New Mutations in the \textit{KVLQT1} Potassium Channel That Cause Long-QT Syndrome

Hua Li, PhD; Qiuyun Chen, PhD; Arthur J. Moss, MD; Jennifer Robinson, MS; Veronica Goytia, BS; James C. Perry, MD; G. Michael Vincent, MD; Silvia G. Priori, MD; Michael H. Lehmann, MD; Susan W. Denfield, MD; Desmond Duff, MD; Stephen Kaine, MD; Wataru Shimizu, MD; Peter J. Schwartz, MD; Qing Wang, PhD; Jeffrey A. Towbin, MD

\textbf{Background}—Long-QT syndrome (LQTS) is an inherited cardiac arrhythmia that causes sudden death in young, otherwise healthy people. Four genes for LQTS have been mapped to chromosome 11p15.5 (\textit{LQT1}), 7q35–36 (\textit{LQT2}), 3p21–24 (\textit{LQT3}), and 4q25–27 (\textit{LQT4}). Genes responsible for \textit{LQT1}, \textit{LQT2}, and \textit{LQT3} have been identified as cardiac potassium channel genes (\textit{KVLQT1}, \textit{HERG}) and the cardiac sodium channel gene (\textit{SCN5A}).

\textbf{Methods and Results}—After studying 115 families with LQTS, we used single-strand conformation polymorphism (SSCP) and DNA sequence analysis to identify mutations in the cardiac potassium channel gene, \textit{KVLQT1}. Affected members of seven LQTS families were found to have new, previously unidentified mutations, including two identical missense mutations, four identical splicing mutations, and one 3-bp deletion. An identical splicing mutation was identified in affected members of four unrelated families (one Italian, one Irish, and two American), leading to an alternatively spliced form of \textit{KVLQT1}. The 3-bp deletion arose de novo and occurs at an exon-intron boundary. This results in a single base deletion in the \textit{KVLQT1} cDNA sequence and alters splicing, leading to the truncation of \textit{KVLQT1} protein.

\textbf{Conclusions}—We have identified LQTS-causing mutations of \textit{KVLQT1} in seven families. Five \textit{KVLQT1} mutations cause the truncation of \textit{KVLQT1} protein. These data further confirm that \textit{KVLQT1} mutations cause LQTS. The location and character of these mutations expand the types of mutation, confirm a mutational hot spot, and suggest that they act through a loss-of-function mechanism or a dominant-negative mechanism. \textit{(Circulation. 1998;97:1264-1269.)}

\textbf{Key Words:} arrhythmias \textbullet\ long-QT syndrome \textbullet\ potassium \textbullet\ death, sudden \textbullet\ \textit{KVLQT1}

Sudden death from cardiac arrhythmias is thought to account for 11% of all natural deaths.\textsuperscript{1,2} LQTS is an inherited cardiac disorder that causes syncope, seizures, and sudden death, usually in young and otherwise healthy individuals.\textsuperscript{3-8} In many cases, the first symptom is sudden death. Individuals with LQTS usually have prolongation of the QT interval on electrocardiograms, an indication of abnormal repolarization.\textsuperscript{5,10} The clinical features of LQTS result from episodic ventricular tachyarrhythmias, specifically torsade de pointes and ventricular fibrillation.\textsuperscript{5-11}

Inherited LQTS can result from at least five different genes. Four genes were mapped to chromosome 11p15.5 (\textit{LQT1}),\textsuperscript{12,13} 7q35–36 (\textit{LQT2}),\textsuperscript{14} 3p21–24 (\textit{LQT3}),\textsuperscript{14} and 4q25–27 (\textit{LQT4}).\textsuperscript{15} Several other families with autosomal dominant LQTS are not linked to any known LQTS loci (unpublished data), indicating that additional LQTS locus heterogeneity exists. Three LQTS genes (\textit{LQT1}, \textit{LQT2}, and \textit{LQT3}) were identified either by the candidate gene approach or positional cloning. These include the cardiac potassium channel genes \textit{KVLQT1} (\textit{LQT1}),\textsuperscript{16,17} \textit{HERG} (\textit{LQT2}),\textsuperscript{17} and the cardiac sodium channel gene \textit{SCN5A} (\textit{LQT3}).\textsuperscript{18,19} In addition, mutations in \textit{KVLQT1} were shown to result in both Romano-Ward syndrome (heterozygous mutations) and Jervell and Lange-Nielsen syndrome (homozygous mutations).\textsuperscript{16,20} Wang et al\textsuperscript{16} identified 11 different types of \textit{KVLQT1} mutations (one 3-bp deletion and 10 missense mutations) in 16 LQTS families with Romano-Ward syndrome and, more recently, Neyroud et al\textsuperscript{20} identified a homozygous insertion-deletion mutation in Jervell and Lange-Nielsen syndrome.\textsuperscript{16,20} Here, we report identification of new \textit{KVLQT1} mutations in affected members of seven families with Romano-Ward syndrome. We identified two identical missense mutations (one in a
were classified as unaffected, (2) symptomatic individuals with a QTc of $0.47$ seconds were considered affected, and (3) symptomatic individuals with a QTc of $\geq 0.47$ seconds were considered affected, and (3) symptomatic individuals with a QTc of $< 0.47$ seconds were considered unaffected.

