Association of 25 bp Deletion in MYBPC3 Gene with Left Ventricle Dysfunction in Coronary Artery Disease Patients

Anshika Srivastava¹, Naveen Garg², Tulika Mittal¹, Roopali Khanna², Shipra Gupta³, Prahlad Kishore Seth³, Balraj Mittal¹*

¹Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences Lucknow (UP), Lucknow, India, ²Department of Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences Lucknow (UP), Lucknow, India, ³Bioinformatic Center, Biotech Park Lucknow (UP), Lucknow, India

Abstract

Rationale: Mutations in MYBPC3 encoding cardiac myosin binding protein C are common genetic cause of hereditary cardiomyopathies. An intronic 25-bp deletion in MYBPC3 at 3’ region is associated with dilated (DCM) and hypertrophic (HCM) cardiomyopathies in Southeast Asia. However, the frequency of MYBPC3 25 bp deletion and associated clinical presentation has not been established in an unrelated cohort of left ventricular dysfunction (LVD) secondary to coronary artery disease (CAD) patients.

Objective: We sought to determine the role of MYBPC3 25 bp polymorphism on LVD in two cohorts of CAD patients.

Methods and Results: The study included 265 consecutive patients with angiographically confirmed CAD and 220 controls. MYBPC3 25 bp polymorphism was determined by polymerase chain reaction. Our results showed that carrier status of MYBPC3 25 bp deletion was associated with significant compromised left ventricle ejection fraction (LVEF ≤ 45) in CAD patients (p value = <0.001; OR = 4.49). To validate our results, we performed a replication study in additional 140 cases with similar clinical characteristics and results again confirmed consistent findings (p = 0.029; OR = 3.3). Also, presence of the gene deletion did not have significant association in CAD patients with preserved ejection fraction (LVEF > 45) (p value = 0.1; OR = 2.3).

Conclusion: The frequency of MYBPC3 DW genotype and D allele was associated with compromised LVEF implying that genetic variants of MYBPC3 encoding mutant structural sarcomere protein could increase susceptibility to left ventricular dysfunction. Therefore, 25 bp deletion in MYBPC3 may represent a genetic marker for cardiac failure in CAD patients from Southeast Asia.

Introduction

Coronary artery disease (CAD) is defined as atherosclerotic blockage[1] of arteries, supplying oxygen rich blood to the myocardium (heart muscle), is the most common cause of fatality, disability and economic loss, particularly in industrialized countries. While the signs and symptoms of coronary artery disease are noted in advanced state of disease, most individuals remain asymptomatic for decades. Furthermore, major complication that some CAD patients face over time is the development of Left Ventricle dysfunction (LVD). The LVD usually leads to heart failure, arrhythmias and other cardiovascular complications. Besides poorly developed collaterals, extensive atherosclerotic disease and large myocardial infarction are traditional predictors of LVD. However, in clinical practice, it is observed that some CAD patients who do not have such complications but still develop LVD, whereas others with well-defined predictors do not develop LV dysfunction, which is quite puzzling.

Earlier genetic studies have correlated LVD with dilated cardiomyopathy (DCM).[2] Mutations in several genes including those encoding sarcomeric proteins such as myosin heavy polypeptide (MYH7), cardiac troponin I (TNNI3),[3] cardiac myosin binding protein C (MYBPC3)[4] have been identified in DCM.[5,6,7]

Myosin-binding protein C (MyBP-C) is a key constituent of cardiac muscle thick filaments, which by interacting with myosin,[8,9,10] titin[11] and actin[12] contributes to the structural integrity of the sarcomere and regulates cardiac contractility in response to adrenergic stimulation.[13,14] Mutations in MYBPC3 result in disorganized sarcomeric structure, thus MYBPC3 has emerged as an important gene for increased risk of heart failure through cardiomyopathies (DCM and HCM).[13,15,16,17,18]

