Meta-Analyses of KIF6 Trp719Arg in Coronary Heart Disease and Statin Therapeutic Effect

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Abstract

Aims: The goal of our study is to assess the contribution of KIF6 Trp719Arg to both the risk of CHD and the efficacy of statin therapy in CHD patients.

Methods and Results: Meta-analysis of 8 prospective studies among 77,400 Caucasians provides evidence that 719Arg increases the risk of CHD (P<0.001, HR = 1.27, 95% CI = 1.15–1.41). However, another meta-analysis of 7 case-control studies among 65,200 individuals fails to find a significant relationship between Trp719Arg and the risk of CHD (P = 0.642, OR = 1.02, 95% CI = 0.95–1.08). This suggests that the contribution of Trp719Arg to CHD varies in different ethnic groups. Additional meta-analysis also shows that statin therapy only benefit the vascular patients carry 719Arg allele (P<0.001, relative ratio (RR) = 0.60, 95% CI = 0.54–0.67).

Conclusions: Our meta-analysis demonstrates the role of Trp719Arg of KIF6 gene in the risk of CHD in Caucasians. The meta-analysis also suggests the role of this variant in statin therapeutic response in vascular diseases. Our case-control study suggests that Trp719Arg of KIF6 gene is associated with CHD in female Han Chinese through a post hoc analysis.

Introduction

Severe coronary artery disease (CAD), also called coronary heart disease (CHD), is characterized by occlusive epicardial coronary artery stenosis. CHD complications such as myocardial infarction (MI) are the leading causes of death in the United States [1] and worldwide, with over 500,000 and 7,000,000 deaths per year in the United States and worldwide, respectively [2]. The most generally accepted hypothesis is that CHD is a complex disease, resulting from the interaction of multiple genes and together with environmental factors [3]. Current genome-wide association studies (GWAS) have identified a handful of genetic variants underlying the risk of CHD. However, over 95% of the genetic variants in disease risk remains unknown and warrant further investigation [4,5].
Han Chinese case-control study sample collection

A total of 289 CHD patients and 193 non-CHD patients are collected between May of 2008 and November of 2011 from the Lihuili Hospital in Ningbo city of Zhejiang province, China. Patients are differentiated into case and control group by 719Arg genotypes, or had sufficient published data on ORs or HRs and 95% CIs, or genotype and allele frequencies to determine an estimate of relative risk [22].

Study selection

Data extraction is carried out by at least two reviewers (PP and LMX) on a standard protocol, and the consensus data are established by discussion. In the meta-analyses, the following data collection is included: name of the first author, publication year, country, ethnic population, study stage, numbers of individual in the case and the control groups and prospective studies, OR, RR, HR and 95% CI. The meta-analyses are performed by Stata software (version 11.0, Stata Corporation, College Station, TX) [23]. Publication bias is visualized by funnel plots and Egger regression plot [24].

Materials and Methods

Retrieval of published studies

To perform meta-analysis, we systematically search for available articles in English or Chinese from 2005 to 2011 in multiple electronic databases, including PubMed, Embase, China National Knowledge Infrastructure (CNKI), Wanfang Chinese Periodical Database and Web of Science. The search keywords apply the MeSH (Medical Subject Headings in the US National Library of Medicine) terms that include “coronary heart disease” or “coronary artery disease” or “myocardial infarction” combined with “KIF6” or “kinesin like protein 6” or “rs20455” or “719Arg”, “polymorphism”, “genetic association” and/or “statin” [20]. We read the full text articles to collect the relevant information. The related articles in the MEDLINE option as well as reference lists of all retrieved studies are also checked for citations of other relevant publications that are not identified initially [21]. The included studies have to satisfy the following criteria: 1) they have been published as articles or letters in peer-reviewed journals, 2) had a case-control design or a nested case-control design within a prospective study and reported their results by genotype, or had sufficient published data on ORs or HRs and 95% CIs, or genotype and allele frequencies to determine an estimate of relative risk [22].

