Notched T Waves on Holter Recordings Enhance Detection of Patients With LQT2 (HERG) Mutations

J.M. Lupoglazoff, MD; I. Denjoy, MD; M. Berthet; N. Neyroud, PhD; L. Demay; P. Richard, PhD; B. Hainque, PhD; G. Vaksman, MD; D. Klug, MD; A. Leenhardt, MD; G. Maillard, MD; P. Coumel, MD; P. Guicheney, PhD

Background—The 2 genes KCNQ1 (LQT1) and HERG (LQT2), encoding cardiac potassium channels, are the most common cause of the dominant long-QT syndrome (LQTS). In addition to QT-interval prolongation, notched T waves have been proposed as a phenotypic marker of LQTS patients.

Methods and Results—The T-wave morphology of carriers of mutations in KCNQ1 (n=133) or HERG (n=57) and of 100 control subjects was analyzed from Holter ECG recordings. Averaged T-wave templates were obtained at different cycle lengths, and potential notched T waves were classified as grade 1 (G1) in case of a bulge at or below the horizontal, whatever the amplitude, and as grade 2 (G2) in case of a protuberance above the horizontal. The highest grade obtained from a template defined the notch category of the subject. T-wave morphology was normal in the majority of LQT1 and control subjects compared with LQT2 (92%, 96%, and 19%, respectively, P<0.001). G1 notches were relatively more frequent in LQT2 (18% versus 8% [LQT1] and 4% [control], P<0.01), and G2 notches were seen exclusively in LQT2 (63%). Predictors for G2 were young age, missense mutations, and core domain mutations in HERG.

Conclusions—This study provides novel evidence that Holter recording analysis is superior to the 12-lead ECG in detecting G1 and G2 T-wave notches. These repolarization abnormalities are more indicative of LQT2 versus LQT1, with G2 notches being most specific and often reflecting HERG core domain missense mutations. (Circulation. 2001; 103:1095-1101.)

Key Words: long-QT syndrome ■ genetics ■ electrocardiography

Genetic studies have shown that long-QT syndrome (LQTS) is a primary electrical disease caused by mutations in specific ion channels.1 Five LQTS genes have been identified, including the potassium channel genes HERG; KCNH2 (LQT2),2 which encodes the α-subunit of the channel that underlies the rapidly activating delayed rectifier potassium current Ikr; and KCNQ1 (LQT1),3 which encodes the slowly activating delayed rectifier potassium channel Ik. Most mutations have been identified in the core domain, constituted by the transmembrane domains and the pore, of KCNQ1 and HERG. Mutations in the N- and C-terminal domains, however, have also been reported.4–7 LQTS patients exhibit QT prolongation on the ECG and are at risk of arrhythmogenic syncope and sudden death. In addition to duration, T-wave morphology is often abnormal, and notched T waves have been included in diagnostic criteria.8 Furthermore, this pattern has been associated with a poor prognosis.9 Moss et al10 reported that patients with ion channel defects often display different T-wave patterns. LQT3 patients show distinctive T waves of late onset, whereas LQT1 or LQT2 patients display broad-based or low-amplitude T waves, respectively. We suggested that notched T waves on ECGs and Holter recordings in family members with a HERG A561T mutation were linked to the presence of that mutation.11 Paying attention to these ECG aspects, we reviewed all the ECG and Holter recordings of our carriers of mutations in KCNQ1 and HERG to determine the relevance of the T-wave anomalies for diagnostic and genetic testing purposes.

Methods

Study Population
Clinical evaluation and blood samples were obtained after written consent in accordance with the guidelines set down by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de la Pitié-Salpêtrière (Paris, France). Subjects underwent detailed clinical and cardiovascular examination, including a 12-lead ECG and a 24-hour Holter recording, which were analyzed by 2 experienced observers (J.M.L., I.D.) without knowledge of the localization of the mutation. QT interval was measured on the surface ECG in lead II or V5, and corrected for heart rate (QTc) according to Bazett’s formula. All probands of the families had

Received August 14, 2000; revision received October 13, 2000; accepted October 16, 2000.

From Cardiologie, Hôpital Robert Debré (J.M.L., I.D., G.M.); Cardiologie, Hôpital Lariboisière (I.D., A.L., P.C.); INSERM U523, Institut de Myologie, IFR “Coeur, Muscle et Vaisseaux” n°14, Hôpital Pitié-Salpêtrière (M.B., N.N., P.G.); and Biochimie, Hôpital Pitié-Salpêtrière (L.D., P.R., B.H.), Paris; and Cardiologie, Hôpital Cardiologique, Lille (G.V., D.K.), France.

