedisinske fag
Seksjon for rettsmedisinske undersøkelser av barn
Postboks 4950 Nydalen
0424 Oslo
Norway

Tel: +47 2301 3120
Email: trognum@ous-hf.no

Abstract We report a case of a woman who experienced intrauterine fetal death at full term pregnancy, and then died suddenly soon after learning about the death of her fetus. At autopsy, previously undiagnosed neurofibromatosis and an adrenal gland pheochromocytoma were discovered in the mother. Genetic screening also revealed a novel *KCNH2* mutation in both fetus and mother indicating type 2 congenital long-QT syndrome (LQTS). A catecholamine surge was suspected as the precipitating event of fetal cardiac arrhythmia and sudden fetal death, while the addition of emotional stress provoked a lethal cardiac event in the mother. This case illustrates the potential for lethal interactions between two occult diseases (phenochromacytoma, LQTS).

Keywords Sudden intrauterine death • Sudden maternal death in pregnancy • Long QT syndrome • pheochromocytoma • neurofibromatosis

Introduction

Case report

The patient was a 33 year old woman, with one previous spontaneous abortion, who was in the 40th week of an uncomplicated pregnancy. She was previously healthy with no known inherited diseases. Five days prior to term, she experienced a nocturnal headache with nausa, vomiting and tachycardia. She was hospitalised the following morning.

On examination, she was awake and alert, but appeared ill and pale. Her skin was dry and warm. Systolic blood pressure was 110 mmHg. The uterus was slightly hypertonic, but the patient did not feel pain. The patient had not felt fetal movements since her illness began. Fetal heart beat could not be detected and fetal cardiac arrest was diagnosed. An ultrasound examination demonstrated the fetus in a normal head down position but with a non-contracting fetal heart. There were no signs that could explain fetal cardiac arrest.

Although the patient's initial grief reaction was appropriate, within 30 minutes she became progressively more affected, and complained of abdominal pain and shortness of breath. She was given meperidine (Pethidine, 75 mg i.m.) for pain relief. Subsequently, her blood pressure fell to 100 mm Hg while she had a regular pulse at 86 beats per minute. Intravenous saline and supplemental oxygen were started. An abdominal and vaginal ultrasound examination was done to exclude abdominal bleeding, and there were no signs of fluid in the abdomen or pouch of Douglas. In route to the operating room where the patient was to undergo an emergency Cesarean section, she lost consciousness and had cardiac arrest. Despite resuscitation efforts lasting 25 minutes, she was pronounced dead. ECG recordings during resuscitation showed pulseless electrical activity (PEA).

Maternal autopsy

Several small (≤ 1 cm) skin tumours were observed on her abdomen, chest and back that resembled neurofibromas. In addition, there were several pigmented areas consistent with cafè-au-lait spots, and by histological examination with routine hematoxylin-azofloxin-safranin (HAS) staining and immunohistochemical demonstration of S-100 positive cells the diagnosis neurofibromatosis was made (Figs. 1a, b). Within the abdomen, there was a 4×4×6 cm adrenal tumour, weight 65 g, with haemorrhage. Histological examination showed pheochromocytoma (Fig. 2)

The heart weight was 376 g. Histological examination showed normal heart tissue architecture with mainly normal looking muscle fibers. In some areas muscle fibers showed contraction bands and in some areas the capillaries were filled with granulocytes. The findings are suspicious of acute hypoxic damage.Neuropathological examination of the brain showed moderate oedema and focal gliosis in the left cerebral hemisphere possibly consistent with neurofibromatosis. Blood chemistries demonstrated therapeutic levels of meperidine, and blood cultures grew β-haemolytic group B *streptococcus*, *staphylococcus aureus* and another *streptococcus* species.

In retrospect, signs of neurofibromatosis were evident in first degree relatives. The patient's 40 year old brother was healthy, but had small skin tumours on his arms and chest similar to those found at autopsy. He also had one syncopal episode at age 17 years. Her father died at age 72 years from myocardial infarction, but he also had several skin, and subcutaneous tumours. The patient's mother was alive at age 62 years and healthy. The mother had experienced syncope twice within the last two years.

