Relationship between dilated cardiomyopathy and the E23K and I337V polymorphisms in the Kir6.2 subunit of the $K_{ATP}$ channel

H.L. Xi¹*, J.F. Liu¹*, L. Li² and J. Wan¹

¹Institute of Cardiovascular Research, Renmin Hospital of Wuhan University, Wuhan, China
²Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL, USA

*These authors contributed equally to this study.
Corresponding author: J. Wan
E-mail: wanjun1963@hotmail.com

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ABSTRACT. ATP-sensitive potassium channels play an important role in myocardial electrical activity. Genetic disruption of these channels predisposes the myocardium to cardiac diseases. Herein we investigated whether two polymorphisms, E23K and I337V, located in the Kir6.2 subunit of ATP-sensitive potassium channels are associated with dilated cardiomyopathy (DCM) in a Chinese population. Blood was collected from DCM patients and controls. DNA was extracted for polymerase chain reaction, which was followed by DNA sequencing. The 2 polymorphisms were present in both DCM patients and normal controls. The frequencies of both the E23K and the I337V polymorphisms were not significantly different between DCM patients and normal controls. However, in DCM patients carrying the E23K polymorphism, the left ventricular end diastolic dimension (LVEDD) and the left atrial dimension (LAD) were significantly greater than those in DCM patients without the E23K polymorphism. Moreover, the occurrence of ventricular arrhythmias in DCM patients was
also slightly increased in the presence of the E23K polymorphism (P < 0.05). We failed to identify an association between the I337V polymorphism and LVEDD, LAD, or ventricular arrhythmias in patients with DCM. The Kir6.2 E23K polymorphism in DCM patients of Han ethnicity may increase the risk of negative outcomes such as congestive heart failure and sudden cardiac death by affecting LVEDD and LAD.

**Key words:** E23K; I337V; Polymorphism; Dilated cardiomyopathy; ATP-sensitive potassium channels

**INTRODUCTION**

Dilated cardiomyopathy (DCM) is a leading public health problem in China as well as an important cause of congestive heart failure and sudden cardiac death worldwide. DCM is characterized by left ventricular or biventricular cardiac enlargement,ventricular systolic dysfunction, and chronic heart failure (Richardson et al., 1996). The prognosis of DCM depends on the identification of associated complications such as ventricular arrhythmias and sudden cardiac death. In patients with structural heart disease such as DCM, the left ventricular end diastolic dimension (LVEDD) and left atrial dimension (LAD) are predictors of ventricular arrhythmias and sudden cardiac death (Shen et al., 1992). Although cardiomyocyte dysfunction has been recognized in DCM for decades, the genetic mechanisms underlying these defects remain poorly understood. Recent progress in human genomics has provided an approach for the identification of risk factors, as genetic background likely contributes to the consequences of DCM-induced ventricular tachycardia and sudden cardiac death.

The cardiac ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels are composed of 4 pore-forming Kir6.x subunits and 4 sulfonylurea receptor subunits. Channels consisting of Kir6.2 and sulfonylurea receptor 2A subunits are expressed in cardiac muscles (Inagaki et al., 1995). Cardiac K\(_{\text{ATP}}\) channels regulate cellular membrane potential and action potential durations as well as play an important role in ischemic preconditioning (Gross and Peart, 2003). Recent studies in knockout mice have shown that genetic disruption of K\(_{\text{ATP}}\) channels predisposes the myocardium to arrhythmias after catecholamine challenge (Liu et al., 2004). The myocardium from Kir6.2 knockout mice also shows survival disadvantages; impaired cardiac performance and pathological Ca\(^{2+}\)-dependent structural damage occur in the heart with repetitive exercise (Liu et al., 2004; Tong et al., 2006; Flagg et al., 2007). These findings suggest that abnormal myocardial K\(_{\text{ATP}}\) channel activity may increase myocardium vulnerability to arrhythmias and thus sudden cardiac death.

The point mutations A1513T and Fs1524 in the sulfonylurea receptor 2A subunit have been identified in patients who died of heart failure and cardiomyopathy (Bienengraeber et al., 2004). Several point mutations have also been identified in the Kir6.2 subunits of patients who died of myocardial infarction or ventricular arrhythmias (Anderson et al., 2002; Delisle et al., 2004). These Kir6.2 subunit mutations include E23K and I337V, which occur in the regions close to the pore-lining subunit of Kir6.2 in the K\(_{\text{ATP}}\) channels that play an important role in myocardium excitability. Therefore, genetic variations in the muscular K\(_{\text{ATP}}\) channels may contribute to abnormalities in cardiomyocyte contraction, thereby impairing the functions of these cells.

