Relationship Between $\beta_1$-Adrenergic Receptor Polymorphisms and Cardiovascular Disease in Peritoneal Dialysis Patients

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Background: Recent studies show that a common gain-of-function polymorphism of $\beta_1$-adrenergic receptor (389 Gly→Arg) plays an important role in the pathogenesis of hypertension and heart failure in patients with normal renal function. We studied the relationship between $\beta_1$-adrenergic receptor polymorphism and cardiovascular disease in peritoneal dialysis (PD) patients.

Methods: We studied 189 new PD patients. The $\beta_1$-adrenergic receptor genotype was determined by polymerase chain reaction-restriction fragment length polymorphism assay. They were then prospectively followed for the development of cardiovascular events. All-cause mortality and duration of hospitalization were also recorded.

Results: There were 95 male cases. The mean age was 56.2 ± 14.8 years. Eighty-six (45.5%) patients were diabetic; 81 (42.9%) received beta-blocker therapy. Only one case was homozygous for the mutant CC genotype. The prevalence of GG, GC, and CC genotypes were 34.9%, 64.6%, and 0.5%, respectively. The genotype distribution was significantly different from that predicted by the assumption of Hardy-Weinberg equilibrium ($p < 0.0001$). There was no difference in the prevalence of pre-existing cardiovascular disease between genotype groups. Actuarial patient survival was 80.2% and 85.1% at 24 months for the GG and GC/CC genotypes, respectively ($p = 0.53$). Event-free survival was 63.6% and 71.5% at 24 months, respectively ($p = 0.26$), and the duration of hospitalization was 15.9 ± 3.0 and 16.6 ± 2.3 days per year, respectively ($p = 0.8$). The results remained similar when patients with and without beta-blocker treatment were separately analyzed.

Conclusion: Our study demonstrates that the $\beta_1$-adrenergic receptor polymorphism is not related to cardiovascular disease in PD patients. Nevertheless, the low prevalence of mutant CC genotype in new PD patients suggests that PD patients represent a highly biased population. [Hong Kong J Nephrol 2008;10(2):58–63]

Key words: cardiovascular disease, peritoneal dialysis, sympathetic system

背景：最近有研究顯示，在腎功能正常的病人間，$\beta_1$-腎上腺素能受體上常見的功能獲得基因多態性（389 Gly→Arg），在高血壓及心衰竭的致病過程中佔有重要角色。本研究在接收腹膜透析（PD）的患者中，調查了$\beta_1$-腎上腺素能受體基因多態性與心血管疾病的關係。

方法：本研究對189位PD新病人，持續監察心血管事件的發生，死亡率，及住院狀況。$\beta_1$-腎上腺素能受體基因型的判斷，乃採用聚合酶鏈反應-限制性片段長度多態性分析法。

結果：研究對象平均年齡為56.2 ± 14.8歲，其中男性佔95人。糖尿病患者有86人（45.5%），接受$\beta$受體阻滯劑療法者則有81人（42.9%）。所有病人中僅1人呈現純合突變之CC基因型，GG、GC、及CC基因型生存率分別為34.9%、64.6%及0.5%。此基因型分佈顯著不同於Hardy-Weinberg平衡的預測結果（$p < 0.0001$）。各基因型患者在先心血管疾病生存率上並無顯著差異。在24個月的追蹤期間，GG及GC/CC基因型患者的存活率分別為80.2%及85.1%（$p = 0.53$）；無事件存活率分別為
INTRODUCTION

End-stage renal disease (ESRD) is one of the most debilitating chronic medical illnesses. In Hong Kong, there were more than 2,500 patients on long-term dialysis in 1997 [1]. Peritoneal dialysis (PD) is the preferred mode of renal replacement therapy in Hong Kong, and accounts for 80% of patients requiring dialysis [1].

Cardiovascular disease is the major cause of mortality and morbidity in PD patients [2,3]. In addition to the classic risk factors of atherosclerosis such as diabetes, hypertension and hyperlipidemia, uremia and possibly the dialysis procedure per se play important roles in the pathogenesis of accelerated atherosclerosis in renal failure patients [4]. However, the pathogenesis of cardiovascular disease in dialysis patients remains incompletely understood.

Recently, a polymorphism found in β1-adrenergic receptor, which was first identified by Mason et al [5], has been implicated as a potent genetic determinant of hypertension and heart failure [6]. Individuals homozygous for this potent and very common [7,8] gain-of-function polymorphism at amino acid position 389 (arginine substituted for glycine) show increased heart responsiveness to adrenergic receptor stimulation [9]. Individuals with this polymorphism are predisposed to heart failure by instigating hyperactive signaling programs leading to depressed receptor coupling and ventricular dysfunction, and which influences the therapeutic response to beta-blockers [10]. These individuals also have enhanced risks of developing hypertension [8] and possibly myocardial infarction [11].