A common polymorphic intronic deletion of 25-base- pair in MYBPC3 at the 3’ region of the gene[19] has been recently reported to be associated with DCM and HCM in populations of Southeast Asia.[20] The deletion in intron 32 causes skipping of
the downstream exon 33 and results in incorporation of mis-sense amino acids at C-terminal region of the protein.[19,20] The 25-bp deletion is present in 2-6% of individuals in Indian populations and high incidence of cardiac diseases has been partly attributed to the existence of this polymorphism. Two community based studies carried out in UK reported significant ethnic differences in left ventricular systolic function and underlying CAD between South Asians and European white subjects.[21,22] Moreover, a close relation between LV ejection fraction (LVEF) and mortality has also been demonstrated. Therefore, we carried out the present study to assess if the common polymorphism in MYBPC3 has any role in LV dysfunction. To the best of our knowledge, it is the first study to report the association of 25 bp polymorphism of MYBPC3 with low left ventricular ejection fraction in CAD patients.

Results

1.1 Patient characteristics

Demographic and lipid profile of healthy subjects is shown in Table 1. Clinical characteristics of CAD patients -Primary, replication and combined cohorts of study are shown in Table 2. In combined cohort, there was no significant difference in the mean age of CAD patients and controls. The male/female ratio was comparable in both CAD cases as well as in controls. Evaluation of the defined risk factors in the cohort showed that 45.9% patients were hypertensive and 28.4% patients were diabetic. Moreover, 26.4% patients were associated with smoking. Patients with stable angina were 31.4% and unstable angina/ Non ST Segment Elevation Myocardial Infarction (STEMI) formed 20.5% of the clinical syndrome. ST Segment Elevation Myocardial Infarction (STEMI) patients with anterior wall myocardial infarction (AWMI) and inferior wall myocardial infarction (IWMI) were 28.1% and 19.5% respectively. Only two patients were found to be affected with lateral wall myocardial infarction (LWMI) (0.5%).

The angiographic profile categorized patients with single vessel disease (SVD), double vessel disease (DVD), and triple vessel disease (TVD) as 68.9%, 24.7% and 6.4% respectively. The mean ejection fraction was 50.0±11.18. The kurtosis and skewness for some patients with LV dysfunction. Therefore, we selected the cut off, level of significance increased but we would have missed considerations. We considered the cut off for LV dysfunction to 50% and at 40%, 50% also. The level of significance decreased when we considered the cut off for LV dysfunction to 50% and at 40% cut off, level of significance increased but we would have missed some patients with LV dysfunction. Therefore, we selected the cut off to 45% to include definite patients with LV dysfunction.

1.2 Influence of MYBPC3 25 bp Deletion on CAD and its clinical characteristics

Distributions of genotypes of MYBPC3 gene deletion in controls were in accordance with Hardy-Weinberg equilibrium (p = 0.98). Considering the low allelic frequency allele, we also applied fisher exact test for conditional probability of heterozygous variants which was found to be p = 0.97.

After this we compared the genotype and allele frequency of MYBPC3 25 bp polymorphism between CAD patients (both stages) and healthy controls. Our results suggested significant association of MYBPC3 gene deletion with CAD [Table 3 (Primary; Replication); DW genotype p value = 0.03; <0.01: D allele p value = 0.03; <0.01]. The frequency of MYBPC3 gene deletion in total (Primary and replication cohorts) CAD patients was also significantly higher (p value = 0.003; Table 4) than healthy controls. As there were no homozygous DD genotyped individuals in both cases and controls, the heterozygous carrier (DW) itself associated with CAD.

Further, we compared the distribution of wild type (WW) genotype of MYBPC3 (no deletion) and DW (Carrier of one deleted allele) with clinical characteristics of CAD. There were no significant differences between frequencies of WW and DW with reference to clinical characteristics of CAD, except with compromised LVEF (Table 5: 23.5% vs. 52.9%; p value = 0.004 OR = 4.32 (1.58 – 11.82). Further, a borderline significance (p value <0.05) was also observed in case of triple vessel disease. When CAD patients were divided on the basis of associated phenotypes like diabetes mellitus, hypertension or risk factors like smoking status of the subjects carrying wild type and deleted allele, the MYBPC3 gene deletion did not modulate the risk of CAD due to these phenotypes or factors.

