Electrophysiological characterization of SCN5A mutations causing long QT (E1784K) and Brugada (R1512W and R1432G) syndromes

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Abstract

Familial long QT syndrome (LQTS) and Brugada syndrome are two distinct human hereditary cardiac diseases known to cause ventricular tachyarrhythmias (torsade de pointes) and idiopathic ventricular fibrillation, respectively, which can both lead to sudden death. Objective: In this study we have identified and electrophysiologically characterized, in patients having either LQTS or Brugada syndrome, three mutations in SCN5A (a cardiac sodium channel gene). Method: The mutant channels were expressed in a mammalian expression system and studied by means of the patch clamp technique. Results: The R1512W mutation found in our first patient diagnosed with Brugada syndrome produced a slowing of both inactivation and recovery from inactivation. The R4132G mutation found in our second patient who also presented Brugada syndrome, resulted in no measurable sodium currents. Both Brugada syndrome patients showed ST segment elevation and right bundle-branch block, and had experienced syncopes. The E1784K mutation found in the LQTS showed a persistent inward sodium current, a hyperpolarized shift of the steady-state inactivation and a faster recovery from inactivation. Conclusion: The different clinical manifestations of these three mutations most probably originate from the distinct electrophysiological abnormalities of the mutant cardiac sodium channels reported in this study. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Familial long QT syndrome (LQTS) [1–3] and Brugada syndrome [4], are two distinct hereditary cardiac diseases causing sudden death related to torsade de pointes and ventricular fibrillation (VF), respectively.

Brugada syndrome combines a right bundle-branch block (RBBB), an elevation of the ST segment in V1 through V3 without prolongation of the QTc interval on the surface ECG and VF [4]. LQTS is characterized by abnormal prolongation of the QTc interval and torsades de pointes [5,6].

Two forms of LQTS have been identified, the Jervell Lange-Nielsen [1] and the Romano–Ward syndromes [2,3]. Mutations in at least five different genes produce the Romano–Ward form: KvLQT1 encodes for a potassium channel located on chromosome 11p15.5 [7]; KCNE1 encodes for a KvLQT1 modulating subunit (hminK), located on chromosome 21q15 [8]; HERG which encodes also for a potassium channel located on chromosome 7q35 [9]; KCNE2 encodes for a HERG modulating subunit (MiRP1) located on chromosome 21q22.1 [10] and SCN5A which encodes for a cardiac sodium channel located on chromosome 3p21 [11]. For all these genes, point muta-

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tions or deletions have been identified and cause the clinical manifestations of the disease. Genetic linkage analysis recently showed that another gene located on chromosome 4q25–27 is also implicated in LQTS [12]. However, the product of this gene is as yet unknown.

Six mutations in the cardiac sodium channel gene (SCN5A) [13] have been linked to the LQTS (Fig. 1). Four of these mutations (ΔKPQ, N1325S, R1644H, E1784K) induce a late component of sodium current sufficient to delay repolarization by reopenings of the channel during prolonged depolarization [14–16]. The D1790G mutant affects voltage-gated cardiac sodium channel activity by interfering with the α and β1-subunit interaction [17], however, it is not clear how the electrophysiological abnormalities could lead to QT-prolongation. Finally an R1623Q mutant delays the ventricular repolarization by uncoupling activation from inactivation [18].

In 1992, a familial idiopathic ventricular fibrillation (IVF) associated with RBBB, ST segment elevation and sudden death, now known as Brugada syndrome, was described [4], however the familial aspect was not described for all patients. Missense, splice-donor and frameshift mutations were identified in SCN5A, in patients with Brugada syndrome [19,20] (Fig. 1).

In this study, three mutations in SCN5A identified in a LQTS patient (E1784K) and in two Brugada syndrome patients (R1512W and R1432G) were analyzed. We report the characterization of these mutations on hH1 (human heart 1 sodium channel) expressed in a mammalian cell line, using the patch clamp technique [21].