**Methods**

**Identification of LQTS Patients**

LQTS patients were identified throughout North America, Europe, and Asia, with the majority of patients being identified from the International LQTS Registry established by the National Institutes of Health at the University of Rochester, NY. Informed consent was obtained from participants in 115 families in accordance with standards established by local institutional review boards. For each individual, historical data (the presence of syncope, the number of syncopal episodes, the presence of seizures, the age of onset of symptoms, and the occurrence of sudden death) and the length of the QTc were obtained. Phenotypic criteria used were as follows: (1) Individuals without any symptoms and with a QTc of $\leq 0.41$ second were classified as unaffected, (2) symptomatic individuals with a QTc of $\leq 0.45$ second and asymptomatic individuals with a QTc of $\geq 0.47$ seconds were considered affected, and (3) symptomatic individuals with a QTc of $< 0.44$ second and asymptomatic individuals with a QTc of $\geq 0.41$ and $0.47$ second were classified as uncertain.

**Genomic DNA Samples and Linkage Analysis**

Genomic DNA was prepared from peripheral blood lymphocytes or cell lines derived from Epstein-Barr virus–transformed lymphocytes by standard procedures. Genomic DNA was amplified from lymphocytes or cell lines derived from Epstein-Barr virus–transformed lymphocytes by standard procedures. 12,14,16,18

PCR amplification was carried out in a Perkin-Elmer System 9600 thermocycler using the following profile: 1 cycle of denaturation at 94°C for 5 minutes; 5 cycles at 94°C for 20 seconds, 64°C for 20 seconds, 72°C for 30 seconds; and 25 cycles of 94°C for 20 seconds, 62°C for 20 seconds, 72°C for 30 seconds; followed by a 5-minute extension at 72°C.

**SSCP Analysis**

SSCP analysis was performed on genomic DNA samples from 115 LQTS families. The partial genomic structure of KVLQT1 was previously determined. 16 Primers (intronic sequences) that can PCR-amplify exons encoding trans-membrane domains S2-S6 were defined previously from the partial genomic structure and used in this study for SSCP analysis. 16 PCR was carried out in a 10-μL volume containing 50 ng genomic DNA, 0.52 μmol/L of each primer, 75 μmol/L of each dNTP, 1 μCi [α-32P]dCTP, 0.24 mmol/L spermidine, 1.5 mmol/L MgCl2, 10 mmol/L Tris (pH 8.3), 50 mmol/L KCl, and 1 U Taq DNA polymerase (Promega and Gibco-BRL). PCR amplification was carried out in a Perkin-Elmer System 9600 thermodenaturizing using the following profile: 1 cycle of denaturation at 94°C for 5 minutes; 5 cycles at 94°C for 20 seconds, 64°C for 20 seconds, 72°C for 30 seconds; and 25 cycles of 94°C for 20 seconds, 62°C for 20 seconds, 72°C for 30 seconds; followed by a 5-minute extension at 72°C.

**DNA Sequencing**

**Results**

**KVLQT1 Splicing Mutations Associated With LQTS in Four Families**

Aberrant SSCP conformers were identified in affected members of four families (F1002, F1003, F1004, and F1005: Fig 1); these SSCP anomalies were not observed in DNA samples from unaffected members of these families (Fig 1) or from more than 150 control subjects (data not shown). The pattern of aberrant banding appeared to be similar in all four LQTS families (Fig 1). Sequence analysis of the aberrant bands revealed the presence of an identical splicing mutation, a G-to-A substitution, in all four families. This substitution occurs at the third position of codon A249 (SP/A249/g-a) and

---

**Selected Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS</td>
<td>long-QT syndrome</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>QTc</td>
<td>QT interval on ECG corrected for heart rate</td>
</tr>
<tr>
<td>SSCP</td>
<td>single-strand conformation polymorphism</td>
</tr>
</tbody>
</table>