1.3 Influence of MYBPC3 25 bp Deletion on CAD patients with reduced and preserved ejection fraction

In case-only analysis, first we applied linear model on all CAD patients and found 25 bp gene deletion to be significantly associated with compromised LVEF (p value = 0.002 [B = 6.3] Table 6). Then we segregated CAD patients on the basis of compromised (<45%) and preserved (≥45%) left ventricular ejection fraction and compared with their status of MYBPC3 gene polymorphism. We found that higher percentage of CAD patients carrying heterogeneous DW genotype had compromised ejection fraction as compared to the patients with preserved ejection fraction. This difference was statistically significant (p value = 0.004 OR = 4.32 (1.58 – 11.82) Table 7). In addition, we performed a replication study on 140 more CAD patients and compared with primary cohort. The effect was consistently stronger in the CAD patients with reduced ejection fraction than CAD patients with preserved ejection fraction subgroups (p value = 0.029 OR = 6.208 (3.3 (1.1–10.1))]. Furthermore, we calculated the z and p values to look into the overall significance and the results again confirmed the association of 25 bp gene deletion with compromised ejection fraction (Table 7).

We looked for the association of MYBPC3 gene deletion on LVD by changing the cut off values for LVEF to <40% and <50% also. The level of significance decreased when we considered the cut off for LV dysfunction to 50% and at 40% cut off, level of significance increased but we would have missed some patients with LV dysfunction. Therefore, we selected the cut off to 45% to include definite patients with LV dysfunction.

1.4 Frequency Distribution of MYBPC3 25 bp deletion polymorphism in CAD patients with preserved and compromised LVEF and Controls

To further evaluate whether MYBPC3 gene deletion is associated with CAD or LVEF, we analyzed the status of gene deletion in CAD patients.
patients with preserved and compromised LVEF. Of the total 405 CAD patients, 105 CAD patients had ejection fraction ≤45 while remaining 300 had preserved LVEF (>45). On comparing the genotype frequency distribution of these two groups of patients with that of healthy controls it was observed that only carriers of DW genotype were associated with LVEF [DW genotype p value = <0.001 OR = 11.6 (3.9–34.2); D allele p value = <0.001 [9.8 (3.5–27.6)] while no risk of DW genotype was observed in CAD patients with preserved ejection fraction [Table 8; DW genotype p value = 0.09; OR = 2.3 (0.8–6.6); D allele (p = 0.1;OR = 2.3