Although a role for K\(_{\text{ATP}}\) channels in ventricular arrhythmia has been suggested, its role in patients with DCM has not been studied, especially in Chinese populations. We per-
formed this study to elucidate a possible contribution of polymorphisms in the Kir6.2 subunit of the myocardial K\textsubscript{ATP} channels to DCM in Chinese patients.

MATERIAL AND METHODS

Subjects

We studied 89 unrelated patients with DCM (59 males and 30 females aged 60.7 ± 2.5 years old) and 80 normal control subjects (57 males and 23 females aged 59.8 ± 2.5 years old) who visited the Renmin Hospital of Wuhan University from January 2009 to March 2011. All subjects enrolled in this study were of Han ethnicity. DCM was diagnosed according to the diagnostic standards revised by the World Health Organization/International Society and Federation of Cardiology in 1995 (Richardson et al., 1996). Because the polymorphisms in our study are also associated with diabetes in white populations (Nielsen et al., 2003), patients with diabetes were excluded from the study to rule out confounding effects. Patients with secondary DCM due to ischemia, alcohol use, autoimmune, electrolyte, and acid-base disorders were also excluded. All participants underwent resting echocardiography testing and Holter monitoring. Normal control subjects were selected from populations who came to Renmin Hospital during the same time period. We obtained informed consent from all patients and controls. Our protocol followed the guidelines of the ethics committee of Wuhan University and the Declaration of Helsinki. All subjects were examined the morning after an overnight fast. Blood samples were drawn for biomedical measurements and for DNA extraction and analysis.

DNA sequencing

Genomic DNA was extracted from peripheral white blood cells using a genomic DNA extraction kit (Tiangen Biochemical Company, Beijing, China). The primers were designed by Primer Premier 5.0 based on the human Kir6.2 messenger RNA sequence (GenBank accession No. BC064497). The forward primer 5'-GAC TCT GCA GTG AGG CCC TA-3' and reverse primer 5'-GCC GGG CTA CAT ACC ACA T-3' were used to amplify a 1390-bp fragment. The primers were synthesized by the Shanghai Saibaisheng Gene Technology Co. Ltd (China). Polymerase chain reaction (PCR) was carried out in 25 μL including 100 ng genomic DNA, 10 pM each primer, 1.5 mM Mg\textsuperscript{2+}, 200 μM each deoxyribonucleotide triphosphate, and 1.0 U Taq DNA polymerase. After a 5-min pre-denaturation at 95°C, DNA fragments were amplified for 35 cycles as follows: denaturation for 30 s at 95°C, annealing for 45 s at 67°C, and extension for 1 min at 72°C and a final extension for 10 min at 72°C. All PCR amplifications were processed in a Biometra TGradient thermocycler (Biometra, Goettingen, Germany). After amplification, purified PCR products were sequenced with an ABI 3730 XL DNA sequencer (Harlow Scientific, USA). Sequence alignments were analyzed using the GenBank Basic Local Alignment Search Tool online provided by the National Center for Biotechnology Information.

Echocardiography

LVEDD and LAD measurements in all patients with DCM were determined using 2-dimensional echocardiography.
**Statistical analysis**

Data are presented as means ± SD. Statistical Product and Service Solutions 13.0 for Windows (IBM, New York, USA) was used to analyze all data. The Student t-test was applied for parametric variables, and the chi-square test was used for nonparametric variables. A value of $P < 0.05$ indicated statistically significant difference.

**RESULTS**

**E23K and I337V polymorphisms in Chinese populations**

PCR analysis and DNA sequencing were applied to identify whether the E23K and I337V polymorphisms in the Kir6.2 subunit of $K_{ATP}$ channels occurred in patients with DCM and normal controls. The E23K polymorphism has been found in more than 50% of whites and is associated with 15% of type 2 diabetes and 50% of cardiovascular disease cases (Nielsen et al., 2003; Reyes et al., 2008, 2009). By contrast, the I337V polymorphism is present in 12% of whites (Sakura et al., 1996). No reports of E23K and I337V polymorphisms have been published for Chinese populations. We found that 53% of normal controls and 62% of patients with DCM carried the E23K polymorphism. In contrast to the E23K variant, the I337V polymorphism was present at an essentially equal degree (approximately 66%) in both normal controls and patients with DCM. To our surprise, the frequencies of both the E23K and I33V variants did not differ significantly between DCM patients and normal controls (Figures 1 and 2).