---

Figure 1. KVLQT1 splicing mutation cosegregating with LQT in families F1002, F1003, F1004, and F1005. Pedigree structures are shown. Individuals with characteristic features of LQT, including prolongation of QT interval on ECG and history of syncope or aborted sudden death are indicated by solid circles (females) or squares (males). Unaffected individuals are indicated by open symbols, and individuals with an equivocal phenotype by hatched symbols. Deceased individuals are indicated by a slash. Results of SSCP analyses are shown below each pedigree. All SSCP conformers are indicated by arrows. Sequence analyses of normal (left) and aberrant (right) conformers revealed that all four families had an identical change, a G to A substitution (SP/A249/g-a). This mutation occurs in splice-donor sequence of exon in S6 domain. PCR primers 9 and 10 in Wang et al were used: 9, CCCCAGGACCCCAGCTGTCCAA; and 10, AGGCTGACCACCTGTCCTCCTCT.
KVLQT1 Missense Mutations Associated With LQTS in Two Families

SSCP analysis with a pair of primers in the S6 domain revealed aberrant bands in affected members of families F1006 and F1007 (Fig 2). These abnormal SSCP bands were not seen in DNA samples from unaffected members of these families (Fig 2) or from more than 150 control individuals (data not shown). DNA sequence analysis of the normal and aberrant conformers revealed that both F1006 and F1007 had an identical missense mutation, a single base substitution (C to T) (Fig 2). This mutation results in substitution of an alanine residue by a valine (A246V). F1006 is a Japanese family and F1007 a white family. Same mutation was previously reported in six other families.16,25 PCR primers 9 and 10 in Wang et al16 were used (see Fig 1).

De Novo Intragenic Deletion of KVLQT1 in a Sporadic Case of LQTS

SSCP analysis with a pair of primers within the pore region of KVLQT1 identified an aberrant conformer in an affected individual in F1008 (Fig 3). This SSCP anomaly was not observed in DNA samples from unaffected members of this family or from more than 150 control subjects (data not shown). Direct sequencing of the abnormal SSCP band identified a 3-bp deletion (SP/V212/ΔGtG) spanning an exon-intron boundary in the pore region. This deletion results in a frame shift in KVLQT1 cDNA sequence, leading to a nonfunctional protein. Genotypic analysis of this kindred using more than 15 polymorphic markers confirmed paternity. Qc intervals for proband, proband’s father, and proband’s mother are 0.50, 0.39, and 0.36 second, respectively. PCR primers 7 and 8 in Wang et al16 were used: 7, TCCTGGAGCCCGAACTGTGTGT; and 8, AGGCTGACCACCTGTCCTCCTG.

Phenotype-Genotype Correlation

Despite the genotypic differences found in these seven families, the phenotype was fairly similar in all affected individuals (Table 1). In six of seven families, the QTc was >0.500 second; the seventh family had QTc measured in the range of 0.490 to 0.493. In addition, six of seven families were symptomatic, with episodes of syncope. Only the family in which the QTc was <0.500 second was without symptoms (Table 1). T-wave alternans, ventricular tachycardia, and torsade de pointes were uncommon; only one family had evidence of T-wave alternans, and two families were noted to have episodes of torsade de pointes (Table 1).

Discussion

The potassium channel gene KVLQT1 was initially identified by positional cloning,16 and 11 different types of missense mutations of KVLQT1 were identified in 16 LQTS families16 in the original report (Table 2). Later, Tanaka et al24 reported...
four missense mutations, and Russell et al. reported two additional missense mutations in three LQTS families, including a previously reported mutation, A246V (previously named A212V) (Table 2). In this report, we found the same

A246V mutation in affected members of two more LQTS families, including one Japanese family (Table 2 and Fig 4). To date, of 30 families with KVLQT1 mutations, alanine at position 246 was mutated 10 times (33%) (Table 2).