Fetal autopsy

By the autopsy a fully developed male baby (weight 4198 g, length 53 cm, head circumference 37 cm) was found. There were no signs of trauma. There was a small amount of blood in the adrenal marrow consistent with hypoxia. The brain was oedematous without obvious signs of pathology. However, microscopic investigation of medulla oblongata showed slightly increased number of astroglial cells in both white and grey matter. In cerebellum, the number of astroglial cells in white matter was strikingly high. The findings may be part of a systemic disease, such as neurofibromatosis, the same systemic disease found in the mother. The microbiological examination did not disclose any bacterial invasion, and there were no alcohol or drugs in the fetal blood. The heart weight was 36 g and had normal configuration. The left ventricle wall was 7 mm thick and the right ventricle wall 3 mm thick. Histological examination showed normal tissue architecture and normal looking heart muscle fibers. Examination of the conduction system did not disclose abnormalities.

Methods

Molecular genetic testing

Five genes associated with the congenital long-QT syndrome (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*) were screened by DNA sequencing for mutations in translated exons and flanking intron sequences using methods that have been described previously [1].

Functional testing of the novel mutation

The *KCNH2*-R62Q mutation was engineered in a recombinant HERG cDNA using PCR site-directed mutagenesis (primer sequences available on request). The final construct was

assembled in a mammalian expression plasmid (pIRES2-EGFP, BD Biosciences-Clontech, Mountain View, CA, USA) in tandem with an internal ribosome entry sequence (IRES) and the coding sequence of enhanced green fluorescent protein (EGFP) for use as an indicator of successful transfection. All constructs were fully sequenced to verify the mutation and to exclude polymerase errors.

Cell culture and electrophysiology

A Chinese hamster ovary (CHO-K1, American Type Culture Collection, Manassas, VA, USA) cell line stably expressing IKS was generated utilizing the Flp-In System (Invitrogen-Life Technologies), as previously described [2]. Stable clones were identified by hygromycin (600 µg/ml) resistance and tested by whole-cell patch clamp recording. The cells were grown at 37°C in 5% CO2 in F-12 nutrient mixture medium supplemented with 10% fetal bovine serum, 2mM L-glutamine, penicillin (50 units/ml)-streptomycin (50 µg/ml), and 600 µg/ml hygromycin [2]. Thereafter, cells were transiently transfected using FuGENE®6 (Roche Applied Science, Indianapolis, IN, USA) with 3 µg plasmid DNA. Following transfection (48 hours), fluorescent cells were selected by epifluorescence microscopy for use in whole-cell patch clamp recording experiments [2]. Non-transfected CHO cells grown under these conditions did not exhibit measurable endogenous currents with the recording conditions employed for this study

Whole-cell currents were measured in the whole-cell configuration of the patch clamp technique using an Axopatch 200B amplifier (Molecular Devices Corp., Sunnyvale, CA, USA). The bath solution consisted of (in mM): NaCl 145, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10, HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanosulfonic acid) 10, glucose 10, adjusted to pH 7.35 with NaOH, ~275 mosmol/kg. The pipette solution consisted of (in mM): KCl 110, ATP-K_2 5, $MgCl_2$ 2, EDTA (ethylenediaminetetraacetic acid) 10, HEPES 10,

adjusted to pH 7.2 with KOH, ~265 mosmol/kg. Patch pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, USA) with a multistage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co., San Rafael, CA, USA) and heat-polished with a Micro Forge MF 830 (Narashige, Japan). After heat polishing, the resistance of the patch pipettes was 3-5 MΩ in the standard extracellular solution. As reference electrode, a 1-2% agar-bridge with composition similar to the bath solution was utilized. Whole-cell current traces were filtered at 1 kHz and acquired at 2 kHz.

Whole-cell currents were measured from –80 to +60 mV (in 10 mV steps) 1990 ms after the start of the voltage pulse from a holding potential of –80 mV, and at the peak amplitude of the currents elicited upon repolarization to –50 mV (tail currents). The access resistance and apparent membrane capacitance were estimated as described by Lindau and Neher [3]. Pulse generation, data collection and analyses were done with Clampex 8.1 (Molecular Devices Corp.). Statistical comparisons were made using Student's t-test and statistical significance was assumed for $P < 0.05$.