| Table 2. Clinical Characteristics of CAD patients. |
|-------------------------------|-----------------|-----------------|
| **CLINICAL CHARACTERISTICS**  | **Primary Stage** | **Replication Stage** | **Combined** |
| Total Subjects                | 265             | 140             | 405          |
| *Age – yr*                    | 56.52±10.20     | 54.36±8.179     | 55.78±9.59   |
| Male sex                      | 225 (84.9)      | 123 (87.9)      | 348 (85.9)   |
| **RISK FACTORS**             |                 |                 |              |
| Hypertension                  | 119 (44.9)      | 67 (47.9)       | 186 (45.9)   |
| Diabetes                      | 77 (29.1)       | 38 (27.1)       | 115 (28.4)   |
| Smoking                       | 67 (25.3)       | 40 (28.6)       | 107 (26.4)   |
| Lipid Levels                  |                 |                 |              |
| a) High Density Lipoprotein (mg/dl) | 32.3±7.91      | 31.8±5.86      | 32.19±7.45   |
| b) Low Density Lipoprotein (mg/dl) | 100.1±24.33    | 103.7±22.90    | 100.97±24.40 |
| c) Triglycerides (mg/dl)      | 155.6±69.19     | 125.4±35.54    | 149.11±62.25 |
| d) Total cholesterol (mg/dl)  | 170.3±21.98     | 141.7±39.61    | 163.7±30.53  |
| **CLINICAL SYNDROME**         |                 |                 |              |
| Stable angina                 | 82 (30.9)       | 45 (32.1)       | 127 (31.4)   |
| Unstable angina/Non ST Elevation Myocardial Infarction (NSTEMI) | 59 (22.3)       | 24 (17.1)       | 83 (20.5)   |
| ST Elevation Myocardial Infarction (STEMI) | 124 (46.8)     | 71 (50.7)       | 195 (48.1)   |
| Anterior wall myocardial infarction (AWMI) | 72 (27.2)       | 42 (30)         | 114 (28.1)   |
| Inferior wall myocardial infarction (IWMI) | 51 (19.2)       | 28 (20)         | 79 (19.5)   |
| Lateral wall myocardial infarction (LWMI) | 1 (0.4)        | 1 (0.7)         | 2 (0.5)     |
| **ANGIOGRAPHIC PROFILE**      |                 |                 |              |
| Single vessel disease (SVD)   | 165 (62.3)      | 114 (81.4)      | 279 (68.9)   |
| Double vessel disease (DVD)   | 77 (29.1)       | 23 (16.4)       | 100 (24.7)   |
| Triple vessel disease (TVD)   | 23 (8.7)        | 3 (2.1)         | 26 (6.4)     |
| **LEFT VENTRICULAR FUNCTION** |                 |                 |              |
| *Mean Left Ventricle Ejection Fraction (LVEF) >45 | 50.7±11.65      | 48.5±10.2       | 50.0±11.18   |
| ≤45                           | 206 (77.7)      | 94 (67.1)       | 300 (74.1)   |

*Values are mean ± SD.
doi:10.1371/journal.pone.0024123.t002

| Table 3. Distributions for 25 bp deletion in MYBPC3 in CAD patients and Healthy controls. |
|--------------------------------------|-----------------|-----------------|
| **Genotypes/Alele**                  | **HC (220)**    | **CADb (265)**  | **CADa (140)** |
| WW                                   | 215 (97.7)      | 248 (93.6)      | 123 (87.9)     |
| DW                                    | 5 (2.3)         | 17 (6.4)        | 17 (12.1)      |
| W                                    | 435 (98.9)      | 513 (96.8)      | 263 (93.9)     |
| D                                    | 5 (1.1)         | 17 (3.2)        | 17 (6.1)       |
| **p-value**                          | 1(reference)    | 1(reference)    | 1(reference)   |
| **OR (95% CI)**                      | 1(reference)    | 1(reference)    | 1(reference)   |
| **p-value**                          | 0.03 ; 3.0 (1.1–8.5) | <0.01 ; 6.8 (2.3–19.3) |
| **OR (95% CI)**                      | 0.03 ; 3.0 (1.0–8.2) | <0.01 ; 6.3 (2.2–17.5) |

CAD-Coronary artery disease, HC-Healthy control, OR-Odds Ratio, CI-Confidence interval.
D, allele with deletion; W, wild-type allele;
*CAD patients in Primary cohort; *CAD patients in Replication cohort.
a = represents the p value for the comparison of carriers (D,W + D,D) and non-carriers (W,W) in CAD patients (primary cohort) and HC.
b = represents the p value for the comparison of carriers (D,W + D,D) and non-carriers (W,W) in CAD patients (replication cohort) and HC.
Significant values are shown in BOLD.
doi:10.1371/journal.pone.0024123.t003
between these two groups. It suggests that MYBPC3 25 bp deletion does not show any association with CAD patients having preserved LVEF.

Generally, CAD patients with ST elevation MI are more prone to develop LV dysfunction which was also evident in the present study. Therefore, we looked for any significant interaction of STEMI with MYBPC3 deletion status and low Ejection fraction but found borderline significance (p value = 0.04) whereas in case of CAD patients with low LVEF and no history of STEMI, the association with MYBPC3 gene deletion was highly significant (p value = 0.001). It suggests that MYBPC3 gene deletion is influencing development of LVD in CAD patients without history of STEMI.