Correspondence to J.M. Lupoglazoff, Cardiologie, Hôpital Robert Debré, 48, Boulevard Sécurier, 75019 Paris, France. E-mail jean-marc.lupoglazof@rdh.ap-hop-paris.fr

© 2001 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

1095
by guest on April 13, 2017 http://circ.ahajournals.org/ Downloaded from

Lehmann et al, 13 with T waves assigned to 1 of 3 categories: grade above the apex (G2)9,13 (Figure 1a). The highest grade observed apex, whatever the amplitude (G1); and grade 2, a distinct protuber-
wave.9,13 Classification of T-wave morphology was adapted from
sleeping hours with lowest heart rate. The totality of these templates
hours with fastest heart rate and the second of the 4 consecutive
were defined according to the mean hourly heart rate and the
in the occurrence of notched T waves, 2 different periods of analysis
account a possible role of heart rate or the autonomic nervous system
provide a wide range of RR intervals for analysis and to take into
account a possible role of heart rate or the autonomic nervous system

Examples of QRS-T templates from leads X and Y of Holter
recordings are shown: at shorter and mean diurnal RR intervals
(ms) and mean and longer nocturnal RR intervals (ms). LQT1
patient depicts a broad-based T wave without any notches (G0)
and a similar positive T-wave pattern in both leads. In contrast,
LQT2 patient displays a G2 notched T-wave pattern in lead X at
all heart rates, associated with an inverted T wave in lead Y.


categories as in text. Typical QRS-T templates of symp-
tomatic untreated LQT1 (b) and LQT2 (c) patients carrying a
mutation in core domain, V254M and T613M, respectively.

syncope or documented torsade de pointes and a QTc >440 ms. Diagnosis was confirmed by mutation identification. Thirty-two
LQT1 and 21 LQT2 families formed the basis of this study.

Mutation Identification

After identification of the mutation by polymerase chain reaction–
single-strand conformation polymorphism analysis and direct se-
quencing of polymerase chain reaction products,5,7 all the family
members were screened and separated into carriers and noncarriers
of the mutation. The mutated nucleotides for the 9 new HERG
missense mutations were G131T, G172A, C340T, C1001T, G1704T,
A1724G, G1825A, G1862A, and G2879A for C44F, E58K, P114S,

Twenty-Four-Hour Holter Recordings

All subjects underwent a 24-h Holter ECG recording (Delmar
Avionics) with an XYZ configuration. Analog data from leads X
(ECG lead I equivalent) and Y (ECG lead V2 equivalent) were
digitized at 200 Hz and analyzed by the Elatec Holter System (Ela
Medical). QRS-T templates were obtained as previously described.12
Briefly, QRS-T complexes were classified according to the value of
the preceding RR interval every 25 ms and then averaged. To
provide a wide range of RR intervals for analysis and to take into
account a possible role of heart rate or the autonomic nervous system
in the occurrence of notched T waves, 2 different periods of analysis
were defined according to the mean hourly heart rate and the
patient’s diary. The first period consisted of the 8 consecutive diurnal
hours with fastest heart rate and the second of the 4 consecutive
sleeping hours with lowest heart rate. The totality of these templates
was considered for morphological analysis. Notched T waves, also
called bifid waves or humps, were defined as a bulge or protuberance
just above the apex or on the descending limb of an upright T
wave.9,13 Classification of T-wave morphology was adapted from
Lehmann et al,13 with T waves assigned to 1 of 3 categories: grade
0, no deflection (G0); grade 1, a perceptible bulge at or below the
apex, whatever the amplitude (G1); and grade 2, a distinct protuber-
ance above the apex (G2)9,13 (Figure 1a). The highest grade observed
over the range of templates for an individual defined the notch
category for that individual. QRS-T-wave templates were consid-
ered to be inverted if the T wave was negative and to be broad-based
when the base of the T wave was >50% of the QT interval duration
(working definition).

Statistical Analysis

Univariate analyses were carried out with the t test for quantitative
data. To take into account familial correlations within the same
family, multivariate analyses were carried out by the hierarchical
mixed models (SAS Proc Mixed) for quantitative data. A random-
ized familial effect was introduced at the intercept level. Multivariate
analyses were performed by generalized estimating equations (SAS
Proc Genmod) for qualitative data with the logit function. Results
from quantitative data analyses are expressed as mean±SD and from
qualitative data as odds ratios (OR) and their 95% confidence
interval (95% CI). A significant level of P<0.05 was required for an
explanatory variable to remain in the models.