2. Methods

2.1. Subjects

Subjects underwent a detailed clinical and cardiovascular examination, including a 12-lead electrocardiogram (ECG) and a 24-h Holter recording. The QT interval was measured in lead II or V5 of the ECG and corrected for heart rate (QTc) using Bazett’s formula. Subjects were considered as affected by LQTS if they showed (i) syncope or documented torsades de pointes; (ii) QTc > 460 ms, or (iii) QTc > 440 ms associated with bradycardia or abnormal T-wave pattern as previously reported. Brugada syndrome was identified on the basis of ECG changes: (i) RBBB and (ii) an elevation of the ST segment in V1 through V3 (>0.10 mV) [4]. No drug challenge was performed in these patients.

Informed consent was obtained from all subjects in accordance with the guidelines set down by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale du Groupe Hospitalier de la Pitié-Salpêtrière (Paris). The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. SSCP analysis and direct sequencing of the PCR products

Previously published primer pairs were used to amplify all the exons of SCN5A from genomic DNA [22]. For SSCP (single strand conformation polymorphism) analysis,
the PCR products were denatured for 5 min at 96°C in a LIS (low ionic strength) solution which generates stable single-stranded DNA [23], kept on ice for 5 min, loaded onto a 10% polyacrylamide gel, and run at 8 mA, at 25°C and 7°C, in a Hoeffer apparatus. The bands were visualized after silver staining of the gels (Bio-Rad). When abnormal patterns were observed, PCR products were sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an ABI-Prism 377 DNA sequencer (Perkin-Elmer/Applied Biosystems).

2.3. Mutagenesis

Mutants hH1/E1784K, hH1/R1432G and hH1/R1512W were generated according to QuickChange™ site-directed mutagenesis kit instruction manual from Stratagene (La Jolla, CA, USA). The mutations were performed using the following mutagenic sense and antisense primers:

5′-CGGAAGGACCCAGCCCTGAGTGAGG-3′ and 5′-CCTCACTCAGGGGCTTGGTCTCCTCCTCCG-3′ for hH1/E1784K; 5′-CCCCAGAAGCCCATC-CCAAAGCCCCCTAAGACAAGTACC-3′ and 5′-GGTAC- TTGTTCAGGGCCCTGGGTGGGCTTCTGGGG-3′ for hH1/R1512W. 5′-GGCTGCTCTTCATA-CCTCACTCAGGGGCTTGGTGCTCTCCTCCTCCG-3′ and 5′-GGCTGCTCTTCATACCCAGAAGGAGCACC-3′ for hH1/R1432G.

The underlined nucleotides represent the mutation sites.

Mutants and wild-type hH1 in pcDNA1 construct were purified using Qiagen columns (Qiagen Chatsworth, CA, USA).

2.4. Transfections of tsA201 cell line

The tsA201 is a mammalian cell line derived from human embryonic kidney HEK 293 cells by stable transfection with SV40 large T antigen. Cells were grown in DMEM high glucose supplemented with FBS (10%), L-glutamine (2 mM), penicillin G (100 U/ml) and streptomycin (10 mg/ml) (Gibco Life Technologies, Burlington, Canada). Cells were incubated in a 5% CO₂ humid atmosphere. Transfections of tsA201 cells were carried out using the calcium phosphate method [24]. These cells were cotransfected with an expression plasmid for a lymphocyte surface antigen (CD8-a), to facilitate the identification of individual transfected cells [25]. A 10-μg amount of cDNAs encoding for wild-type or mutant hH1 sodium channels [21] with 10 μg of CD8-a were used. For patch clamp experiments, the cells were used 2–3 days post-transfection. The cells were incubated in a medium containing anti-CD8-a-coated beads for 2 min (Dynabeads M-450 CD8-a) and the non-attached beads were washed away. Beads were prepared according to the manufacturer’s instruction (Dynal, Oslo, Norway). Cells expressing CD8-a on their surface fixed the beads and were visually distinguishable from non-transfected cells.

The human sodium channel β1 subunit in pRc/CMV vector was a gift from Dr. A.L. George, Vanderbilt University, Nashville, TN, USA.

