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Genotyping and meta-analysis of *KIF6* Trp719Arg polymorphism in South Indian Coronary Artery Disease patients: A case–control study

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ABSTRACT

The *KIF6* 719Arg allele is an interesting genomic variant widely screened in various populations and is reported to be associated with the risk of Coronary Artery Disease (CAD) and statin treatment outcome. Recent population based clinical studies and large-scale meta-analyses pondered over the role of 719Arg variant in CAD risk and treatment response. We screened the *KIF6* Trp719Arg polymorphism (rs20455) in south Indian CAD patients in a case–control approach. A total of 1042 samples (510 CAD patients and 532 controls) were screened for the *KIF6* Trp719Arg SNP by TaqMan SNP genotyping assay, followed by meta-analysis of the genotype data of non-Europeans reports. The 719Arg risk genotype (GG) was observed in 29.6% of CAD cases and in 30.1% of controls with an odds ratio (OR) of 1.07 (95% CI: 0.76–1.50), p value = 0.709. No significant difference in the genotype frequency was observed between CAD and controls in both dominant model (AG + GG *vs* AA) and allelic model (719Arg vs 719Trp) with an OR of 1.11 (p = 0.491) and 1.03 (p = 0.767), respectively. The covariate analysis indicated that smoking & alcohol consumption increased the risk for MI among CAD patients. Meta-analysis showed that the *KIF6* 719Arg allele is not associated with CAD risk in both fixed effect (p = 0.515, OR = 1.023, 95% CI = 0.956–1.094) and random effect (p = 0.547, OR = 1.022, 95% CI = 0.953–1.096). The symmetrical shape of the Egger's funnel plots revealed that there is no publication bias. These results suggest that there is no association of *KIF6* 719Arg allele with CAD risk in South Indian population and the meta-analysis confirms the same among non-European population.

1. Introduction

Coronary Artery Disease (CAD) is the leading cause of death and disability worldwide with 80% of CAD related deaths being reported from low and middle-income countries like India. As per the Disease Control Priorities in Developing Countries report in 2006, CAD mortality rates in India between 2000 and 2030 would be about 35% and in 2016 Asian Indians would account for 40–60% of global CAD burden. In comparison to Western population, Indians are prone to early onset of CAD mainly among age group of 35 to 64, which leads to increased rate of premature-CAD related mortality (Gaziano et al., 2006; Sharma and Ganguly, 2005). Various reports have confirmed the CAD prevalence and mortality rate in South India (Gupta et al., 2012, 2013). The conventional risk factors for development of CAD are age, sex, obesity, smoking status, high blood pressure, plasma lipid concentrations, diabetes, physical inactivity and mental stress. Twin and family studies established the heritability of CAD to be in the range of 40–60%. Genome Wide Association studies (GWAS) have identified nearly 54 chromosomal loci and several single nucleotide polymorphisms (SNPs) associated with the risk of CAD (Deloukas et al., 2013; Holdt and Teupser, 2013).

The KIF6 protein is one of several molecular components that mediate intracellular transport of organelles, protein complexes, and mRNAs. A common Trp719Arg (rs20455) SNP in exon 19 of the *KIF6* gene has been identified as a potential risk factor for CAD (Bare et al., 2007; Morrison et al., 2007). The KIF6 protein belongs to the kinesin superfamily, which is involved in the intracellular transport in a microtubule and ATP-dependent manner (Miki et al., 2001). The rs20455 polymorphism replaces the nonpolar 'Trp' residue in codon 719 with a basic 'Arg' amino acid. This SNP lies near the putative cargo binding tail domain and may alter the cargo activity of KIF6 (Li et al., 2010). During acute Myocardial Infarction (MI), the endothelial progenitor cells (EPC)

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mobilize from bone marrow and initiate neovascularization through Endothelial colony-forming cells (ECFC) resulting in reduction of infarct size. The patients with homozygous *KIF6* 719Arg status were negative for ECFC cells and this phenomenon may be due to low mobilization of ECFC from bone marrow (Davani et al., 2010).

A strong association of the *KIF6* 719Arg allele towards the statin treatment outcome has been reported widely in various clinical trials (Iakoubova et al., 2008a,b). Analysis of *KIF6* SNPs from CARE, WOSCOPS and PROVE IT TIMI 22 studies identified three highly linked SNPs (rs20455, rs9462535, rs9471077) that predict differential reduction of coronary events from statin therapy (Li et al., 2011). Meta-regression analysis on the association between *KIF6* 719Arg allele, LDL cholesterol and the risk of CAD involving 144,931 participants have shown that the 'Arg' allele increases vulnerability to LDL cholesterol and thereby influences the expected clinical benefit of statin therapy (Ference et al., 2011).

Till date, many large-scale clinical studies have reported the association of *KIF6* 719Arg allele with CAD risk and benefit over statin treatment (Iakoubova et al., 2008a,b; Li et al., 2010; Morrison et al., 2007; Shiffman et al., 2008a,b). A large scale meta-analysis suggested that *KIF6* 719Arg allele is a risk factor for CAD in Caucasians and carriers are benefitted from statin therapy (Peng et al., 2012).

Although contradictory findings exist in the literature on the association of *KIF6* SNP as a CAD risk biomarker and statin treatment benefit, screening *KIF6* Trp719Arg variant is very important because CAD is the most common type of heart disease, which is the leading cause of mortality among Indians.