Results

Population Characteristics

A total of 190 mutation carriers in KCNQ1 (n=133) and
HERG (n=57) and 100 noncarrier control subjects from the
53 families with LQTS were studied. Holter recordings were
obtained in every subject, and ECG recordings were present
in all but 7 cases (LQT1, n=127; LQT2, n=56; controls,
n=100). LQT patients were similar with regard to female sex,
prior cardiac events, and β-blocking therapy. LQT2 patients
were significantly younger than LQT1 patients, with 42%<15 years old versus 28%, respectively. QTc intervals were
longer in LQT2 patients despite a similar proportion of core
domain mutations (47% versus 62%, respectively, P<0.06). The control population was similar in terms of age (30% of
subjects <15 years) and sex (Table 1).

KCNQ1 Mutations

Twenty-three KCNQ1 missense mutations were identified in
133 subjects. Most of them have been reported previously by
us or other groups.4 These missense mutations changed
conserved amino acid residues. Nineteen were found in the
core domain (25 families, n=83) and 2 in the C-terminal

| TABLE 1. Clinical Characteristics and T-Wave Morphology of LQT Patients and Control Subjects |
|---|---|---|
| Age, y | 33±20 | 24±19* | 28±19 |
| Female sex | 74/133 (56) | 31/57 (54) | 48/100 (48) |
| QTc, ms | 472±30 | 485±40† | 394±24‡ |
| Prior cardiac event | 49/133 (37) | 22/57 (39) | 0 |
| β-Blocking therapy | 34/133 (26) | 22/57 (39) | 0 |
| Core missense mutations | 83/133 (62) | 27/57 (47) | 0 |
| G0 notches | 122/133 (92) | 11/57 (19§) | 96/100 (96) |
| G1 notches | 11/133 (8) | 10/57 (18¶) | 4/100 (4) |
| G2 notches | 0/133 (0) | 36/57 (63§) | 0/100 (0) |

Values are n (%) except as given.
*P<0.007, †P<0.05 vs LQT1 patients.
‡P<0.01 vs LQT1 and LQT2 patients.
§P<0.001, ¶P<0.01 vs LQT1 and control subjects.
domain (4 families, n=43). In addition, 2 frameshift mutations were evidenced in 3 families (n=7).

**HERG Mutations**

Eighteen HERG mutations were identified in 57 subjects: 15 missense mutations and 3 mutations leading to a premature truncation of the protein. Among the missense mutations, 8 were in the core domain (11 families, n=27) and 7 in the C- and N-terminal domains (7 families, n=23) (Figure 2). Six have been described previously, and 9 were novel: C44F, E58K, P114S, P334L, W568C, E575G, D609N, S621N, and S960N. All these mutations were absent in 150 unrelated control subjects. In addition, a novel 1-bp insertion (1009insT, n=2) in the N-terminal domain (codon 336) induced a premature stop codon at position 355 before the S1 transmembrane domain. 7 splicing mutations (2592+1G→A, n=3) and a 1-bp insertion (3108insG, n=2) induced a putative premature truncation of the C-terminal end of the channel. 7

**Holter Recordings in Control Subjects**

The majority of control subjects had normal T-wave morphology (96%). G1 notched T waves were found in only 4% of the patients, all older than 15 years. No G2 notches were detected.

**Holter and ECG Recordings in LQT1 Patients**

Figure 1b displays the typical T-wave morphology obtained in leads X and Y at all cycle lengths in LQT1 patients. In the majority of cases, T-wave morphology was normal (122 of 133, 92%). G1 notches were found in only 11 of 133 (8%), with only 5 of 11 patients <15 years old (Table 1). No correlation was found between notch grade and age, sex, QTc, type or localization of the mutation, treatment, and prior cardiac event (Figure 3a). No G2 was evidenced in any heart rate or period analyzed. The T wave was broad-based in all cases but 1 (132 of 133) and was always positive in both leads X and Y.

**Holter Recordings in LQT2 Patients**

A notched T-wave pattern (G1 or G2) was found in lead X in a high proportion of LQT2 patients (46 of 57, 81%) and was associated with inverted T waves in lead Y in 83% of them (38 of 46) (Figure 1c). G1 notches were present in 10 of 57 (18%), with only 2 patients <15 years old. G1 notches were relatively more frequent in LQT2 (18% versus 8% [LQT1])

### TABLE 2. T-Wave Morphology Classification in LQT2 Patients According to the Types of Mutations and Their Localization

<table>
<thead>
<tr>
<th>Mutations</th>
<th>QTc, ms</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core domain missense</td>
<td>498±45†</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>25 (92)§</td>
<td>27</td>
</tr>
<tr>
<td>N- or C-terminal missense</td>
<td>473±34</td>
<td>6 (26)</td>
<td>7 (30)</td>
<td>10 (44)¶</td>
<td>23</td>
</tr>
<tr>
<td>Frameshift</td>
<td>463±9</td>
<td>4 (57)</td>
<td>2 (29)</td>
<td>1 (14)¶</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are n (%).