2.5. Patch clamp methods

Macroscopic sodium currents from transfected cells were recorded using the whole-cell method of patch clamp technique [26]. Patch electrodes were made from 8161 Corning glass and coated with Sylgard (Dow-Corning, Midland, MI, USA) to minimize their capacitance. Low resistance electrodes (<2 MΩ) were used, and a routine series resistance compensation of an Axopatch 200 amplifier was performed to values >80% to minimize the voltage-clamp errors. To ensure the quality of voltage clamp we measured the time constant of membrane charge capacitance (τ = R_series · C_m). The C_m value was obtained from the capacitance telegraph and the R_series value was obtained from the dial of the Axopatch 200. Voltage-clamp command pulses were generated by microcomputer using pClamp software v5.5 (Axon Instruments, Foster City, CA, USA). Recorded membrane currents were filtered at 5 kHz. To stabilize the current, experiments were performed 10 min after entering whole-cell configuration.

2.6. Solutions and reagents

For whole-cell recording, the patch pipette contained (mM): 35, NaCl; 105, CsF; 10, EGTA; and 10, Cs-Hepes (pH 7.4). The bath solution contained (mM): 150, NaCl; 2, KCl; 1.5, CaCl₂; 1, MgCl₂; 10, glucose; and 10, Na-Hepes (pH 7.4). A correction of the liquid junction potential of −7 mV between patch pipette and bath solutions was made.

Experiments were performed at room temperature, 22–23°C.

2.7. Statistical analysis

Data were expressed as mean±S.E.M. When indicated, t-test analysis was assessed using statistical software in Sigmaplot (Jandel Scientific Software, San Rafael, CA, USA). Differences were considered to be significant at a P value <0.05.

3. Results

3.1. Family 13415 with Brugada syndrome

The proband was a 43-year-old man who experienced his first syncopal attack while bicycling at slow speed. When admitted to the hospital, the patient was completely conscious, and had normal arterial pressure. Physical examination revealed no apparent abnormality in his...
health. The initial ECG recorded few hours after admission showed a sinus rhythm at 68 bpm and an RBBB pattern with a major coved shape in ST segment elevation (≥0.10 mV) in leads V1–V3, a normal QTc (QT: 380 ms; RR: 900 ms) and a slightly prolonged PR interval (240 ms) (Fig. 2A). The coronary angiogram was completely normal and no ventricular arrhythmia could be induced during electrophysiological testing, the HV interval was measured at 55 ms. Late potentials were positive (for two criteria) at signal averaged ECG with a 25-Hz filter (RMS: 42.7 mV; LAS 37 ms and filtered QRS duration: 117 ms). No familial history was noted. The ECG strongly suggested a Brugada syndrome. In view of the recent syncope, an ICD was inserted. Blood samples were obtained from the proband only.

3.2. Family 13416 with Brugada syndrome

The proband experienced a first syncope at age 31 which proved to be ventricular fibrillation (VF). The ECG revealed a typical Brugada syndrome with RBBB pattern, an ST segment elevation (≥0.10 mV) in the right precordial leads and a slightly prolonged PR interval (240 ms) (Fig. 2B). An ICD was implanted. Three to four times, the patient continued to experience VF which were successfully converted by the ICD. After numerous episodes (100) of VF that led to premature failure of the ICD it was replaced. Once amiodarone treatment was started, the patient became asymptomatic and no arrhythmia has since been recorded. Blood samples were obtained from the proband only.

No drug challenge was given as in each case a typical ECG pattern of Brugada syndrome was recorded (Fig. 2A and B).

3.3. Family 9959 with LQTS

The proband of this family was a boy who presented his first syncope at age 12 after running in the forest. He was found lying unconscious on the ground, and needed rescue intervention to regain consciousness. A second syncope occurred 2 days later, while he was taking a hot bath.

![Fig. 2. ECGs from patients with Brugada syndrome (A) family 13415 and (B) family 13416 and with LQTS (C) family 9959. ECG traces are displayed at 25 mm/s. PR values were 200, 240 and 160 ms for A, B and C, respectively.](image-url)
Neurological examination and EEG proved normal. A slow heart rate (42 bpm) was noted. The ECG confirmed the slow heart rate and uncovered a prolonged QT interval (QT = 600 ms, RR = 1400 ms, QTc = 509 ms). Furthermore, the T wave morphology was abnormal, with a prolonged ST segment and high amplitude (0.4 mV) and narrow based (160 ms) T wave (Fig. 2C). Long QT syndrome was diagnosed and the child was given propranolol (160 mg) and has remained asymptomatic. The rest of the family was asymptomatic. The mother presented a QTc value of 440 ms with normal morphology, while the father and the brother of the proband showed normal QTc values (<400 ms). Blood samples were obtained from the proband and his mother.