Several lines of evidence indicate the presence of a sympathetic hyperactivity in renal failure patients [12, 13], which is related to arterial hypertension and atherosclerosis. It is suggested that diseased kidneys send afferent nervous signals to central integrative sympathetic nuclei, thus contributing to the development and maintenance of arterial hypertension [14]. However, the relation between β1-adrenergic receptor and cardiovascular disease in renal failure patients has not been studied. In the present study, we examined the relationship between β1-adrenergic receptor polymorphisms and cardiovascular disease in PD patients.

PATIENTS AND METHODS

Study population

We studied 189 consecutive new PD patients. We excluded patients who planned to have elective living donor transplant or transfer to another renal center within 6 months. The presence of diabetes, hypertension, and a history of cardiovascular disease were recorded. Hypertension was defined according to the Joint National Committee VII criteria [15]. Blood pressure, number of antihypertensive agents, and the use of beta-blocker for all reasons were recorded. The definition of cardiovascular disease used was as previously described [16], which included angina, class III to IV congestive heart failure, a history of previous myocardial infarction, cerebrovascular accident, or amputation for vascular disease.

Polymorphism detection

Genomic DNA was extracted from whole blood samples by standard techniques. The method of polymorphism detection has been described previously [7,11]. Briefly, polymerase chain reaction analysis was performed with 200 ng DNA as template in a final volume of 50 μL. The primer pair used to amplify the DNA was 5’-CGC TCT GCT GGC TGC CCT TCT L. The primer pair used to amplify the DNA was 5’-CGC TCT GCT GGC TGC CCT TCT TCC-3’ (sense) and 5’-TGG GCT TCG AGT TCA CCT GCT ATC-3’ (antisense), as described by Maqbool et al [7] for the given polymorphism site. Amplified DNA was digested with restriction endonuclease Bcg I (New England Biolabs, Beverly, MA, USA). After restriction enzyme digestion of the amplified DNA, genotypes were identified by electrophoresis on 1.5% agarose gels and visualized with ethidium bromide staining ultraviolet illumination.

Clinical follow-up

All patients were planned to be followed for at least 2 years. The clinical management and dialysis regimen were decided by individual nephrologist and not affected by the study. The primary end point was a composite one that consisted of cardiovascular death, non-fatal myocardial infarction or stroke, hospital admission for unstable angina, coronary intervention, congestive heart failure, transient ischemic attack, or lower limb ischemia. Secondary end points included duration of hospitalization (all-cause) and all-cause
mortality. Censoring events for survival analysis included transfer to long-term hemodialysis, kidney transplant, recovery of renal function, loss to follow-up, and transfer to other dialysis centers.

**Statistical analysis**
Statistical analysis was performed using SPSS version 10.0 software (SPSS Inc., Chicago, IL, USA) for Windows. Data were expressed as mean ± standard deviation. Hardy-Weinberg equilibrium was calculated by the standard method with the \( \chi^2 \) test. Comparisons of the prevalence of hypertension and pre-existing cardiovascular disease between genotype groups were performed by the \( \chi^2 \) test, Student’s \( t \) test or one-way analysis of variance as appropriate. Kaplan-Meier analysis and the log rank test were used to explore the effect of \( \beta_1 \)-adrenergic receptor genotype on the composite cardiovascular end point as well as the actuarial patient survival. A \( p \) value below 0.05 was considered statistically significant. All probabilities were two-tailed.

**RESULTS**
We studied 189 consecutive new PD patients. Their demographic and baseline clinical information are summarized in Table 1. Amongst the 189 patients, 181 (95.8%) had pre-existing hypertension, and 71 (37.6%) had underlying cardiac or vascular disease before initiation of dialysis.

**Genotype and pre-existing vascular disease**
Of the 189 patients, 66 (34.9%) had GG, 122 (64.6%) had GC, and only 1 (0.5%) had CC genotype. The values predicted by the assumption of the Hardy-Weinberg equilibrium in the whole group of studied subjects (GG, GC and CC groups are 46.2%, 44.1% and 10.8%, respectively) were significantly different from those that were observed (\( \chi^2 \) test, \( p < 0.0001 \)), indicating that the studied subjects represented a highly biased population. For the convenience of analysis, patients were categorized into GG and GC/CC groups.