†P<0.03 vs N- or C-terminal missense; †P<0.04 vs frameshift.

‡P<0.001 vs N- or C-terminal missense; §P<0.0001 vs frameshift.

¶P<0.001 vs frameshift.
and 4% [control], P<0.01). G2 notches were found in 36 of 57 (63%) (Table 1). Furthermore, taking into account the type and localization of the mutation, these abnormalities were significantly more frequent in case of core missense mutations in the core domain, with a G2 pattern evidenced in 25 of 27 (92%) of the carriers (Table 2, Figures 1c and 4). A consistent G2 pattern at all cycle lengths of the Holter recording was found in the majority of patients carrying core mutations (20 of 27, 74%).

In contrast, in LQT2 patients with missense mutations localized in the N- or C-terminal domain or frameshift mutations, T-wave anomalies were significantly less frequent (Table 2, Figure 5). In addition, among the 11 patients with G2, only 5 had a consistent G2 pattern at all cycle lengths.

Grade classification was independent of QTc duration (Figure 3b). From univariate analysis, younger age, missense mutation, and core domain mutation were significant predictors of the presence of G2, at variance from prior cardiac events, β-blocking therapy, and female sex (Table 3). At multivariate analysis, only a core missense domain mutation (OR 30.1; 95% CI 3.8 to 238.8; P<0.002) and young age (OR 8.8; 95% CI 1.7 to 46.1; P<0.02) remained significantly and positively associated with the presence of G2 on the Holter recordings in lead X.

Taking into account the control group and the LQT1 and LQT2 populations, the specificity of G2 was very high for LQT2 (100%), with a sensitivity of 63% and a positive predictive value of 100%. In contrast, G1 and G2 T waves gave a lower specificity (94%) and positive predictive value (75%) but a higher sensitivity (80%) for LQT2.

Comparison Between ECG Findings and Holter Recordings in LQT2 Patients

On the surface ECG, the T-wave morphology was normal (G0) in 43% of the LQT2 patients (n=24 of 56). G1 (n=6, 11%) and G2 (n=26, 46%) T waves were found mainly in the 3 left precordial leads. A subset of the 24 patients with G0 at baseline ECG had higher grade classification at Holter recording: G1 (n=7) or G2 (n=11). In addition, 4 of 6 patients with G1 at ECG had G2 morphology at Holter. Altogether, G2 notches were evidenced at Holter recording in 15 of 30 patients who did not have G2 at surface ECG, thus demonstrating the superiority of Holter recording over ECG for detection of T-wave notches.

Discussion

This retrospective study provides evidence that Holter recording, by exploring a wide range of cycle lengths, is superior to the 12-lead ECG in detecting G1 and G2 T-wave notches. These repolarization abnormalities are more indicative of LQT2 versus LQT1, with G2 notches being most specific and often reflecting HERG core domain missense mutations.
However, and although our data are highly suggestive of G2 notches being more likely to manifest with core missense mutations and G1 notches with other types and localizations of mutations, further studies with a larger number of subjects are needed to confirm our preliminary results. In addition, the absence of Holter data from LQT3 subjects and rare variants of LQTS is a limitation to our findings.

It is noteworthy that G2 notches were less frequent in patients with frameshift mutations or missense mutations in the N- or C-terminal domains and could be detected on Holter recordings only at certain heart rates. For these particular cases, Holter recording, which explores a wide range of heart rates, improved detection of notched T-waves compared with ECG. To better characterize such patterns obtained on Holter recording, however, quantitative and more refined methods, such as wavelet analysis, may be more informative.

The strong association of this phenotypic trait with a genetic defect was foreseen by Lehmann et al., who, in a study of nongenotyped LQTS families, identified notched T waves in a significant proportion of blood relatives with prolonged QTc intervals. We found it interesting to report that the notched T-wave pattern in lead X is associated with inverted T waves in lead Y in a large proportion of the LQT2 patients. This combination was never found in our LQT1 patients or in control subjects and could contribute to identification of true G1 notches in LQT2 versus false-positive G1 notches in LQT1 and control subjects.