### 3.4. SSCP analysis

SSCP analysis of SCN5A revealed aberrant bands for exon 28 in the LQTS patient and his mother. For the Brugada syndrome patients, aberrant bands were found in exon 26 for family 13415 and in exon 24 for family 13416 (data not shown). These SSCP abnormalities were absent in DNA samples from more than 100 control subjects (data not shown). Sequence analysis of the aberrant DNA fragment of the first Brugada syndrome patient (family 13415) revealed a single base transition (C → T) at position 4534 (CGG → TGG) in exon 26. The mutation resulted in a replacement of an arginine by a tryptophan at codon 1512 within the III–IV linker of the sodium channel (Fig. 3). In

![SCN5A missense mutation R1512W](image1)

![SCN5A missense mutation R1432G](image2)

Fig. 3. Sequence analysis obtained form automated sequencing of DNA fragments. Top: family 13415; bottom: family 13416, both with Brugada syndrome.
the second patient affected by Brugada syndrome (family 13416), a single base transition (A→G) at position 4295 (CAG→CGG) resulted in a replacement of an arginine by a glycine at codon 1432 within the C-terminal domain III of the channel (Fig. 3). In the LQTS patient, a single base transition (G→A) was found at position 5350 (AAG→GAG) and resulted in a replacement of a glutamic acid by a lysine at codon 1784 within the C-terminal domain.

3.5. Characteristics of hH1/R1512W

To study the electrophysiological characteristics of mutant hH1/R1512W, cDNA carrying this mutation was expressed in the tsA201 cell line. Large sodium currents were recorded from the hH1/R1512W mutant channel (Fig. 4A), indicating that the level of expression of the resultant mutation had not diminished. The hH1/R1512W mutation did not induce a persistent inward current (Fig. 4). This was also shown by using 20 μM TTX, where the current at the end of the 20 mV depolarization pulse in presence of TTX was indistinguishable from the control current (Fig. 4C). Similar parameters of the G–V curves were obtained for hH1/WT and hH1/R1512W; hH1/WT (V_{1/2} = −53.6±2 (half-activation voltage), K = 6.4±0.9 (slope factor), n = 5), hH1/R1512W (V_{1/2} = −52.7±2, K = 7.7±0.6, n = 4) (Fig. 5C). The kinetics of current decay were assessed by fitting the decay of sodium current using a biexponential function, with τ_{h,slow} and τ_{h,fast}, for the slow and the fast component of current decay, respectively. The kinetics of current decay were slowed; for example, at −30 mV τ_{h,fast, hH1/R1512W} = 1.23±0.09 ms (n = 6) and τ_{h,fast, hH1/WT} = 0.73±0.02 ms (n = 5) (P = 0.0017) (Fig. 5A). Steady-state inactivation was studied using a two-pulse protocol. The normalized peak sodium current was plotted versus 500 ms pre-pulse potential and fitted with a Boltzmann equation (Fig. 5B). The steady-state inactivation was not significantly affected; V_{1/2}^{hH1/R1512W} = −97.4±1.1 mV (n = 6) (half-inactivation voltage) with k = 6.3±0.3 mV (n = 6) (slope factor) compared to hH1/WT: V_{1/2}^{hH1/WT} = −100.0±1.4 mV (n = 7) with k = 5.7±0.2 mV (n = 7). The recovery from inactivation was also assessed using a double-pulse protocol, with 40 ms pre-pulse duration at −140, −130, −120 and −100 mV. The current recovery was plotted versus the duration between the pre-pulse and the test pulse of −20 mV, and a monoexponential function was used to fit the data (Fig. 6A). For example, time constants of recovery from inactivation at −100 mV were found to be significantly slower than wild-type hH1 (P=0.04): τ_{rec}^{hH1/R1512W} = 70.9±7.5 ms (n = 5); τ_{rec}^{hH1/WT} = 51.9±5.8 ms (n = 5). The use of different holding potentials of −140, −130, −120 and −100 mV revealed that τ_{rec} was voltage-dependent and significantly slower than hH1/WT at all studied voltages (Fig. 6B). In addition, coexpression of R1512W with β-subunit did not change the electrophysiological characteristics of this mutant channel (data not shown).