Results

A total of 14 published articles [6,8,9,10,11,13,14,15,18,19,29,33,34,35] are eligible for the meta-analysis of Trp719Arg to the risk of CHD. Among these studies, 8 are prospective studies with 77,400 individuals who are Caucasians in Europe and North America; while the other 6 studies are case-control studies with 64,400 individuals from various ethnic groups, comprising Europeans or European descendents, Hispanics, African American and Asians. As shown in Figure 1, we have found a significant contribution of Trp719Arg to the risk of CHD. Among these studies, 8 are prospective studies with 77,400 individuals who are Caucasians in Europe and North America; while the other 6 studies are case-control studies with 64,400 individuals from various ethnic groups, comprising Europeans or European descendents, Hispanics, African American and Asians. As shown in Figure 1, we have found a significant contribution of 719Arg allele to the risk of CHD ($P<0.001$, the overall HR = 1.27, 95% CI = 1.15–1.41) and a high heterogeneity among the 8 prospective studies ($I^2=64.4\%$, $P=0.002$, $\chi^2=28.11$, $df=10$). In contrast, a low heterogeneity is observed in the meta-analysis of the six case-control studies and our study ($I^2=18.6\%$, $\chi^2=15.98$, $df=13$, $P=0.25$). The latter meta-analysis has not found a significant association between 719Arg and the risk of CHD ($P=0.642$, the overall OR = 1.02, 95% CI = 0.95–1.08) despite of the large sample size (65,200 individuals). This observation along with the mixed ethnicity in this meta-analysis, we speculate that the contribution of Trp719Arg to the risk of CHD varies in different ethnic groups. No publication bias is observed among the involved studies in the two meta-analyses (Figure 2a and 2b).

To assess the role this genetic variant in statin response, a meta-analysis has also been performed using data from eight association studies [8,10,13,15,16,17,36,37]. As shown in Figure 3, a significant reduction of the number of deaths or major cardiovas-
cular events in the 719Arg carriers is observed (P<0.001, overall RR = 0.60, 95% CI = 0.54–0.67). High heterogeneity (I^2 = 56.5%, P = 0.011, x^2 = 23, df = 10) is found among the eight studies. Random effects analysis model is used for the meta-analysis. No publication bias is observed among the involved studies by the funnel plot (Figure 2c).

To test the specific role of Trp719Arg in CHD risk in Han Chinese, we have conducted a case-control study focusing on this ethnic group. Genotypic and allelic comparison of \textit{KIF6} Trp719Arg between CHD cases and different controls are shown in Table 1. No departure of HWE is observed for Trp719Arg. The 719Arg allele frequencies are 0.503 in CHD cases, 0.482 in non-CHD controls, and 0.483 in healthy controls and 0.483 in total controls. These are similar to the allele frequency reported by HapMap in Asian populations (0.570 in HapMap-CHB and 0.477 in HapMap-JPT). No significant differences are observed in the genotype and allele distribution between CHD cases and each of three controls (Table 1) regardless the genetic model evaluated (Table S1 and S2). Furthermore, when we stratify the data analysis into each sex group with respect to allele and genotype frequencies, we haven’t found any significant association of \textit{KIF6} Trp719Arg with the risk of CHD either (Table 2). However, on post hoc analysis we observe a deviation from HWE in female CHD cases with an excess of heterozygotes (P = 0.041). Interestingly, an association test in females shows significant different distribution of 719Arg-containing genotypes between CHD cases and healthy controls under the dominant model (Table 3, P = 0.04, \chi^2 = 4.231, df = 1, OR = 2.015, 95% CI = 1.024–3.964). This finding remains true between CHD cases and total controls under the dominant model for females (P = 0.04, \chi^2 = 4.228, df = 1, OR = 1.979, 95% CI = 1.023–3.828). Note that post hoc analysis of our results generates the hypothesis that Trp719Arg tends to have a dominant effect on the risk of CHD (OR = 2.012) only in female Eastern Han Chinese (Table 3 and Table S3).

A power calculation shows that our study only has a 20.6% power to detect a relative risk of 719Arg at a significant level of 0.05, suggesting that a lack of power is likely to explain our failure to find a significant association (Table 1 and 2).

### Discussion

Several lines of evidence have shown that 719Arg is likely to increase the risk of CHD [14]. In the Cardiovascular Health Study (CHS), a population-based investigation of 3,849 white Americans has found that 719Arg is associated with the risk of cardiovascular disease [14]. Two prospective trials comprising the Cholesterol and Recurrent Events (CARE) and the West of Scotland Coronary Prevention Study (WOSCOPS) have revealed 719Arg as a CHD risk factor among a total of over 4,000 Caucasian participants [10]. Another investigation among 25,283 initially healthy Caucasian women, namely Women’s Health Study (WHS), has found that females with 719Arg allele of \textit{KIF6} have 34% higher risk of AMI and 24% higher risk of CHD [6]. Under a dominant model, our post hoc analysis reveals the contribution of 719Arg to the higher risk of CHD in females (P = 0.04, \chi^2 = 4.231, df = 1, OR = 2.015, 95% CI = 1.024–3.964). This finding remains true between CHD cases and total controls under the dominant model for females (P = 0.04, \chi^2 = 4.228, df = 1, OR = 1.979, 95% CI = 1.023–3.828). Note that post hoc analysis of our results generates the hypothesis that Trp719Arg tends to have a dominant effect on the risk of CHD (OR = 2.012) only in female Eastern Han Chinese (Table 3 and Table S3).
OR = 2.015, 95% CI = 1.024–3.964). This female-specific finding agrees with the observations in a total of 25,283 Caucasian women enrolled in the WHS [6]. Our meta-analyses among 77,400 Caucasians provides evidence that 719Arg increases the risk of CHD (P<0.001, HR = 1.27, 95% CI = 1.15–1.41). This result agrees with a previous meta-analysis that has found a 20% increase in the risk of CHD for the 719Arg carriers [12].