![Figure 1](image-url)

**Figure 1.** E23K polymorphism in patients with dilated cardiomyopathy (DCM) and normal controls. A. Positive sequence alignment with sequence from *Homo sapiens*. The position of the mutation (634 G to A) is highlighted in red. The following graph shows the corresponding position of the peak map, and the position is marked with an arrow. B. Protein sequence of the E23K polymorphism is aligned with *H. sapiens*. The amino acid changed is highlighted in red.
Clinical characteristics of DCM patients

The clinical characteristics of DCM patients in the study are shown in Table 1. These patients were divided into 2 groups based on the presence or absence of the E23K polymorphism. Values of fasting plasma glucose, systolic blood pressure, total cholesterol, triglyceride, alanine transaminase, and aspartate transaminase were not significantly different between these two patient groups. Therefore, the E23K polymorphism did not affect biomedical measurements in the DCM patients in the current study.

<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>E23K</th>
<th>Non-E23K</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td>5.33 ± 0.24</td>
<td>5.15 ± 0.27</td>
<td>0.633</td>
</tr>
<tr>
<td>TC (mM)</td>
<td>3.71 ± 0.17</td>
<td>4.06 ± 0.16</td>
<td>0.182</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.11 ± 0.06</td>
<td>1.16 ± 0.10</td>
<td>0.640</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>35.60 ± 9.80</td>
<td>31.24 ± 4.34</td>
<td>0.739</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>43.19 ± 8.19</td>
<td>31.24 ± 4.34</td>
<td>0.265</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride; ALT = alanine transaminase; AST = aspartate transaminase.

Effects of E23K and I337V polymorphisms on LVEDD and LAD in DCM patients

DCM is characterized by cardiac ventricular or atrial enlargement, which is an im-
important risk factor that influences clinical outcomes in patients with DCM (Hammermeister et al., 1979; White et al., 1987; Galderisi et al., 1992; St John et al., 1994; Eriksson et al., 1995). Thus, we measured LVEDD and LAD in DCM patients and studied the effects of the E23K and I337V polymorphisms on these 2 critical parameters. The LVEDD in DCM patients carrying the E23K polymorphism was significantly elevated compared with that measured in DCM patients without the E23K polymorphism (65.5 ± 6.3 vs 61.3 ± 3.6, P = 0.017; Figure 3A, C, and D). Similarly, LAD was also increased in DCM patients with the E23K polymorphism (46.2 ± 6.9 vs 42.5 ± 4.2, P = 0.04; Figure 3B-D). However, we failed to identify an effect of the I337V polymorphism on either LVEDD or LAD in patients with DCM (data not shown).

**Figure 3.** A. and B. Grouped data of the left ventricular end diastolic dimension (LVEDD) and the left atrial dimension (LAD) measured from patients with dilated cardiomyopathy with or without the E23K polymorphism, *P < 0.05. C. and D. Representative echocardiography images from two DCM patients. The positions marked with arrows indicate left ventricle and left atrium, respectively.
Effects of E23K and I337V polymorphisms on ventricular arrhythmias in DCM patients

Enlargements in LVEDD and LAD have been linked with congestive heart failure, ventricular arrhythmias, and sudden cardiac death (St John et al., 1994). Thus, we examined whether the incidence of ventricular arrhythmias in patients with DCM was associated with the presence of the E23K and I337V polymorphisms. In our study, ventricular arrhythmia was a broad term describing any arrhythmia that originated in 1 of the ventricles of the heart. We found that the E23K polymorphism dramatically increased the likelihood of ventricular arrhythmias in patients with DCM. In the group of DCM patients with ventricular arrhythmias, the frequency of the E23K polymorphism was 69.4%. By contrast, the frequency of the E23K polymorphism was only 38.5% in DCM patients without ventricular arrhythmias (P = 0.02; Figure 4A). The presence of the I337V polymorphism did not affect the frequency of ventricular arrhythmias in patients with DCM (Figure 4B). The E23K polymorphism, rather than I337V polymorphism, seems to be a risk factor for ventricular arrhythmias, indicating that the effects of the E23K polymorphism are specific.

Figure 4. Grouped data of the occurrence of the E23K or I337V polymorphisms in patients with dilated cardiomyopathy (DCM). The occurrence of E23K in DCM patients with ventricular arrhythmias (VA) is statistically greater than that in DCM patients without VA (P < 0.05). The occurrence of I337V is not statistically different between DCM patients with or without VA.

DISCUSSION

DCM, one of the most common indications for cardiac transplantation (Kaye, 1992; Mudge et al., 1993), is characterized by ventricular dilation and reduced contractile function, which lead to congestive heart failure, arrhythmia, and sudden cardiac death (Pye et al., 1994;...
Cowie et al., 1997; Sharpe and Doughty, 1998). Recent studies have found that pathophysiology and disease phenotypes are modulated by genetic variations ranging from risk-conferring polymorphisms to disease-causing polymorphisms. Human molecular genetic studies have discovered more than 25 distinct genes linked to the pathophysiology of DCM (Schönberger and Seidman, 2001), among which K_ATP channels are implicated. The genetic variations identified in the subunit of the sulfonylurea receptor have established a previously unrecognized mechanism of channel malfunction in human cardiomyopathy. These variants are believed to disrupt catalysis-dependent gating and impair metabolic decoding (Bienengraeber et al., 2004).