Results

Discovery of a Novel *KCNH2* mutation

DNA sequencing of the translated exons with flanking intron sequences of the long-QT syndrome (LQTS) genes *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2* revealed that the index patient and her child both were heterozygous for a novel *KCNH2* mutation (c.185G>A, ref. seq.: NM_000238.2) in exon 2 predicting substitution of arginine at position62 with glutamine (designated R62Q) in the encoded HERG potassium channel. The mutation was not identified in 630 unrelated subjects referred for genetic testing for LQTS.

Functional consequences of *KCNH2*-R62Q mutation

To determine the functional consequences of the novel mutation, we performed whole-cell patch clamp recordings of recombinant HERG potassium channels heterologously expressed in cultured mammalian (CHO-K1) cells. Expression of either wildtype (WT) or mutant HERG channels resulted in rapidly inactivating currents evoked by membrane depolarization and large tail currents upon repolarization to –50 mV (Fig. 3a), typical of the rapid cardiac delayed rectifier current, I_{Kr}. However, cells expressing the R62C mutation exhibited significantly smaller current density over a range of positive test potentials (Fig. 3b, c) indicating a partial loss-of-function. Further, the voltage dependence of channel activation was significantly shifted in the hyperpolarized direction ($V_{½}$: WT-HERG, 15.8 ± 3.4 mV, n = 4; R62Q, –4.3 ± 4.9 mV, n = 5; p = 0.016) without a significant difference in slope factor or time constants for deactivation. The degree of reduced current density observed for the mutant HERG channel is similar to the level reported for *KCNH2* mutations identified in two cases of sudden infant death syndrome and this was deemed sufficient to evoke cardiac arrhythmias [4]. These findings suggest that mutation *KCNH2*-R62Q confers reduced I_{Kr} and this is a plausible explanation for maternal and fetal predisposition to a life-threatening cardiac arrhythmia consistent with type 2 LQTS (*LQT2*).

Discussion

We report an unusual and tragic case of sequential sudden death of a fetus and mother likely related to undiagnosed LQTS and an occult phenochromocytoma. Intrauterine fetal death was associated with cardiac arrest possibly provoked by catecholamines. Maternal death may also have been related to high levels of circulating catecholamines originating from the

pheochromocytoma and aggravated by emotional stress. Lethal cardiac arrhythmias in both fetus and mother, both carriers of a novel *KCNH2* mutation with functional properties consistent with a loss-of-function and type 2 LQTS.

As described by Seth et al. [5] women with LQTS in a 9-month postpartum time have a 2.7-fold increased risk of experiencing a cardiac event and a 4.1-fold increased risk of experiencing a life-threatening event when compared to the preconception time period. After this transient high-risk postpartum period, the risk of cardiac events reverts to the pre-pregnancy risk. They also found that women with mutations in the HERG gene were at a considerable higher risk for cardiac events during the postpartum period than those with mutations in the *KCNQ1* or *SCN5A* genes. These findings confirm the report of Khositseth et al. [6] who identified a particularly high postpartum cardiac risk in 14 women with mutations in HERG gene, and also the findings of Heradien et al. [7] who reported a low rate of cardiac events during pregnancy in 36 women with mutations in the KCNQ1 gene.

To date, only a few case reports of a fetus with LQTS have been published. Hayashi et al. [8] described in 2000 the first report of identical twins with long QT syndrome due to a mutation in the HERG gene. Then, Miller et al. [9] reported a mother with recurrent third-trimester fetal loss and one surviving child who was heterozygous for a mutation in the gene SCN5A (R1623Q). Tomek et al. [10] described a fetus presenting with second-degree atrioventricular block, sinus bradycardia, and transient ventricular tachycardia with ventriculoatrial dissociation, due to long QT syndrome. The impaired relaxation of the left ventricle was explained by the extreme prolongation of the refractory period caused by the prolonged relaxation time. Komarlu et al. [11] reported a fetus presenting with tachycardia and hydrops fetalis, with long QT syndrome diagnosed electrocardiographically soon after birth. Most recently, Murphy, et al. [12] reported discovery of intrauterine ventricular arrhythmia at 19-weeks gestation due to a novel *SCN5A* mutation.

We speculate that heterozygosity for the *KCNH2*-R62Q mutation predisposed to cardiac arrhythmia and this was exacerbated by high levels of catecholamines produced by the pheochromocytoma. Moreover, our study underscores the importance of molecular autopsy in young, deceased subjects without any definite cause of death obtained by regular autopsy.