3.6. Characteristics of hH1/R1432G

The R1432G mutant channel was also expressed in tsA201 cells and no sodium current could be recorded in presence of this mutation (Fig. 7). The effect of this mutation (failure of sodium channels expression) was confirmed in two clones expressed in tsA201 cells.

3.7. Characteristics of hH1/E1784K

Sodium currents from hH1/E1784K were also recorded for this mutant sodium channel, showing fast activation...
and inactivation kinetics with a persistent inward current of about 1.5% at −20 mV (Fig. 8). This persistent inward current can be attributed to late sodium current activity. This was shown by using TTX, a specific sodium channel blocker. As shown in Fig. 7C, TTX at 20 μM reduced this persistent current almost to zero. The G–V curve was shifted to more positive voltages with a less steep slope; hH1/E1784K (V_{1/2} = −44.8±0.9, \(K_o = 11.4±0.4, n=4\)) (Fig. 5C). The biexponential fit of current decay showed that the voltage-dependence of \(\tau_{h}^{\text{slow}}\) and \(\tau_{h}^{\text{fast}}\) of hH1/E1784K were similar to hH1/WT (Fig. 5A). Fig. 5B shows that the steady-state inactivation voltage-dependence is shifted by about 14 mV toward hyperpolarized voltages; the half-inactivation voltage of hH1/E1784K was: \(-114.4±2.2\) mV (\(n=6\)). There is no effect on the steepness of the voltage-dependence of inactivation, \(K_o = 6.3±0.4\) mV (\(n=6\)) (Fig. 5B).

The current recovery at −100 mV was plotted versus the duration between the pre-pulse and the test pulse of −20 mV, and a monoexponential function was used to fit the data (Fig. 6A). hH1/E1784K recovered faster from inactivation, the time constant of recovery from inactivation being 26.2±2.5 ms (n=6) vs. 51.9±5.8 ms (n=5) for hH1/WT at a holding potential of −100 mV (\(P=0.01\)). \(\tau_{\text{rec}}\) was voltage-dependent with larger effect at −100 mV holding potential (Fig. 6B). Expression with the β-subunit resulted in similar electrophysiological properties of this mutant channel (data not shown).
Discussion

We have identified three mutations, two causing Brugada syndrome and one causing LQTS in French subjects. Two of these mutations have also recently been found in other families [16,20]. There are indications that the mode of inheritance of both syndromes is autosomal dominant [19]. We have no direct information about the mode of inheritance in our patients, because there were not sufficient family members wishing to participate in the study.

LQTS and Brugada syndrome cause sudden death related to polymorphic ventricular tachycardia and ventricular fibrillation, respectively. The phenotype of LQTS has been attributed to a prolongation of the QTc interval as a result of a delay in repolarization [5,6]. However, the Brugada syndrome shows a normal QTc interval. It is a distinct syndrome showing RBBB associated with elevation of the ST segment in V1 through V3 and VF.

4.1. Brugada syndrome mutation (R1512W)

The hH1/R1512W mutation resulted in slow time constants of inactivation and of recovery from inactivation. This mutation is localized on the III–IV linker of the voltage-gated sodium channel, known as the inactivation gate of the channel. This region has been a target for mutations inducing several striated muscle diseases (LQTS and paramyotonia congenita) [13,14,27]. The slowing of recovery from inactivation indicates reduced-rate of exiting from the inactivated state. This slowing of recovery from inactivation could reduce the density of sodium channels available for opening after each cardiac cycle, which could generate conduction abnormalities. In addition, coexpression of R1512W with the β-subunit did not change the phenotype of this mutant channel, suggesting that there is...
light of a recent report of this Brugada syndrome mutation, T1620M was shown to be temperature dependent [29]. A detailed temperature study of R1512W mutant is needed, to show whether it also manifests temperature dependence.