We also notice that there is an ethnic difference in the frequency of 719Arg allele. In our healthy controls, it is 0.483 that is similar to 0.570 in HapMap-CHB, 0.477 in HapMap-JPT, and 0.51 in the Indian population [33]. However, much lower frequency of 719Arg allele is observed in Europeans (0.358 in HapMap-CEU) and Japanese (0.386) [33]. Interestingly, the latter is much lower than 0.477 in HapMap-JPT that consists of 90 Japanese individuals. The Costa-Rican population, an admixture of three...
populations, Southern Europeans, Amerindians, and West Africans, has a minor allele frequency of 0.345 [9]. It is interesting that the 719Arg allele frequency is very high in the Sub-Saharan African population (0.908) [33]. These ethnic differences imply that further replication of 719Arg to the risk of CHD in other populations is warranted.

No significant association is found between 719Arg and the risk of CHD in the meta-analysis of 7 case-control studies among 65,200 individuals. The recruited participants in the meta-analysis are from several different ethnic populations including Europeans or European descendents, African descendents in America, East Asians, and South Asians. Among these case-control studies, a large one with a total of 17,000 cases and 39,369 controls failed to replicate the association between Trp719Arg and the risk of clinical CHD in multiple ethnic populations [34]. The contribution of 719Arg to the risk of CHD was unable to be replicated in the Costa Rican [9] and the Western Indian [33]. The conflicting results may be explained by the survival bias and drug interaction that can attenuate the case-control comparisons of Trp719Arg [11], or it could be also due to the lack of genetic effect in certain ethnic groups.

An allele-specific model of 719Arg is observed in the statin therapy of coronary events. Significantly reduced coronary events and other major vascular events have been observed in 719Arg carriers but not in non-carriers [10,12,13], although a large primary prevention trial JUPITER study with 8,781 Caucasian trial participants has found no difference in the rosuvastatin therapeutic outcomes between carriers (P=0.007, HR = 0.61, 95% CI = 0.43–0.87) and non-carriers (P=0.009, HR = 0.59, 95% CI = 0.39–0.88) of 719Arg [17]. Our meta-analysis has found that statin therapy receives significant benefit only in the carriers of 719Arg (P<0.001, overall RR = 0.60, 95% CI = 0.54–0.67).

Mechanistically, the structure of KIF6 protein consists of a conserved motor domain and a non-conserved tail domain. The conserved motor domain can propel the kinesin along microtubules in an ATP-dependent manner. The non-conserved tail domain binds to its cargoes such as membrane organelles, protein complexes, and mRNAs [10]. Trp719Arg is located in a predicted coiled-coil structure of the non-conserved tail domain. This variant causes a basic arginine residue to be replaced with a nonpolar tryptophan residue, and thus it might affect the cargo binding of the kinesin [37]. The higher risk estimates for heterozygotes could indicate that there is a functional difference between heterodimers and homodimers of the KIF6 protein, possibly because the Arg-Trp heterodimers differ from Arg-Arg and Trp-Trp homodimers in their stability or in their ability to transport cargo [16]. KIF6 may play a role in cell shape remodeling, however, the pathophysiologic role of Trp719Arg of KIF6 gene in CHD risk and coronary event reduction from statin therapy has yet to be clearly elucidated [12,14].

There are several limitations in our case-control study focused on Han Chinese. Firstly, sample size in our study is comparatively small and it has only 20.6% power to detect the association of Trp719Arg with CHD at a significant level of 0.05. In addition, only non-fatal CHD cases are recruited in the present study. The 719Arg allele is hypothesized to increase the risk of incident fatal

Table 1. Genotype and allele of Trp719Arg in case and control groups.

| Group(rs20455) | Genotype | Allele | OR(95%CI) | HWE |
|---------------|----------|--------|-----------|-----|
| CHD cases     | 719Arg/  | 719Arg | 0.642     |
| non-CHD controls | 197/199 | 69     | 0.570     |
| Healthy controls | 74/170 | 85     | 0.769     |
| Total controls | 121/262 | 139    | 0.679     |

Table 2. Genotype and allele of Trp719Arg in male and female subgroups.

| Group(rs20455) | Total Genotype | Allele | OR(95%CI) | HWE |
|---------------|----------------|--------|-----------|-----|
| Male          | CHD cases 208  | 719Arg | 1.159     | 0.684 |
| non-CHD controls | 96  | 719Arg | 0.824–1.631 | 0.684 |
| Healthy controls | 85/170 | 0.760 | 0.740–1.511 | 0.387 |
| Total controls | 182/38 | 93    | 0.530     | 0.769 |