Discussion

The main finding of the present study suggests close relationship between the MYBPC3 25 bp polymorphic deletion, a common MYBPC3 variant, and significantly higher risk of severe left ventricular dysfunction (LVD) in CAD patients.

Notably, LVD is a complex condition that emerges as a common pathway for a host of cardiac disorders. The LVD results from the changes in the structure and function of heart muscle as well as changes in collagen and other cardiac proteins.

Previously, a number of mutations in the genes for sarcomeric proteins have been found to be associated with the risk of DCM [7] and some of them are shown to cause sarcomeric disorganization, which is believed as one of the mechanisms by which pathogenesis is triggered in the heart.[8], [23] In addition to genetic predisposition, viral infection, molecular mimicry, and oxidative stress are potential contributing factors to dilated cardiomyopathy. These factors are believed to be present before DCM and lead to severe LV dysfunction. Individuals, who are genetically predisposed, when exposed to any of these contributory factors, may develop LVD.

Similarly, we believe that CAD patients carrying the 25 bp polymorphic deletion in MYBPC3 when triggered by severe ischemic insult to the cardiac muscle cell (in place of viral infection or other contributory factors for the development of DCM) may be more likely to develop severe LV dysfunction in comparison to CAD patients who are not carrying this mutation.

A large number of studies during last ten years have clearly confirmed that cardiac myosin binding protein C plays pivotal role in the genesis of cardiac muscle disorders.[20] The 25 bp intronic deletion causes skipping of exon 33 and incorporation of mis-sense of amino acids at the C-terminal.[19,20] The mutated protein has been shown to incorporate in the myofibrils[20] and may cause break down of sarcomere.

Several mechanisms have been proposed to explain pathogenesis of cardiac muscle due to truncated and mis-sense myosin binding protein C. These include poison peptides, haplo-insufficiency caused by nonsense-mediated mRNA decay and impairment of ubiquitin-proteasome system (UPS). The missense MYBPC3 mutations have been reported to destabilize its proteins through UPS and it may contribute to cardiac dysfunction through impairment of the ubiquitin-proteasome system.[24] It has been shown that mutant cMyBP-C protein is preferentially degraded by the ubiquitin proteasome system (UPS), which, in turn, may competitively inhibit breakdown of other UPS substrates. Also, taking into consideration the decline in function of the UPS with age and oxidative stress [25,26] the altered protein may simply accumulate, disrupt the cellular homeostasis and initiate LVD.

A recent study from UK explored any ethnicity-related differences in left ventricular function, structure and geometry in a population based study of UK Indian Asian and European Whites [22] and they observed significant differences in cardiac structure and sensitive parameters of LV function. Earlier, another UK based study did not observe differences in incidence of LVSD but reported that majority of patients originating from Indian subcontinent had CAD as underlying cause of LVSD.[21] As MYBPC3 25 bp deletion is mainly confined to Southeast Asia,[20] it is possible that some of the differences in two groups (Southeast Asians and Whites) might be associated with the common MYBPC3 25 bp deletion.

So far, the influence of 25 bp MYBPC3 intronic deletion on LVD in patients with DCM was known.[27] However, our results show that CAD patients with MYBPC3 25 bp deletion also become more prone to LVD. Presently there are no such methods or tests available to pinpoint CAD patients who are at higher risk of developing severe cardiac disorder in later stages. The late onset symptoms and influence of secondary risk factors may cause a lasting threat to the carriers. However, the gene-based insights into pathophysiology may allow more subtle clinical manifestations to be identified and additional phenotypes associated with the variant

Table 4. Overall genotypic and allelic frequency of MYBPC3 in total CAD patients.

| Genotypes/Allele | HC (220) N (%) | CAD (405) N (%) | p–value OR (95% CI) |
|------------------|----------------|----------------|---------------------|
| WW              | 215 (97.7)     | 371 (91.6)     | 1 (reference)       |
| DW              | 5 (2.3)        | 34 (8.4)       | 0.003 ; 4.3 (1.6–11.2) |
| W               | 435 (98.9)     | 776 (95.8)     | 1 (reference)       |
| D               | 5 (1.1)        | 34 (4.2)       | 0.003 ; 4.1 (1.5–10.6) |