In addition, two other mutations causing the Brugada syndrome resulted in a different phenotype, where they showed loss of sodium channel function [19]. This was consistent with our second Brugada syndrome mutation R1432G which failed to express in our mammalian cell line, suggesting that the level of expression is directly implicated in the Brugada syndrome. The R1432G is the first missense mutation which has shown to abolish sodium channel expression.

The mechanism by which ST segment elevation occurs is not well understood. It was recently suggested that reducing sodium currents amplitude either by drugs (class IC antiarrhythmics) [30] or by reduced expression (genetic defects) [31] could result in a pronounced early repolarization in the epicardium due to I(to) (transient outward potassium current), where the channel responsible for this current are preferentially expressed. This would result in a shortening of action potential (AP) in the epicardium without any effect on AP of the endocardium, creating potential disparities between these two regions of the heart, thus leading to ST segment elevation [32]. Our results obtained with the hH1/R1512W mutant (decreased channel density during the cardiac cycle) or with hH1/R1432G mutant (loss of function) are consistent with this idea.

### 4.2. LQTS mutation (E1784K)

The LQTS mutation (E1784K) was characterized by a persistent inward sodium current and a faster recovery from inactivation indicating a destabilization of the inactivation state. The persistent current is related to sodium channel activity, since it is greatly reduced by TTX, a potent and specific sodium channel blocker. This persistent current could delay the repolarization of the action potential and predispose patients to torsade de pointes leading to syncope and sudden death. It appears from several studies that the persistent inward current is a common electrophysiological effect for mutations in SCN5A causing the LQTS. In fact, at least four other mutations found in patients with LQTS (ΔKPQ, N1325S, R1644H and R1623Q) were reported to induce a persistent current [14,15,18]. However, the persistent inward sodium current is not the only mechanism underlying LQTS. It was recently shown for another LQTS mutation that changes in the interaction between α- and β-subunits could also account for the LQTS [17]. Further studies are required to investigate how changes in α- and β-interaction could lead to QT-prolongation. The E1784K mutation is located near the previously reported LQTS mutation (D1790G) [17]; the authors report that this mutation, when coexpressed with the β1-subunit, eliminates the steady-state inactivation shift seen when the wild-type channel was also

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Fig. 8. (A) Family of sodium currents recorded from cells expressing hH1/E1784K mutation. Currents were generated from a holding potential of −140 mV from −90 to +50 mV in 10-mV increments. The dashed line represents the zero current. (B) Normalized sodium currents from hH1/WT and hH1/E1784K recorded from HP= −140 mV to a voltage test of −20 mV are superimposed. (C) Effect of 20 μM TTX on sodium current recorded from a cell expressing hH1/E1784K. Sodium currents were recorded from HP value of −140 mV to a test value of −20 mV.

no interaction of R1512 with this subunit. Our data indicate that the R1512W mutation makes the inactivation gate move in (inactivation) and out (recovery from inactivation) more slowly. This suggests that the energy level required for reaching the inactivated state is not changed but rather that the energy barrier is higher. Moreover the R1512W mutation could interfere with the effects of other modulating factors such as protein kinases [28].

However, Brugada syndrome can also be produced by other changes in the function of sodium channel. Another Brugada syndrome missense mutation (T1620M), when expressed in Xenopus oocytes, results in a shift of the steady-state inactivation (h∞) curve to the right and a recovery from inactivation that is faster [19]. It was not clear, from this study how these parameters could explain the clinical manifestations of the disease. However, in the
coexpressed with this accessory subunit. Expression with the β-subunit resulted in similar electrophysiological properties of this mutant channel, suggesting that the β-subunit is not interacting with the E1784 residue. Our data also showed that steady-state inactivation of E1784K mutant is affected at more hyperpolarized voltages where sodium channels are normally closed and the activation curve is slightly shifted to the more positive voltages. Replacing E1784 with aspartate, another negatively charged amino acid, resulted in a wild-type phenotype. Importantly there was an absence of a persistent sodium current (Fig. 9), indicating that a negative charge at this position is essential for a normal channel function.

In summary, we have investigated the effect of two mutations found in patients with LQTS and Brugada syndrome. Our results suggest that those divergent clinical phenotypes are due to the different electrophysiological effects of these three mutations.

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