From a practical point of view, consistent G2 T waves at all heart rates were found in 74% of LQT2 patients with core missense mutations and in 17% in the other group and can be detected on all types of Holter analyzer, provided that signal averaging according to heart rate is available. Holter recordings of related family members, especially children, can often be helpful to improve G2 T-wave detection.

**Notched T Waves and HERG Mutations**

Mutations in HERG lead to a decreased contribution of $I_K$ to cardiac repolarization by multiple mechanisms. It is con-
receivable that missense mutations in functional domains of HERG, like the core domain, induce a major decrease in \( I_{Kr} \), due to dominant-negative effects and/or altered gating properties and produce a more severe ECG phenotype with a greater prolongation of QTc interval and T-wave morphology alteration, such as G2 notches. In contrast, frameshift mutations or missense mutations in the N- or C-terminal domains, which may result in protein processing defects leading to failure of the channel protein to undergo normal transport to the cell surface, induce a milder phenotype with less consistent morphological alterations of the ECG and milder QTc prolongation. In our patients, G2 notched T waves were found in only 11 of 30 such cases, and QTc prolongation was milder (471 ± 31 ms).

Notched T Waves in HERG and Electrophysiological Mechanism

Because the presence of notched T waves appears to be an indicator of genetic defects in HERG, one can speculate that this pattern is related to \( I_{Kr} \) decrease. In an arterially perfused wedge of canine ventricle allowing the simultaneous recording of transmural ECG and transmembrane action potentials, \( d \)-sotalol and chromanol 293B were used to mimic LQT2 and LQT1 defects, respectively. In the LQT2 model, the \( I_{Kr} \) blocker \( d \)-sotalol caused a preferential prolongation of the M-cell action potential duration and resulted in the interruption of the descending limb of the T wave, consistent with the high prevalence of notched T waves in our patients who were carriers of genetic defects in HERG. Such notched T waves have also been observed in vivo in humans after \( I_{Kr} \) block, the slope of phase 3 of the action potential is reduced, leading to much smaller transmural voltage gradients. These changes have 2 effects: a smaller T-wave amplitude and a transient crossing over of the opposing voltage gradient, thus generating the notched T wave. In contrast, chromanol 293B, a specific \( I_{Kr} \) blocker used to mimic defects in \( KCNQ1 \), prolonged the action potential duration homogeneously, resulting in a prolonging QT interval with a broad-based T wave characteristic of our LQT1 population and others.

Prognosis and Notched T Waves

A higher prevalence of notched T waves on 12-lead ECGs in symptomatic than in asymptomatic nongenotyped LQTS patients has been reported. In that study, notched T waves were found in 62% of LQTS patients and could possibly reflect a high proportion of LQT2 patients. Interestingly, in our study, G2 notches were found only in the LQT2 group and were associated with a missense mutation in the core domain of HERG and a Holter recording at a young age. These conditions could represent potentially severe forms of LQT2. We could not observe any relation between prior cardiac events and the presence of G2, however, which in turn signifies no additional risk of events. This apparent discrepancy could be explained by the fact that we analyzed data from all family members (including numerous asymptomatic carriers) in the setting of an identified HERG mutation, at variance from other studies in which patients were included and characterized according to their clinical status. Thus, detection of G2 or G1 T-wave notches by Holter recordings in known or suspected LQTS patients, especially in case of a borderline QTc, suggests the presence of a HERG mutation causing LQTS. In addition, we did not evidence G2 in our LQT1 population, even in patients with a history of cardiac events.

In conclusion, this study provides novel evidence that Holter recording analysis is superior to the 12-lead ECG in detecting G1 and G2 T-wave notches. These repolarization abnormalities are more indicative of LQT2 versus LQT1, with G2 notches being most specific and often reflecting HERG core domain missense mutations. This confirms pathophysiological approaches. From a practical point of view, detection of notched T waves can be used as a diagnostic tool as well as to direct genetic testing in favor of HERG.

Acknowledgments

This work was supported by the Institut National de la Santé et de la Recherche Médicale (Progrès No. 4P009D), the Association Française contre les Myopathies (AFM, France), and the Fondation Leducq. We are indebted to the family members, without whose participation this work could not have been done. We thank Drs J.M. Davy, V. Lucet, and I. Durand for their participation in clinical examinations and Drs K. Schwartz and J. Weissenburger for helpful discussions.

References


Notched T Waves on Holter Recordings Enhance Detection of Patients With LQT2 (HERG) Mutations


Circulation. 2001;103:1095-1101
doi: 10.1161/01.CIR.103.8.1095

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/103/8/1095

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/