The prevalence of pre-existing vascular disease, use of cardioprotective medications, peritoneal transport characteristics, dialysis adequacy and nutritional status were comparable between the genotype groups (Table 2). During the study period, the average systolic blood pressure was 145.7 ± 20.7 mmHg, diastolic blood pressure was 77.1 ± 13.7 mmHg, total cholesterol was 5.1 ± 1.3 mmol/L, triglyceride was 2.0 ± 1.3 mmol/L, low-density lipoprotein cholesterol was 2.8 ± 1.5 mmol/L, and HbA1c (for diabetic subjects) was 6.5 ± 1.2%. There was no significant difference in blood pressure, serum lipid or diabetic control between genotype groups during the study period (details not shown). There were 39.4% and 36.6% patients of the GG and GC/CC groups, respectively, with pre-existing cardiac or vascular disease (\( p = 0.4 \)). There were marginally more patients with previous cerebrovascular disease in the GG than in the GC/CC group (13 in 66 vs. 10 in 123 cases, \( p = 0.034 \) without adjustment for multiple comparison).

**Clinical outcome**
The average follow-up was 30.2 ± 17.7 months. Eighty-seven (46.0%) patients developed the primary composite end point. The events contributing to the primary composite end point were fatal myocardial infarction (7 cases), fatal stroke (1 case), non-fatal myocardial infarction (6 cases), non-fatal stroke (7 cases), hospitalization for congestive heart failure (53 cases), and hospitalization for acute coronary syndrome (13 cases). The event-free survival was 63.6% and 71.5% at 24 months for GG and GC/CC genotypes, respectively (\( p = 0.26 \) (Figure 1)).

During the follow-up period, 62 (32.8%) patients died. The causes of death were myocardial infarction (11 cases), sudden cardiac death (11 cases), cerebrovascular accident (5 cases), peripheral vascular disease (1 case), peritonitis (10 cases), non-peritonitis infection (10 cases), termination of dialysis (5 cases), malignancy (3 cases), liver failure (2 cases), and other specific causes (4 cases). During the study period, 30 patients had kidney transplantation, 15 changed to hemodialysis for peritoneal failure, and five were transferred to other centers. The actuarial patient survival was 80.2% and 85.1% at 24 months for GG and GC/CC genotypes, respectively (\( p = 0.53 \) (Figure 2)). There was no difference in cardiovascular mortality between the groups (details not shown).

During the follow-up period, there were 959 hospital admissions, with a total of 5,658 days of hospitalization. The number of hospital admissions for all causes was similar between the GG and GC/CC groups (2.34 ± 0.36 vs. 2.73 ± 0.34 admissions per year, \( p = 0.3 \)). The duration of hospitalization for all causes was 15.9 ±
3.0 and 16.6 ± 2.3 days per year for the GG and GC/CC groups, respectively ($p = 0.8$). The results remained similar when patients with and without beta-blocker treatment were separately analyzed, and when hospitalizations for cardiac cause were separately analyzed (details not shown).

### Table 2. Comparison of baseline blood pressure, pre-existing vascular disease, peritoneal transport, dialysis adequacy and nutritional status between the genotype groups

<table>
<thead>
<tr>
<th></th>
<th>GG ($n = 66$)</th>
<th>GC / CC ($n = 123$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>143.6 ± 25.6</td>
<td>146.9 ± 23.3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.5 ± 14.0</td>
<td>80.1 ± 13.4</td>
</tr>
<tr>
<td>Cardioprotective medication, $n$ (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>28 (42.4)</td>
<td>53 (43.1)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>16 (24.2)</td>
<td>34 (27.6)</td>
</tr>
<tr>
<td>ACE inhibitor or ARB</td>
<td>28 (42.4)</td>
<td>58 (47.2)</td>
</tr>
<tr>
<td>Statin</td>
<td>15 (22.7)</td>
<td>37 (30.1)</td>
</tr>
<tr>
<td>Pre-existing vascular disease, $n$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>65</td>
<td>116</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Peritoneal transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D/P creatinine at 4 hr</td>
<td>0.62 ± 0.15</td>
<td>0.61 ± 0.13</td>
</tr>
<tr>
<td>MTAC creatinine (mL/min/1.73 m²)</td>
<td>9.54 ± 4.24</td>
<td>8.95 ± 4.75</td>
</tr>
<tr>
<td>Dialysis adequacy and nutritional status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weekly total Kt/V</td>
<td>1.97 ± 0.52</td>
<td>2.06 ± 0.57</td>
</tr>
<tr>
<td>Residual GFR (mL/min/1.73 m²)</td>
<td>2.93 ± 2.19</td>
<td>3.16 ± 2.87</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>27.2 ± 4.1</td>
<td>29.1 ± 5.2</td>
</tr>
<tr>
<td>NPNA (g/kg/day)</td>
<td>1.05 ± 0.18</td>
<td>1.08 ± 0.31</td>
</tr>
<tr>
<td>FEBM (%)</td>
<td>42.9 ± 11.6</td>
<td>46.5 ± 14.1</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; D/P = dialysate-to-plasma ratio; MTAC = mass transfer area coefficient; GFR = glomerular filtration rate; NPNA = normalized protein nitrogen appearance; FEBM = fat-free edema-free body mass.

