Association between Beta Adrenergic Receptor Polymorphism and Ischemic Stroke: A Meta-Analysis

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Background and Purpose  The purpose of this meta-analysis was to determine the precise association between beta-2 adrenergic receptor (β2AR) polymorphism and ischemic stroke.

Methods  Published case control studies on association between β2AR and ischemic stroke were searched from electronic databases. Pooled Odds ratio and 95% Confidence interval were calculated by using software RevMan version 5.2.

Results  A total of three studies involving 1,642 cases and 1,673 controls, which were published from 2007 to 2014, were subjected to meta-analysis for allelic association and 518 cases and 510 controls for genotypic association. Pooled analysis of two studies for genotypic association suggested that subjects carrying Gln27Glu polymorphism of β2AR had an increased risk for ischemic stroke under recessive model (OR 2.09; 95% CI; 1.20 to 3.64) and under dominant model (OR 1.47; 95% CI 1.14 to 1.90). Pooled analysis of three studies for allelic association showed a significantly higher Glu27 allele of β2AR in the patients with ischemic stroke (OR 1.58; 95% CI; 1.38 to 1.81).

Conclusions  The present meta-analysis suggests that Gln27Glu polymorphism of β2AR gene is associated with increased risk for ischemic stroke.

Keywords  β2-adrenergic receptor gene; β2AR; Ischemic stroke; Cerebral infarction; Polymorphism; Meta-analysis

Introduction

Stroke is the second common cause of death following ischemic heart disease.1 Stroke has accounted for nearly 5.7 million deaths globally and 87% of these deaths take place in low and middle income nations.2 Stroke is a multi-factorial disease and epidemiological and animal studies have robustly recommended genetic influences in the pathogenesis of ischemic stroke.3 The genetic influences are probably polygenic whereby multiple genes exert a small influence or risk on phenotype. Beta adrenergic receptors are members of a family of receptors known as G-protein coupled receptors (GPCR) and have a seven membrane spanning domain structure, an extracellular amino terminus, three intracellular and three extracellular loops, and an intracellular carboxyl terminus.4 These are receptors for neurohormone epinephrine and nor-epinephrine.

Several mechanisms contribute to loss of receptor activity including uncoupling of the receptor from adenylyl cyclase activity, internalization of the receptor and phosphorylation of internalized receptors.5 Several studies support the role of cyclic Adenosine Monophosphate (cAMP) in atherogenesis by modulating the function of vascular endothelium, the production of reactive oxygen species, the recruitment of circulating monocytes to the artery wall and their differentiation into macrophages-foam cells, by controlling the expression of pro-and anti-inflammatory interleukin, and regulating serum level of triglycerides and cholesterol.6 7 CAMP is also a possible target for prevention and treatment of atherosclerosis.8 The major non-synonymous SNPs of β2AR
have been recognized at nucleotides 46 (A > G) (rs1042713) and 79 (C > G) (rs1042714) causing changes in amino acid residues at position 16 (Arg > Gly) and 27 (Gln > Glu) of the amino terminus respectively of the fourth intracellular loop. An enhanced agonist-mediated receptor down-regulation for the Gly16 variant of β2AR and a resistance to down-regulation for the Glu27 variant of β2AR has been observed. It is hypothesized that due to the polymorphism in β2AR, functional alteration in the receptor function occurs which influences a certain intermediate mechanism for the predisposition of cardiovascular and cerebrovascular diseases. We conducted a meta-analysis with available published studies to precisely determine the association of β2AR with ischemic stroke in order to offer early diagnosis of the susceptible subjects.

**Methods**

**Literature search**

PubMed database was comprehensively searched from 2007 to 2014 to identify all relevant studies. The following search keywords were applied: ‘BAR’ OR ‘BAR gene variant’ OR β2AR, ‘Beta-adrenergic receptor’ OR ‘Beta adrenergic receptor polymorphism’ AND ‘cerebral infarction’ OR ‘cerebrovascular accident’ OR ‘Ischemic stroke’ OR ‘brain infarction’.

**Criteria for considering the studies**

*Inclusion criteria:* (i) Case control study studying the association between β2AR polymorphism and stroke; (ii) Studies published in English language with full text; (iii) Stroke Confirmed by MRI or CT. *Exclusion Criteria:* (i) Studies other than case control design; (ii) Studies did not report genotypic and allelic frequencies; (iii) Duplicate publication.