Table 2. Summary of KVLQT1 Mutations

<table>
<thead>
<tr>
<th>Nucleotide Change</th>
<th>Coding Effect</th>
<th>Mutation Denotation (Old)</th>
<th>Region</th>
<th>Mutation Denotation (New)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔTCG</td>
<td>Missense</td>
<td>F38W/G39Δ</td>
<td>S2</td>
<td>F72W/G73Δ</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GCC to CCC</td>
<td>Missense</td>
<td>A49P</td>
<td>S2-S3</td>
<td>A83P</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GCC to ACC</td>
<td>Missense</td>
<td>A49T</td>
<td>S2-S3</td>
<td>A83T</td>
<td>Tanaka et al 24</td>
</tr>
<tr>
<td>GGG to AGG</td>
<td>Missense</td>
<td>G60R</td>
<td>S2-S3</td>
<td>G94R</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>CCG to AGG</td>
<td>Missense</td>
<td>R61Q</td>
<td>S2-S3</td>
<td>R95Q</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GTG to ATG</td>
<td>Missense</td>
<td>V125M</td>
<td>S4-S5</td>
<td>V159M</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>CTC to TTC</td>
<td>Missense</td>
<td>L144F</td>
<td>S5</td>
<td>L178F</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to AGG</td>
<td>Missense</td>
<td>G177R</td>
<td>Pore</td>
<td>G211R</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>ΔGGT</td>
<td>Deletion</td>
<td></td>
<td>Pore</td>
<td>SP/V212/ΔGGT</td>
<td>This study</td>
</tr>
<tr>
<td>ACG to ATC</td>
<td>Missense</td>
<td>T1831</td>
<td>Pore</td>
<td>T2171</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>AC to ATG</td>
<td>Missense</td>
<td>I184M</td>
<td>Pore</td>
<td>I218M</td>
<td>Tanaka et al 24</td>
</tr>
<tr>
<td>GCC to AGC</td>
<td>Missense</td>
<td>G185S</td>
<td>Pore</td>
<td>G219S</td>
<td>Russel et al 25</td>
</tr>
<tr>
<td>GCC to AGC</td>
<td>Missense</td>
<td>G185S</td>
<td>Pore</td>
<td>G219S</td>
<td>Russel et al 25</td>
</tr>
<tr>
<td>GGG to AGG</td>
<td>Missense</td>
<td>G196R</td>
<td>S6</td>
<td>G230R</td>
<td>Tanaka et al 24</td>
</tr>
<tr>
<td>GGG to AGG</td>
<td>Missense</td>
<td>A212E</td>
<td>S6</td>
<td>A246E</td>
<td>Wang et al 26</td>
</tr>
<tr>
<td>GGG to AGG</td>
<td>Missense</td>
<td>A212E</td>
<td>S6</td>
<td>A246E</td>
<td>Wang et al 26</td>
</tr>
<tr>
<td>GGG to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GCT to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Russsel et al 25</td>
</tr>
<tr>
<td>GCT to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>Russsel et al 25</td>
</tr>
<tr>
<td>GCT to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>This study</td>
</tr>
<tr>
<td>GCT to GTG</td>
<td>Missense</td>
<td>A212V</td>
<td>S6</td>
<td>A246V</td>
<td>This study</td>
</tr>
<tr>
<td>GGG to GCA</td>
<td>Splicing</td>
<td>S6</td>
<td>SP/A249(g-a)</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>GGG to GCA</td>
<td>Splicing</td>
<td>S6</td>
<td>SP/A249(g-a)</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>GGG to GCA</td>
<td>Splicing</td>
<td>S6</td>
<td>SP/A249(g-a)</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>GGG to GCA</td>
<td>Splicing</td>
<td>S6</td>
<td>SP/A249(g-a)</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>GGG to GAG</td>
<td>Missense</td>
<td>G216E</td>
<td>S6</td>
<td>G250E</td>
<td>Wang et al 16</td>
</tr>
<tr>
<td>GGG to GCG</td>
<td>Missense</td>
<td>R237P</td>
<td>S6</td>
<td>R271P</td>
<td>Tanaka et al 24</td>
</tr>
<tr>
<td>CAGTACT to GTGGAGAT</td>
<td>Deletion and Insertion</td>
<td>C-terminal</td>
<td>G415 Y416 S417 G418 G419 to V415 E416 I417 A418 G419 X422</td>
<td>Neyroud et al 10</td>
<td></td>
</tr>
</tbody>
</table>

*The previously reported KVLQT1 sequence by Wang et al.16 lacked 34 amino acids at the N-terminal end, which has been cloned recently.16 The new mutation denotation system is based on the complete amino acid sequence of KVLQT1.
frequent occurrence of A246 mutations and its presence in both white and Japanese populations indicate that the alanine residue at position 246 is a mutational hot spot in KVLQT1.