2. Materials and methods

2.1. Cases and controls

In the present study, 510 Coronary Artery Disease (CAD) patients and 532 controls were included. All the CAD patients clinically diagnosed by Echo/Electro Cardiogram and/or angiogram were recruited from the Intesive and Coronary Care units (ICU/CCU) of the Department of Cardiology, Madras Medical College, Chennai. Of the 510 CAD cases 483 were known for their MI status by angiogram (with or without MI). Age, sex and ethnicity matched healthy adults were used as controls. All the controls have normal electrocardiogram (ECG) records and no evidence of any systemic disease and cardiac complaints were documented. The ethical guidelines of Indian Council of Medical Research (ICMR), India (2006) for biomedical research on human participants were appropriately followed. The Institutional Ethics Committee (IEC) of Madras Medical College, Chennai has approved the study and all the participants gave informed consent prior to sample collection. A volume of 5 ml blood sample was collected from all CAD patients into EDTA coated tubes.

2.2. Genomic DNA isolation and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Genomic DNA from the blood sample was isolated by the standard Phenol:Chloroform:Isoamyl alcohol (PCI) extraction procedure (Sambrook and Russell, 2005). The quantity and quality of genomic DNA was ascertained by NanoDrop2000 UV-Spectrophotometer [Thermo Scientific, USA] and agarose gel electrophoresis. The genomic DNA was diluted to 100 ng/μl and used subsequently for PCR.

As a pilot study we designed a PCR-RFLP protocol to screen the *KIF6* Trp719Arg SNP and tested it on 100 CAD samples. The PCR was carried out in 20 μl reaction volume using 100 ng of DNA, 80 nM of each primers (forward: 5′-CTC CTT CTG GGG CCA ACA GG-3′; reverse: 5′-TCC TGC TGG ATC ATA TGG CTT ATC-3′) [Sigma oligos, India], 100 μM dNTPmix [Takara, Japan], 1.5 mM $MgCl_2$ and 0.5 U of AmpliTaq polymerase enzyme [Applied Biosystems, USA]. Thermal cycling was carried out in GeneAmp Gold 9700 [Applied Biosystems, USA] using the following conditions: 94 °C for 5 min once, 40 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C followed by a final extension of 7 min at 72 °C. The 232 bp PCR amplicons were subjected to overnight digestion with *HpyCH4*III [New England Biolabs, USA] at 37 °C and resolved by electrophoresis on 2% agarose gel. Arg/Arg homozygous genotype remained undigested 232 bp fragment; Trp/Trp homozygous genotype was cleaved into 139 and 93 bp fragments and Arg/Trp heterozygous genotype was partially digested and produces 232, 139 & 93 bp fragments.

2.3. TaqMan SNP genotyping

Based on the PCR-RFLP results a large scale allelic discrimination assay was performed [Applied Biosystems, USA]. The 5 μL PCR reaction mix comprised of 10 ng genomic DNA and 2.5 μL of 2× TaqMan Universal PCR master mix No UNG and 0.125 μL of 40× TaqMan SNP Genotyping assay mix (Probes and Primers) [Assay ID: C___3054799_10; Applied Biosystems, USA]. Absolute Quantification was performed according to the manufacturer's recommendation (2 min at 50 °C, 10 min at 95 °C followed by 15 s at 92 °C and 60 s at 60 °C for 40 cycles) and allelic discrimination with endpoint detection of fluorescence was performed in 7900HT real-time PCR system [Applied Biosystems, USA]. Non-template controls were routinely added in each plate. Genotype calls of >95% quality was scored using Sequence Detection Software (SDS v.2.3) [Applied Biosystems, USA]. The PCR-RFLP genotyped pilot samples were also screened by TaqMan SNP genotyping and the results were 100% matching between both methods.

2.4. DNA sequencing confirmation

Further validation of PCR-RFLP and TaqMan SNP genotyping data were done by sequencing 2% samples of representing each genotypes using commercial service provider (Macrogen Inc, Korea).

2.5. Statistical analysis

Descriptive statistics were presented as mean ± standard deviation [SD] for continuous measures while absolute value and percentages were used for categorical measures. Genotype and allele frequency difference between CAD and controls were estimated by Fisher exact test. Unconditional logistic regression was used to estimate odds ratios [OR] with the 95% confidence intervals [CI] adjusted for age and sex. All tests were two-tailed and a p-value of less than 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS software version 21.0 (IBM, Chicago, IL, USA). Pearson's Chi-square test with simulated p-value (based on 10,000 replicates) estimating Hardy Weinberg Equilibrium (HWE) was performed using Genetics package in R-Statistical computing software. A p-value > 0.05 represented that the genotypes are in HWE.

2.6. Meta-analysis

Meta-analysis of the *KIF6* Trp719Arg genotype from non-European populations (Bare et al., 2010; Bhanushali et al., 2011; Peng et al., 2012; Wu et al., 2012) was performed along with the data of the present study in both dominant and allelic models using fixed and random effects (Helfenstein, 2002). The meta-analysis was carried out by comprehensive meta-analysis software [Biostat, USA]. The association between the carriers of *KIF6* 719Arg (G) allele and CAD risk compared to the 719Trp (A) allele in non-European case–control study groups were examined. The pooled odds ratio (OR) estimates of both allelic (719Arg vs 719Trp) and dominant (GG + GA vs AA) models were calculated by fixed-effects and random effects model. The OR and confidence interval was graphically presented as forest plot. The publication bias was estimated by the asymmetry of the funnel plot assessed by Egger's linear regression test (Egger et al., 1997).