Key points

1. Pregnancy increases the risk of sudden death in persons with Long QT syndrome.
2. Exercise, emotional reactions may induce ventricular fibrillation in Type 2 LQTS.
3. A novel KCNH2 mutation with functional properties consistent with a loss-of-function and type 2 LQTS, was found in a case of sudden intrauterine death in a term male baby and in the mother that had heart arrest as she was informed about the death of her unborn baby.
4. The mother had a pheochromocytoma and high levels of circulating catecholamines originating from the tumour may have induced heart arrest in both baby and mother.
5. Genetic autopsy should be performed in cases of sudden unexplained death.

References

1. Berge KE, Haugaa KH, Früh A, Anfinsen OG, Gjesdal K, Siem G, et al. Molecular genetic analysis of long QT syndrome in Norway indicating a high prevalence of heterozygous mutation carriers. Scand J Clin Lab Invest 2008;68:362-8
2. Lundquist AL, Manderfield LJ, Vanoye CG, Rogers CS, Donahue BS, Chang PA, et al. Expression of multiple KCNE genes in human heart may enable variable modulation of I_{Ks}. J Mol Cell Cardiol 2005;38:277-87.
3. Lindau M, Neher E. Patch-clamp techniques for time-resolved capacitance measurements in single cells. Pflugers Arch 1988;411:137-46.
4. Rhodes TE, Abraham RA, Welch RC, Vanoye CG, Crotti L, Arnestad M, et al. Cardiac potassium channel dysfunction in sudden infant death syndrome. J Mol Cell Cardiol 2007;44:571-81.
5. Seth R, Moss AJ, McNitt S, Wojciech Z, Andrews ML, Qi M, et al. Long QT syndrome and pregnancy. J Am Coll Cardiol 2007;13:1092-8.
6. Khositseth A, Tester DJ, Will ML, Bell CM, Ackerman MJ. Identification of a common genetic substrate underlying postpartum cardiac events in congenital long QT syndrome. Heart Rhythm 2004;1:60-4.
7. Heradien MJ, Goosen A, Crotti L, Durrheim G, Corfield V, Brink PA, et al. Does pregnancy increase cardiac risk of LQT1 patients with KCNQ1-A341V mutation? J Am Coll Cardiol 2006;48:1410-5.
8. Hayashi K, Shimizu M, Ino H, Okeie K, Yamaguchi M, Yasuda T, et al. Identical twins with long QT syndrome associated with a missense mutation in the S4 region of the HERG. Jpn Heart J 2000:41;3:399-404.

9. Miller TE, Estrella E, Myerburg RJ, Garcia de Viera J, Moreno N, Rusconi P, et al. Recurrent third-trimester fetal loss and maternal mosaicism for long-QT syndrome. Circulation 2004;22;3029-34.
10. Tomek V, Skovranek J, Gebauer RA. Prenatal diagnosis and management of fetal Long QT syndrome. Pediatr Cardiol 2009;30:194-6.
11. Komarlu R, Beerman L, Freeman D, Arora G. Fetal and neonatal presentation of long QT syndrome. Pacing Clin Electrofysiol 2012;35:e87-90.
12. Murphy LL, Moon-Grady AJ, Cuneo BF, Wakai RT, Yu S, Kunic JD, et al. Developmentally regulated SCN5A splice variant potentiates dysfunction of a novel mutation associated with severe fetal arrhythmia. Heart Rythm 2012;9:590-7.