An identical splicing mutation was identified in affected members of four unrelated families (one Italian, one Irish, and two American); no unaffected individuals from these families or from more than 150 normal control subjects demonstrate the splicing mutation. In addition, the mutation occurs in a highly conserved region of the gene. Together, these data strongly suggest that the splicing change we identified is the disease-causing mutation. We also identified a 3-bp deletion that arose de novo. The 3-bp deletion, spanning an exon-intron boundary in the pore region, not only alters splicing but also leads to a 1-bp deletion in the coding region, causing a frame shift and truncation of the KVLQT1 protein. These data strongly support the notion that mutations in KVLQT1 cause the chromosome 11–linked LQTS.

Since the original identification of genes for chromosome 3–linked LQTS (SCN5A) and chromosome 7–linked LQTS (HERG), electrophysiological studies have established that the molecular mechanism for chromosome 3–linked LQTS is the presence of a late phase of inactivation-resistant sodium current in the plateau phase of the action potential (a gain-of-function mechanism), whereas HERG mutations cause the loss of If, potassium current through dominant-negative mechanisms or loss-of-function mechanisms. Molecular mechanisms of KVLQT1 mutations are currently unknown. Analysis of the predicted amino acid sequence of KVLQT1 suggests that it encodes a potassium channel subunit. Recent electrophysiological characterization of the KVLQT1 protein in various heterologous systems has confirmed that KVLQT1 is a voltage-gated potassium channel protein. When coexpressed with minK, KVLQT1 forms the slowly activating potassium current (Iks) in cardiac myocytes. A combination of normal and mutant KVLQT1 subunits could therefore form abnormal Iks channels. Thus, LQTS-associated mutations of KVLQT1 could act through a dominant-negative mechanism. The type and location of KVLQT1 mutations described here are consistent with this hypothesis. The missense mutation, A246V, was identified in two families and affects the S6 domain. Two mutations lead to premature termination and truncated proteins (one splicing mutation identified in four unrelated families and one 3-bp deletion that arose de novo). In the first case, the S6 domain and the carboxyl end of the protein are truncated, leaving intact the amino end of the protein and S1 domain to the pore. In the second case, a frame shift and altered splicing cause truncation of the protein in the pore. Alternatively, the latter two mutations could act through a loss-of-function mechanism. In general, these patients had moderate symptomatology, with relatively frequent episodes of syncope and long QT intervals (>0.500 second). It is unclear whether mutations in certain regions of the KVLQT1 gene will cause more malignant disease than mutations in other regions of the gene. Electrophysiological characterization of KVLQT1 mutations will shed light on the molecular mechanisms of these mutations and possibly allow for predictions of clinical outcome.

Neyroud et al demonstrated a homozygous insertion-deletion mutation in the 3′ end of KVLQT1 leading to Jervell and Lange-Nielsen syndrome, which includes QT and deafness. They show that the hearing abnormality occurs in three individuals because of the loss of function of the channel, which is the result of mutation on both alleles (ie, homozygous mutation). When a heterozygous mutation occurs, no matter at which end of the gene, it appears that Romano-Ward syndrome (ie, no deafness) results. Despite the possibility that heterozygous mutations in KVLQT1 act in a dominant-negative mechanism, some functional KVLQT1 potassium channels exist in the stria vascularis of the inner ear. Therefore, deafness is averted.

Identification of SCN5A and HERG as LQTS genes has led to potential new rational gene-specific therapy to prevent life-threatening arrhythmias. Mexiletine, a sodium channel blocking agent, has been shown to markedly shorten the QTc of chromosome 3–linked LQTS patients and to have only a modest effect on chromosomes 7– and 11–linked LQTS patients. By contrast, raising the serum potassium concentration was shown to be effective in shortening the QTc interval for patients with chromosome 7–linked LQTS; however, no corresponding data have yet been reported for chromosomes 3– and 11–linked patients. No effective treatment for patients with chromosome 11–linked LQTS is currently known. Studies with various interventions, for example, potassium channel opening agents, are needed to identify therapeutic strategies aimed at reducing the risk of life-threatening arrhythmias in patients with KVLQT1 mutations.

Acknowledgments

This work was supported by the Abercrombie Cardiology Fund, Texas Children’s Hospital (Dr Wang), NIH grants R01-HL-33843 and R01-HL-51618 (Dr Moss), and The Texas Children’s Hospital Foundation Endowed Chair in Pediatric Cardiac Research (Dr Towbin). Dr Wang is also a Visiting Professor of the China National Rice Research Institute.

References


28. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the 


New Mutations in the *KVLQTI* Potassium Channel That Cause Long-QT Syndrome

*Circulation*. 1998;97:1264-1269
doi: 10.1161/01.CIR.97.13.1264

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/97/13/1264

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/