### Figure 1. Kaplan-Meier estimates of event-free survival of patients with GG and GC/CC genotypes. Log rank test, $p = 0.26$.

### Figure 2. Kaplan-Meier estimates of actuarial survival of patients with GG and GC/CC genotypes. Log rank test, $p = 0.53$. 
DISCUSSION

Although β1-adrenergic receptor polymorphism is widely implicated as a potent genetic determinant of hypertension and cardiovascular disease in the general population [5–11], we found no difference in the incidence of cardiovascular events, all-cause mortality or hospitalization in new PD patients between the genotype groups. The reason for our negative results is not clear. The sample size in our study was determined a priori and was designed to achieve an 80% power to detect a 25% absolute difference in the incidence of the primary composite end point, and may not have been adequate to detect a small effect of the polymorphism. On the other hand, it is possible that renal failure patients have overwhelming sympathetic hyperactivity [12,13] that does not depend on the activity of β1-adrenergic receptor. It should be noted that although our study focused on PD patients, peritoneal dialysis is the first line renal replacement therapy in Hong Kong [1,17] and our study population is therefore a cohort of unselected new dialysis patients.

It is important to note that hospital admission for congestive heart failure was the most common cause of the composite primary end point, and accounted for over 60% of the cases. Although β1-adrenergic receptor polymorphism is associated with ventricular dysfunction and heart failure in subjects with normal renal function [10], the relation may not be present in dialysis patients. More importantly, it is often difficult to distinguish genuine ventricular failure in PD patients from fluid overload secondary to ultrafiltration or compliance problem. The Kaplan-Meier analysis of the primary composite end point remained insignificant if admission for congestive heart failure was not considered as an event (details not shown). However, it is important to note that there might be too few events after congestive heart failure was excluded, making the result insignificant. Theoretically, objective criteria that follow the ventricular function, such as systolic and diastolic heart function by echocardiography, may be more reliable. Unfortunately, these measures are not available due to the limitation of the original design of the study.

In the present study, we did not adjust for the degree of blood pressure control during survival analysis. Nevertheless, the prevalence of pre-existing hypertension was high but similar between genotype groups. It remains unknown whether the effect of β1-adrenergic receptor polymorphisms on cardiovascular disease is due to a difference in prevalence and/or severity of hypertension, or a direct effect of altered sympathetic drive onto the myocardium and vasculature. Bengtsson et al [8] reported that individuals homozygous for the Arg389 allele of the β1-adrenergic receptor gene were at increased risk of developing hypertension, but there was only one case with this genotype in our study. However, subjects with heterozygous Arg389 allele apparently did not have a higher prevalence of hypertension [8] but were still predisposed to congestive heart failure [10] and myocardial infarction [11].

We found no difference in the incidence of cardiovascular events between the genotype groups when patients with and without beta-blocker therapy were separately analyzed. It is possible that our sample size was not sufficient for subgroup analysis. Several studies found that β1-adrenergic receptor polymorphisms are important determinants of antihypertensive response to metoprolol [18,19], although the relation is not unanimously agreed [20]. In mice, hemodynamic responses to beta-blocker were greater in Arg389 strain [10]. Homozygosity for Arg389 was associated with improvement in ventricular function during carvedilol treatment in heart failure patients [10]. Taken together, the cardiovascular effect of the β1-adrenergic receptor gene polymorphism probably has an intricate interaction with beta-blocker treatment, the effect of which probably needs a large study to delineate.

We found that the distribution of β1-adrenergic receptor genotype of our PD patients differed significantly from the values predicted by the assumption of the Hardy-Weinberg equilibrium. In our study, the Arg389 (i.e. C) allele was present in 65% of the PD patients, which is similar to the prevalence of 75% as reported by Xie et al [21] in a Chinese population. The highly skewed genotype distribution in our PD patients is, therefore, not a result of a lower frequency of the Arg389 allele but the distinct rarity of homozygous CC subjects. The explanation is not entirely clear, but there are two possibilities: either the CC genotype is protective for renal failure, or these patients have a high mortality before they progress to a dialysis-dependent state.

In summary, our study shows that the β1-adrenergic receptor polymorphism is not related to cardiovascular disease in PD patients. Nevertheless, the low prevalence of the mutant CC genotype in new PD patients suggests that PD patients represent a highly biased population.

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