Figures

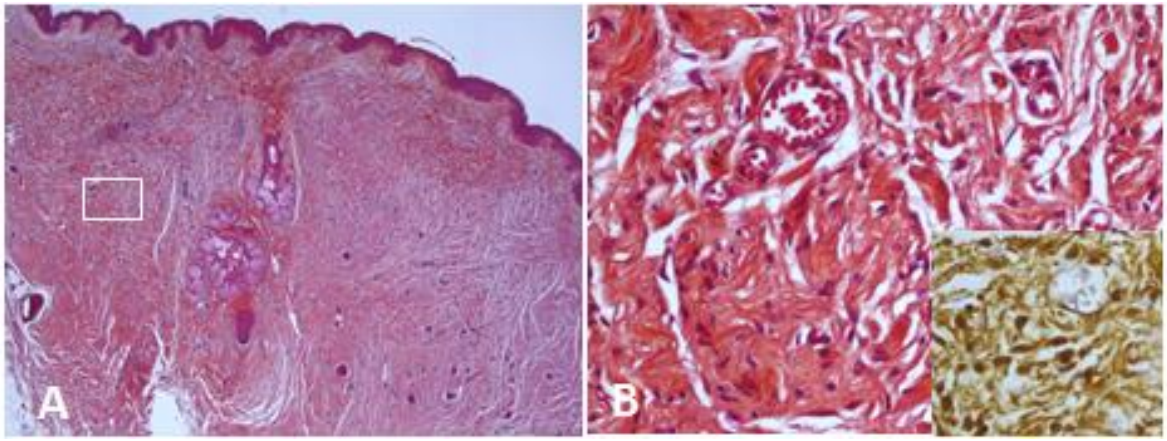

Fig. 1. Neurofibroma from the skin of the mother. (HAS x 25) (A). B Detail (white frame in fig 1 A, x 400) (B). Insert: adjacent section staned positive for the neuromarker Protein S 100 (x 400)