**Selection of relevant articles**

Two investigators (AK and MP) independently evaluated the title, abstracts and search terms for eligibility based on the predetermined selection criteria. All Discrepancies were resolved after rechecking the source papers and further discussion among the two authors.

**Data extraction**

The relevant data from each study were independently extracted by two reviewers (AK and MP) using a standardized, structured form including first author’s name, year of publication, country, genotyping method, no. of cases and controls and frequency distribution of genotype and allele.

**Results**

**Search results**

The literature search through PubMed yielded eight relevant publications. We screened through our inclusion and exclusion criteria. Two studies were excluded because they did not meet the inclusion criteria for study design (they were cohort studies). Two more studies were excluded because they studied another gene polymorphism and one study was excluded for being a pharmacogenetic study. One study did not report the genotype frequency in cases and controls. We requested for the genotype data for this study twice through email. However, we did not receive any reply from them. Therefore, this study could not be included for genotypic data meta-analysis. This study has reported the allelic frequency. Two studies reported deviation from Hardy Weinberg Equilibrium (HWE) in controls. Allelic data was reported in another study. A total three studies were included for allelic association

![Figure 1](image-url)
meta-analysis. Search results were also given in Flow Diagram (Figure 1).

Meta-analysis results

The study characteristics, which are included in the present meta-analysis, are described in Table 1. A total of three studies involving 1,642 cases and 1,673 controls, which were published from 2007 to 2014, were subjected to meta-analysis for allelic association and 518 cases and 510 controls for genotypic association. Meta-analysis results did not show a significant association between Arg16Gly polymorphism of β2AR and ischemic stroke when assuming either recessive model of inheritance (OR 1.20; 95% CI, 0.92 to 1.56) (Figure 2A) or dominant model of inheritance (OR 1.14; 95% CI, 0.68 to 1.91) (Figure 2B). The allelic association also did not show statistically significant association between Arg16Gly polymorphism of β2AR and the risk of ischemic stroke (OR 1.04; 95% CI, 0.85 to 1.28) (Figure 2C). Meta-analysis results did show a significant association between Gln27Glu polymorphism of β2AR and ischemic stroke when assuming either recessive model of inheritance (OR, 2.09; 95% CI, 1.20 to 3.64) (Figure 3A) or the dominant model of inheritance (OR, 1.47; 95% CI, 1.14 to 1.90) (Figure 3B). A significant allelic association was observed between Gln27Glu polymorphism of β2AR and ischemic stroke (OR, 1.58; 95% CI, 1.38 to 1.81) (Figure 3C).

Discussion

The present meta-analysis was conducted to determine the precise estimation of association between polymorphism of β2AR and risk of ischemic stroke. Several studies have shown an independent association of Gln27Glu polymorphism of β2AR gene number of diseases like obesity, dyslipidemia, myocardial infarction, and diabetes. Thus, Gln27Glu polymorphism of β2AR has been suggested to be an independent risk factor for cardiovascular diseases and cerebrovascular diseases. Our meta-analysis suggests a significant association of Gln27Glu polymorphism of β2AR with ischemic stroke. Our results are in agreement with earlier published study. However, two prospective cohort studies failed to show a significant association of β2AR variant with the incidence of stroke. A prospective cohort study including 25,225 women showed that the different haplotypic combination of beta receptor gene variant did not affect the incidence of ischemic stroke in women. Another cohort study reported from same population which included a total of 808 black and 4,441 white participants failed to find significant association of β2AR genotypic with risk of ischemic stroke and combined cardiovascular outcome. These two prospective cohort studies
in which significant associations were not observed, were report-
ed from the American population\textsuperscript{19,20} while the other two stud-
ies,\textsuperscript{11,12} which showed the significant genotypic association, were
reported from India and Italy. As the prevalence of genetics vari-
ants often varies among populations,\textsuperscript{19} this could explain the dis-
crepancy of association of Gln27Glu polymorphism of $\beta_2$AR
and ischemic stroke across the studies. A case control study re-
ported by Zhao et al.\textsuperscript{10} in Chinese population showed significant
allelic association between Glu allele of $\beta_2$AR and risk of isch-
emic stroke (Figure 2C). In all the published case control studies
(three),\textsuperscript{10-12} frequency of the risk allele (Glu27) was higher in
cases as compared to controls. Our meta-analysis of allelic asso-
ciation including three studies with the risk allele versus protec-
tive allele suggested significantly higher risk of ischemic stroke
with Glu27 allele. The meta-analysis for genotypic association
including two studies also suggested a significant association be-
tween Gln27Glu polymorphism and risk of ischemic stroke un-
der both dominant model and recessive model of inheritance.

