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**SHORT REPORT**

# L41Q polymorphism of the G protein coupled receptor kinase 5 is associated with left ventricular apical ballooning syndrome

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## Background

Altered response to acute catecholamine increase in the synaptic cleft is considered to be the mechanism underlying transient left ventricular apical ballooning syndrome (LVABS).[1–3] Cardiac adrenergic receptors (ARs) of the β1 and β2 subtypes[4] activate myocytes by coupling to the Gα subunit of the heterotrimeric Gs protein, but on the other hand they also promote G protein coupled receptor kinase (GRK)-mediated phosphorylation of βAR with the intent to shut-off signalling.[5] The impact on cardiac function of genetic variants of molecules involved in the intracellular pathways of βAR signalling has been extensively investigated.[6–8]

## Aims

We postulated that polymorphisms associated with different βAR responsiveness might play a role in the risk of LVABS, thus allowing the identification of those patients that are prone to LVABS after an adrenergic surge.

## Methods

We prospectively enrolled 22 consecutive LVABS patients (4 men, age 63 ± 13 years) admitted to the Intensive Coronary Care unit of the AOP Federico II, Naples, Italy between 2005 and 2008. Peripheral blood samples were collected from each patient for genetic analysis. The results were compared with those of 740 outpatients (control) aged 50 ± 0.4 years, 57.8% male. To replicate the study in an independent population, we also enrolled 376 normal volunteers who had undergone voluntary screening for cardiovascular disease, hypertension, diabetes, and kidney failure at our outpatient clinic. The study was approved by the Federico II Ethical Committee and all subjects gave written informed consent. Each LVABS patient underwent standard echocardiography at admission, serially before discharge, and at an outpatient visit performed 3–4 weeks after the onset of symptoms. A 16-segment model and a score from 1 (normal) to 4 (dyskinesia) were used for wall motion analysis. Left ventricular ejection fraction was measured using the biplane Simpson's rule.

The following polymorphisms were analysed: rs35230616 and rs1801253 for β1AR; rs1042713, rs1042714, and rs1800888 for β2AR; rs11554276 for Gs-protein alpha subunit (GNAS); rs17098707 and rs34679178 for GRK5. Genomic DNA was extracted from white blood cells using a dedicated workstation (MagRo; BioNobile) and polymerase chain reaction followed by restriction fragment length polymorphism analysis.

Data are expressed as mean ± standard deviation or as a percentage; $\chi^2$ test, the Cochran–Mantel–Haenszel analysis, and one-way analysis of variance (ANOVA) with a Bonferroni *post hoc* test were used as appropriate (SPSS 13.0). A value of $P < 0.05$ was considered statistically significant.

## Results

The demographic and clinical characteristics of the LVABS patients are shown in *Table 1*. Two patients had bronchial asthma and were receiving chronic treatment with long-acting beta stimulating agents. In one patient, LVABS occurred after the intravenous administration of epinephrine during an asthma attack. A stressful event was identified as the precipitating factor in each of the remaining cases (physical, $n = 1$ and emotional, $n = 20$). The results of the genetic analysis are reported in *Table 2*. The prevalence of polymorphisms of β1AR, β2AR, and GNAS were similar between patients and controls. Conversely, the percentage of LVABS patients who presented with the rs17098707 polymorphism of the GRK5 gene was significantly higher. This genetic variant causes a leucine (L) substitution of the glutamine (Q) amino acid at position 41. The OR for GRK5 for the L41 variant to cause LVABS was 4.036 with a confidence interval of 1.443–11.286 ($P < 0.01$).

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**Table 1 Demographic, clinical, and echocardiographic characteristics of left ventricular apical ballooning syndrome patients**