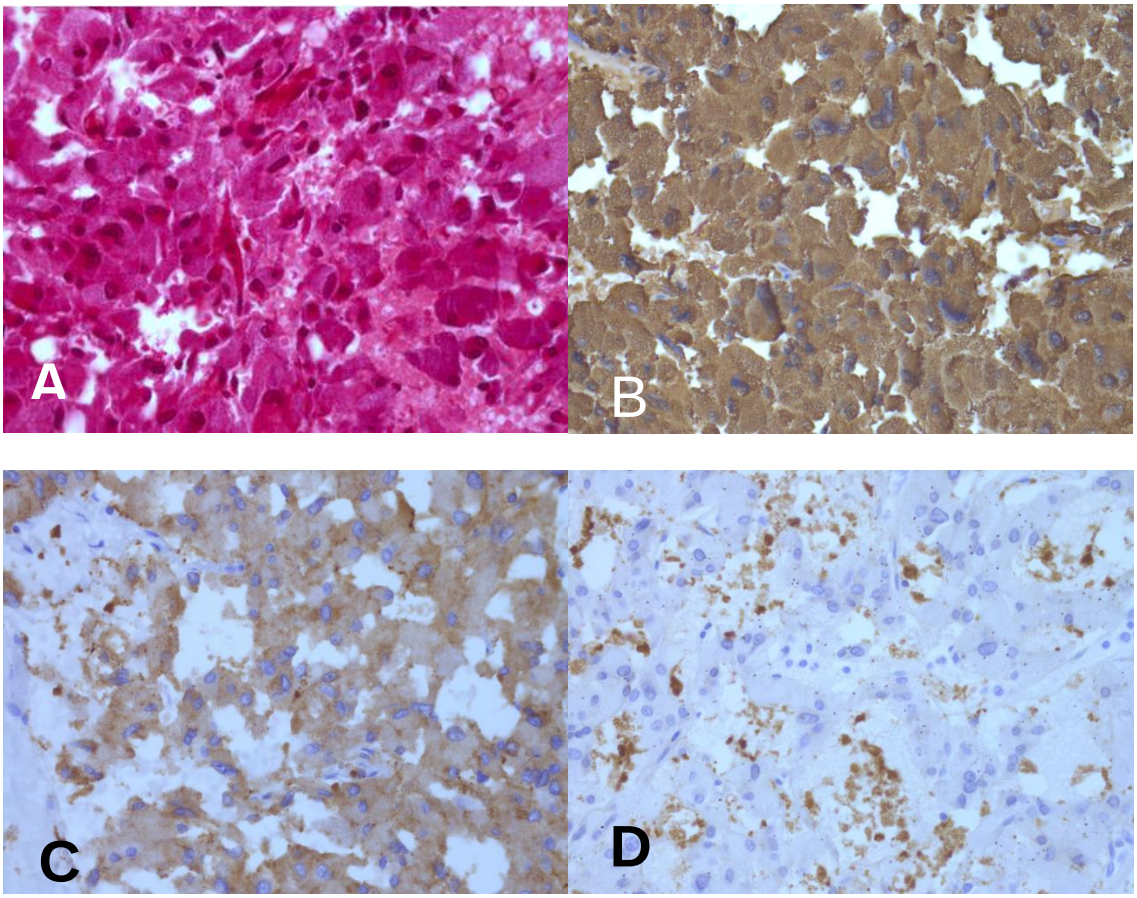

Fig. 2 A, B, C, D. Detail from the pheochromocytoma in the left adrenal of the mother. Haematoxilin-Azofloxine-Saffron stained section (A) showing nests and strands of chromaffin looking cells with abundant basophilic cytoplasm and nuclei with variation in form an size. In some areas the tumour has a nearly alveolar pattern. There are areas with red blood cells between tumor cells. Adjacent section stained positive for the neuroendocrine markers chromogranin (B), synaptophysin (C). This tumor is negative for neuron specific enolase which is not uncommon. Only sustentacular cells are positive (D) (x 400).

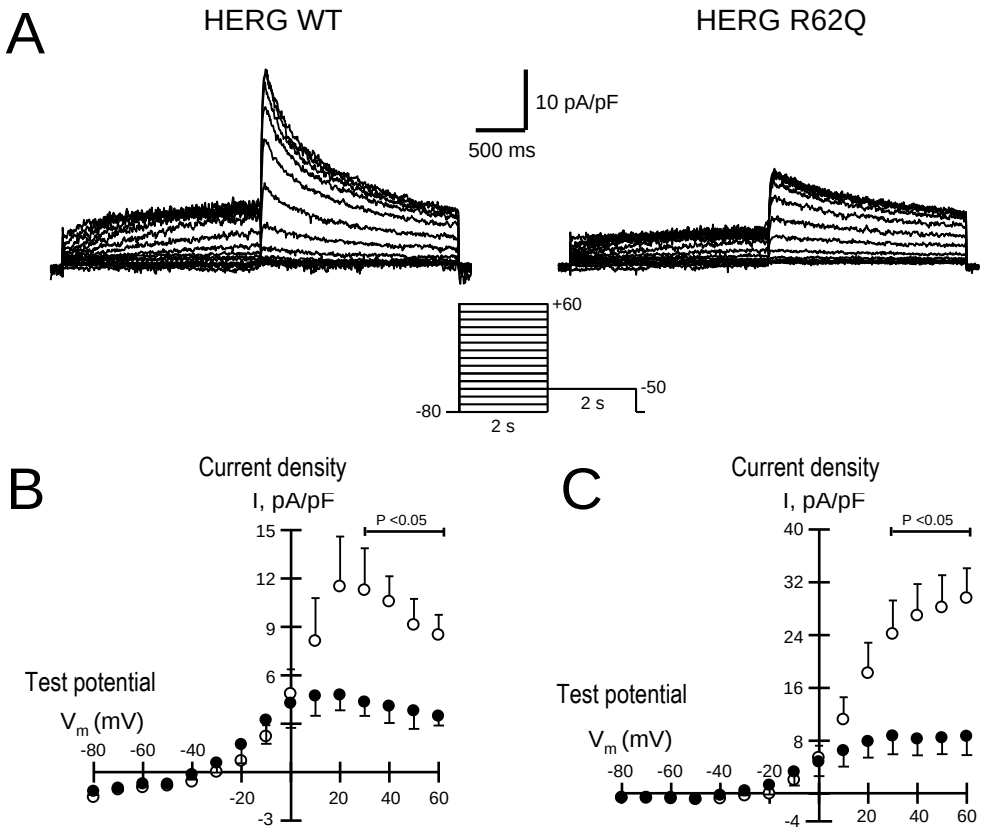

Fig. 3. Functional expression of WT and R62Q HERG in CHO-K1 cells. **A** Representative whole-cell currents normalized for membrane capacitance recorded from cells transiently-expressing WT or mutant HERG channels. Inset shows voltage protocol used. **B** Current-voltage relation for whole-cell currents (normalized to membrane capacitance) measured from CHO cells expressing WT (○, n=4) or R62Q (●, n=5) HERG channels. **C** Current-voltage relation for peak tail current densities after repolarization to –50 mV for WT (○, n=4), or R62Q (●, n=5). A horizontal line marks voltages where differences between WT and mutant current density achieved statistical significance at the $P < 0.05$ level.