Limitations of study

Some limitations exist in the present study, which may have
affected the results of meta-analysis. The haplotype analysis was
not done which plays a crucial role for association studies of com-
plex diseases. The interaction between gene and environment
and gene-gene interaction was not studied in this meta-analysis.
Controls were selected from the hospital, which may have lead to
the selection bias. Multivariate analysis for adjusting the several
confounding factors that could have an effect on results was not
performed as individual data from each study was not available.
Studies were reported from different ethnic population and devi-
Recessive model

| Study or Subgroup | Cases Glu/Glu Total | Control Glu/Glu Total | Weight | Odds Ratio M-H, Random, 95% CI | Odds Ratio M-H, Random, 95% CI |
|-------------------|---------------------|-----------------------|--------|-------------------------------|-------------------------------|
| Kumar A 2014      | 35 224              | 13 224                | 37.7%  | 3.01 [1.54, 5.85]              |                               |
| Stantione R 2007  | 123 294             | 86 288                | 62.3%  | 1.67 [1.19, 2.36]              |                               |
| Total (95% CI)    | 158                 | 99                    |        | 2.09 [1.20, 3.64]              |                               |

Heterogeneity: Tau² = 0.10; Chi² = 2.36, df = 1 (P = 0.12); I² = 58%
Test for overall effect: Z = 2.59 (P = 0.010)

Dominant model

| Study             | Cases Glu/Glu Total | Control Glu/Glu Total | Weight | Odds Ratio M-H, Fixed, 95% CI | Odds Ratio M-H, Fixed, 95% CI |
|-------------------|---------------------|-----------------------|--------|-------------------------------|-------------------------------|
| Kumar A 2014      | 126 224             | 98 224                | 44.5%  | 1.65 [1.14, 2.40]             |                               |
| Stantione R 2007  | 212 294             | 189 286               | 55.5%  | 1.33 [0.93, 1.89]             |                               |
| Total (95% CI)    | 338                 | 287                   |        | 1.47 [1.14, 1.90]             |                               |

Heterogeneity: Chi² = 0.70, df = 1 (P = 0.40); I² = 0%
Test for overall effect: Z = 2.95 (P = 0.003)

Allelic association

| Study             | Cases Glu27 Total | Control Glu27 Total | Weight | Odds Ratio M-H, Fixed, 95% CI | Odds Ratio M-H, Fixed, 95% CI |
|-------------------|-------------------|---------------------|--------|-------------------------------|-------------------------------|
| Kumar A 2014      | 161 448           | 112 448             | 21.5%  | 1.68 [1.26, 2.24]             |                               |
| Nan Zhao 2012     | 247 2248          | 162 2328            | 42.5%  | 1.65 [1.34, 2.03]             |                               |
| Stantione R 2007  | 336 500           | 275 572             | 36.0%  | 1.43 [1.13, 1.80]             |                               |
| Total (95% CI)    | 743               | 549                  |        | 1.58 [1.38, 1.81]             |                               |

Heterogeneity: Chi² = 1.03, df = 2 (P = 0.59); I² = 0%
Test for overall effect: Z = 6.57 (P = 0.00001)

Figure 3. Forest plot of odds ratio (ORs) for association of polymorphism at Gln27Glu (79C > G) position of beta-2 adrenergic receptor with ischemic stroke.

Conclusion

The present study provides preliminary evidence to support the view that carrier of Gln27Glu polymorphism of β2AR demonstrates an increase in risk of ischemic stroke on the basis of meta-analysis of case control studies. Furthermore, well designed larger prospective cohort studies are required to validate this finding and to provide a higher level of evidence. The underlying molecular causal pathways that confer susceptibility to ischemic stroke are warranted to be established.

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