| Patient | Age (years) | Sex | Hypertension | Hyperlipidaemia | Diabetes | Smoking | Wall motion score index | LV ejection fraction (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 40 | m | + | + | − | − | 1.94 | 40 |
| 2 | 58 | f | + | + | − | + | 1.7 | 42 |
| 3 | 74 | f | + | + | + | − | 2.125 | 33 |
| 4 | 52 | m | + | − | − | − | 2.125 | 39 |
| 5 | 45 | f | − | − | − | − | 1.4 | 45 |
| 6 | 62 | m | + | − | − | + | 2.3 | 35 |
| 7 | 54 | f | + | − | − | − | 2.4 | 31 |
| 8 | 66 | f | + | + | + | + | 2.15 | 36 |
| 9 | 78 | f | + | + | − | − | 2.7 | 38 |
| 10 | 55 | f | + | + | − | + | 2.5 | 33 |
| 11 | 77 | f | + | + | − | + | 2.25 | 39 |
| 12 | 80 | m | + | − | − | − | 2.1 | 32 |
| 13 | 46 | f | − | + | − | − | 2.,3 | 40 |
| 14 | 84 | f | + | + | − | − | 2. 5 | 30 |
| 15 | 84 | f | + | − | + | − | 1.84 | 40 |
| 16 | 64 | f | + | + | + | − | 1.9 | 41 |
| 17 | 56 | f | − | + | − | − | 2.3 | 33 |
| 18 | 50 | f | − | − | − | + | 2.1 | 38 |
| 19 | 66 | f | + | + | − | − | 2.5 | 32 |
| 20 | 59 | f | − | − | − | + | 2.25 | 36 |
| 21 | 51 | f | − | + | − | − | 2.3 | 35 |
| 22 | 66 | f | + | + | + | − | 2.1 | 37 |

**Table 2 Frequencies of the studied polymorphisms in the left ventricular apical ballooning syndrome and in the control populations**

| Gene (SNP) | Group | WT (%) | Het (%) | Poly (%) | Pearson $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|
| β1AR Q31R (rs35230616) | Control | 83.2 | 15.0 | 1.7 | 0.42 | n.s. |
| | LVABS | 81.3 | 18.8 | 0.0 | | |
| β1AR G389R (rs1801253) | Control | 68.6 | 31.4 | 0.0 | 8.03 | n.s. |
| | LVABS | 63.6 | 31.8 | 4.5 | | |
| β2AR G16R (rs1042713) | Control | 53 | 45 | 12 | 3.80 | n.s. |
| | LVABS | 58 | 38 | 4 | | |
| β2AR E27Q (rs1042714) | Control | 59.4 | 33.2 | 7.4 | 2.81 | n.s. |
| | LVABS | 45.8 | 50.0 | 4.2 | | |
| β2AR T164I (rs1800888) | Control | 96.8 | 3.2 | — | 0.69 | n.s. |
| | LVABS | 100.0 | 0.0 | — | | |
| Gαs S51F (rs11554276) | Control | 44.6 | 47.4 | 8.0 | 2.82 | n.s. |
| | LVABS | 60.0 | 40.0 | 0.0 | | |
| GRK5 Q41L (rs17098707) | Control | 56.3 | 37.9 | 5.8 | **9.70** | **0.01** |
| | LVABS | 36.0 | 44.0 | 20.0 | | |
| GRK5 T129M (rs34679178) | Control | 83.1 | 16.9 | — | 3.46 | n.s. |
| | LVABS | 100.0 | 0.0 | — | | |

Statistically significant results are highlighted in bold.

## Replication study

The frequency of the GRK5 L41 allele in the replication cohort was similar to that observed in the control population (Q/Q: 57.4%; L/Q: 37.2%; L/L: 5.3%, n.s.). The clinical characteristics of this group are depicted in *Table 3*. Interestingly, a significantly lower heart rate was associated with the presence of one or two L41 alleles of GRK5. This difference held true in the female group (b.p.m.; Q/Q: 75.6 ± 1.4, L/Q: 73.2 ± 1.2, L/L: 67.8 ± 3.0;