Female

| Group(rs20455) | Total Genotype | Allele | OR(95%CI) | HWE |
|---------------|----------------|--------|-----------|-----|
| CHD cases 80  | 719Arg | 0.838–1.472 | 0.769 |
| non-CHD controls | 96  | 719Arg | 0.847–1.690 | 0.914 |
| Healthy controls | 244/57 | 0.861–1.760 | 1.000 |
| Total controls | 340/83 | 0.847–1.690 | 0.914 |

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CHD more than the risk of incident nonfatal CHD [34], the exclusion of fatal CHD cases might attenuate the detection of a significant association between the SNP and CHD. Secondly, the genotype distribution of Trp719Arg in female CHD patients has an excess of heterozygotes (HWE test: \( P = 0.041 \)). This phenomenon may be due to the improperly pair-wised design, the small population size or that the female CHD patients do not obey HWE. Therefore, we need to take caution with the significant association results in females under the dominant model. Thirdly, the information of statin usage is not available in our samples. Our genetic study might underestimate the risk of this genetic variation for a potential bias by the statin therapy. Moreover, CHD is strongly correlated with smoking status, LDL-C/HDL-C, history of hypertension or diabetes, and body mass index. Our genetic testing has not been adjusted with those risk factors. Fourthly, gender has been shown to be an important modifier of cardiovascular disease risk [38]. Our previous study [39] has found a female-dependent association between a \textit{PDGFD} gene variation (rs974819) and CHD risk. In the present meta-analyses, we are unable to make a gender adjustment or stratification for all the involved studies. Finally, our study isn’t designed to test whether \textit{KIF6} variants are associated with the statin therapy outcome.

In conclusion, our meta-analyses over 143,000 individuals have shown that 719Arg is a risk factor of CHD in Caucasians but its effects on CHD may vary in other ethnic populations. Meta-analysis also indicated that statin therapy may selectively benefit patients with \textit{KIF6} 719Arg allele. Despite failing to find a significant relationship between Trp719Arg and CHD in Eastern Han Chinese for the study population as a whole, post hoc analysis reveals a female-specific association. This gender-specific finding should be investigated future studies.

### Supporting Information

#### Table S1
Association of Trp719Arg with CHD in the dominant model.

| Genotype    | Total  | CHD cases | Healthy controls | non-CHD controls | Total controls |
|-------------|--------|-----------|------------------|------------------|---------------|
| 719Arg/719Arg+719Trp | 151    | 208       | 85               | 96               | 244           |
| 719Arg/719Trp    | 57     | 30        | 64               | 72               | 131           |
| 719Trp/719Trp   | 198    | 182       | 24               | 64               | 340           |

\( \chi^2 \) 0.019

| Genotype       | Male | Female |
|----------------|------|--------|
| 719Arg/719Arg+719Trp | 30.431 | 12.541 |
| 719Arg/719Trp    | 0.019 | 0.006  |
| 719Trp/719Trp   | 0.019 | 0.006  |

\( P(d.f. = 1) \) 0.041

OR 95% CI

**Table S2**
Association of Trp719Arg with CHD in the recessive model.

| Genotype    | Total  | CHD cases | Healthy controls | non-CHD controls | Total controls |
|-------------|--------|-----------|------------------|------------------|---------------|
| 719Arg/719Arg+719Trp | 208    | 151       | 85               | 96               | 244           |
| 719Arg/719Trp    | 57     | 30        | 64               | 72               | 131           |
| 719Trp/719Trp   | 198    | 182       | 24               | 64               | 340           |

\( \chi^2 \) 0.041

| Genotype       | Male | Female |
|----------------|------|--------|
| 719Arg/719Arg+719Trp | 30.431 | 12.541 |
| 719Arg/719Trp    | 0.019 | 0.006  |
| 719Trp/719Trp   | 0.019 | 0.006  |

\( P(d.f. = 1) \) 0.041

OR 95% CI

#### Table S3
Association of Trp719Arg with gender risk in the recessive model.

| Genotype       | Male | Female |
|----------------|------|--------|
| 719Arg/719Arg+719Trp | 30.431 | 12.541 |
| 719Arg/719Trp    | 0.019 | 0.006  |
| 719Trp/719Trp   | 0.019 | 0.006  |

\( P(d.f. = 1) \) 0.041

**Flow Diagram S1** PRISMA flow diagram.

**Checklist S1** PRISMA checklist.

## Author Contributions
Conceived and designed the experiments: SD JL JZ. Performed the experiments: PP LX YH. Analyzed the data: CD LZ MY. Contributed reagents/materials/analysis tools: YB XY XH. Wrote the paper: PP SD RSH JL JZ.

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