Aside from genetic variations in the sulfonylurea receptor subunit, several point mutations have been identified in the Kir6.2 subunit, including the common E23K polymorphism (Anderson et al., 2002; Schwanstecher et al., 2002b; Delisle et al., 2004), which is present in approximately 58% of whites. A relatively lower allelic frequency of E23K (34%) has been reported in Japanese populations (Yamada et al., 2001). We found that the frequency of the Kir6.2 E23K polymorphism was approximately 50% in a Chinese population of Han ethnicity. Combining our findings with others suggests that the E23K polymorphism is not rare, and its persistence in populations with different ethnic backgrounds can be explained by a relatively benign effect on K_ATP channel function in a heterozygous state.

Previous research has demonstrated that the K23K polymorphism is associated with left ventricular size in hypertensive individuals (Reyes et al., 2008), demonstrating the impact of this particular position in the Kir6.2 subunit. However, its impact in DCM has not been studied in Chinese patients. We investigated the relationship between the common Kir6.2 E23K polymorphism and DCM and found that the E23K polymorphism was significantly associated with a greater LVDD and LAD in DCM patients. Furthermore, DCM patients carrying the Kir6.2 E23K polymorphism had a relatively high likelihood of developing ventricular arrhythmias, thus demonstrating the impact of the Kir6.2 E23K polymorphism on cardiac structure and function in patients with DCM. The Kir6.2 E23K polymorphism, present in more than half of the DCM patients in our study, may be a risk factor for the transition from DCM to subclinical maladaptive cardiac remodeling.

We also investigated another polymorphism in the Kir6.2 subunit: I337V. However, we were unable to identify any impact of this polymorphism on cardiac structure and function in patients with DCM. The E23K polymorphism results in an amino acid change from glutamic acid to lysine. Glutamic acid is an acidic amino acid with a negative charge, whereas lysine is a basic amino acid with a positive charge. The I337V polymorphism results in the replacement of isoleucine with valine, both of which are non-charged and belong to the same category of amino acids. Previous studies have indicated that the charges on amino acids play essential roles in the ion conduction and gating of K_ATP channels (Xu et al., 2001; Cukras et al., 2002). This relationship could be especially strong for the E23K polymorphism in the Kir6.2 subunit, which is located in a pore-forming area of the K_ATP channel. Therefore, differences in location and amino acid charges between the E23K and I337V polymorphisms may explain their different effects in DCM patients in our study. Our data are consistent with previous reports showing that the E23K polymorphism rather than the I337V polymorphism in the Kir6.2 subunit plays a role in K_ATP channel activity (Cukras et al., 2002; Schwanstecher and Schwanstecher, 2002; Schwanstecher et al., 2002a; Antcliff et al., 2005).

In the Chinese population, we studied the Kir6.2 E23K polymorphism occurred in a similar frequency between DCM patients and normal controls. However, among individuals with documented DCM at the time of echocardiography, the Kir6.2 E23K polymorphism was
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statistically associated with greater left ventricular and atrial enlargement. Our findings, which are consistent with previous human and animal studies (Kane et al., 2006; Olson et al., 2007), further support an interactive $K_{\text{ATP}}$ channel gene-environment substrate that may pose a risk for certain cardiac diseases. Our study might help to establish a genetic marker for impaired cardiac function and structure in patients with DCM as well as underscore the essential role of $K_{\text{ATP}}$ channels in human cardiac pathophysiology. However, the interpretation of our data warrants caution owing to the relatively small sample size and single ethnicity included in our study. Determining the overall impact of Kir6.2 E23K across ethnic groups in Chinese populations and on long-term clinical outcomes, i.e., congestive heart failure and sudden cardiac death, in patients with DCM requires further studies.

In conclusion, 2 polymorphisms, E23K and I337V, in the Kir6.2 subunit of cardiac $K_{\text{ATP}}$ channels were identified in a Chinese population of Han ethnicity. We found that these polymorphisms have different impacts in patients with DCM. The E23K polymorphism is associated not only with greater LVEDD and with LAD in DCM patients but also with a relatively high possibility of ventricular arrhythmias, which might contribute to the susceptibility of DCM patients to negative outcomes such as congestive heart failure and sudden cardiac death.

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