**Table 3 Replication study: effects of GRK5 L41Q on demographic, clinical, and echocardiographic characteristics of volunteers**

| | Q/Q | L/Q | L/L | *P*-value |
|---|---|---|---|---|
| Age (years) | 46.72 ± 0.66 | 47.82 ± 0.73 | 49.72 ± 2.25 | n.s. |
| Male (%) | 66 | 54 | 44 | n.s. |
| BMI | 27.42 ± 0.26 | 26.78 ± 0.29* | 24.99 ± 0.91* | <0.02 |
| SBP (mmHg) | 136.2 ± 1.16 | 134.4 ± 1.30 | 134.9 ± 2.89 | n.s. |
| DBP(mmHg) | 82.89 ± 0.63 | 81.42 ± 0.94 | 81.26 ± 2.48 | n.s. |
| HR (b.p.m.) | 74.63 ± 0.75 | 71.93 ± 0.93* | 69.1 ± 1.98* | <0.03 |
| LVEDd (mm) | 48.84 ± 0.19 | 48.41 ± 0.26 | 48 ± 0.59 | n.s. |
| LVESd (mm) | 30.90 ± 0.16 | 30.55 ± 0.22 | 29.62 ± 0.49 | n.s. |
| EA | 1.065 ± 0.01 | 1.055 ± 0.02 | 1.083 ± 0.05 | n.s. |
| IVS (mm) | 10.33 ± 0.07 | 10.20 ± 0.09 | 9.975 ± 0.26 | n.s. |
| PW (mm) | 9.096 ± 0.04 | 8.989 ± 0.06 | 8.85 ± 0.17 | n.s. |
| LVMi | 103.7 ± 0.76 | 102.6 ± 0.97 | 102.4 ± 2.07 | n.s. |
| EF (%) | 66.27 ± 0.28 | 66.46 ± 0.37 | 68.28 ± 0.85 | n.s. |
| Glucose (mg/dL) | 95.25 ± 1.26 | 94.09 ± 2.28 | 92.11 ± 3.20 | n.s. |
| Creatinine (mg/dL) | 0.960 ± 0.01 | 0.936 ± 0.01 | 0.851 ± 0.03 | n.s. |
| Tryglycerides (mg/dL) | 134.3 ± 6.22 | 135.1 ± 7.08 | 149 ± 19.4 | n.s. |
| Cholesterol (mg/dL) | 202.2 ± 2.67 | 210.4 ± 2.94 | 219.7 ± 11.7 | n.s. |
| HDL (mg/dL) | 48.93 ± 0.88 | 52.21 ± 1.23 | 50 ± 3.68 | n.s. |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVEDd, left ventricle end-diastolic diameter; LVESd, left ventricle end-systolic diameter; IVS, interventricular septum thickness in diastole; PW, posterior wall thickness in diastole; LVMi, left ventricle mass index; EF, ejection fraction; HDL, high density lipoprotein; ANOVA.
*$P < 0.05$ vs. QQ, Bonferroni *post hoc*.

$F = 4.034$, $P < 0.02$), whereas in the male group, due to the small number of cases, significance held true only when the subjects were dichotomized in bearer to not bearer of the mutated allele ($P < 0.05$).

# Conclusion

We did not find any difference in the frequency of β1AR, β2AR, and GNAS S51F polymorphisms between LVABS patients and controls. Conversely, LVABS patients exhibited a higher prevalence of the leucine (L) over the glutamine (N) variant at amino acid 41 of GRK5 non-catalytic regulatory domain. In the replication study, heart rate was reduced in subjects bearing the L41 variant of the GRK5. These findings are consistent with the notion that L41 GRK5 enhances receptor desensitization and impairs βAR response.[6] We envision two scenarios for the pathogenesis of transient LVABS. First of all, the polymorphism might attenuate the inotropic effect of catecholamines on cardiomyocytes. Indeed, both in isolated cells and in transgenic mice, it has been demonstrated that the GRK5 L41 variant causes a negative inotropic effect under conditions of acute catecholamine stimulation.[6] The greater βAR density at the apex when compared with the basal myocardium would account for the characteristic distribution of the wall motion abnormalities in the most common presentation.[9] Concurrently, an impairment in coronary blood flow might contribute to myocardial stunning.[10,11] Increased mechanical wall stress as a consequence of apical ballooning may per se induce coronary microcirculatory abnormalities and GRK5 41L polymorphism, which enhances βAR desensitization, might induce an imbalance between α1 coronary vasoconstriction and βAR vasodilation.[12] This is the first study demonstrating a link between a polymorphism of one of the protein kinases most expressed in the heart and stress-induced acute ventricular dysfunction. The possibility of recurrence and case reports evaluating LVABS in relatives, support a genetic approach to the pathophysiology of this syndrome.[13]


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