Significant values are shown in BOLD; doi:10.1371/journal.pone.0024123.t004

Table 5. Effect of MYBPC3 deletion on clinical characteristics in total CAD patients.

| Variable                        | MYBPC3 (WW) | MYBPC3 (DW) | P value |
|---------------------------------|-------------|-------------|---------|
| Stable Angina                   | 113 (30.5)  | 14 (41.2)   | NS      |
| Unstable Angina                 | 54 (14.6)   | 3 (8.8)     | NS      |
| Anterior wall Myocardial Infarction | 103 (27.8)  | 9 (26.5)    | NS      |
| Inferior wall Myocardial Infarction | 71 (19.1)   | 8 (23.5)    | NS      |
| Single Vessel Disease (SVD)     | 257 (69.3)  | 22 (64.7)   | NS      |
| Double Vessel Disease (DVD)     | 93 (25.1)   | 7 (20.6)    | NS      |
| Triple Vessel Disease (TVD)     | 21 (5.7)    | 5 (14.7)    | <0.05   |
| LVEF <45                        | 87 (23.5)   | 18 (52.9)   | <0.01   |

Significant values are shown in BOLD; NS = Non-significant. doi:10.1371/journal.pone.0024123.t005

Table 6. Parameter Estimate Applying General Linear Model.

| No of Subjects | WW | DW | P-value |
|----------------|-----|----|---------|
| LVEF           | 405 | 371| <0.002  |
| 50.0±11.1      | 50.5±10.92 | 44.26±12.5 | <0.002  |

Significant value is shown in BOLD. doi:10.1371/journal.pone.0024123.t006
to be investigated. Furthermore, genotyping could be used for the identification of persons at risk of severe LV dysfunction and heart failure among various Indian populations and migrants of Indian origin. Thus, could be accompanied by appropriate medications and advice for a lower-risk lifestyle.

Study limitations
The sample size in our present study is limited, therefore it will require confirmation in larger cohorts. Because this is an association study, we cannot rule out the presence of possible linkage disequilibrium with other neighboring genes that might explain the significant association with atherosclerotic phenotype or adverse prognosis.

Moreover, it is a retrospective study, so it must also be carried out prospectively before clinical application. Notably, the study was conducted in patients with severe CAD undergoing angioplasty and it is not certain whether the patients had LVD before ischemic insult or the left ventricular dysfunction developed later.

Conclusion
Our study suggests that MYBPC3 25 bp polymorphic deletion is associated in CAD patients with compromised left ventricular ejection fraction. It implies that a common genetic variant of MYBPC3 encoding structural sarcomere protein could increase susceptibility to left ventricular dysfunction, particularly following an ischemic event. These findings add new evidence to existing data on the linkage between MYBPC3 function and outcome in patients in later stages in the cardiac failure. Therefore, the MYBPC3 25 bp deletion may be explored as biomarker for the development of severe LV dysfunction in CAD patients of persons originating from Indian subcontinent.

Materials and Methods

Ethics Statement
The institutional ethical committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS) approved the study protocol, and the authors followed the norms of World’s
Association Declaration of Helsinki. All the participants were provided with written informed consent for the study.

2.1 Study Population

The present study was carried out in two stages, primary and replication stages. In the primary stage, we studied 265 CAD patients recruited from July 2008 to December 2009. In the replication stage, further 140 cases were enrolled. The diagnostic parameters used in the primary stage were also applied to the replication stage.

Both the primary and replication cohorts had significant coronary artery disease, (diagnosis, confirmed by coronary angiography and further all these subjects underwent coronary angioplasty) recruited from the Department of Cardiology of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India. The detailed clinical history of CAD patients was based on hospital investigations including angiography. Angiographically identified stenoses >70% in the major coronary vessels at the time of the study were used to classify patients as having single-vessel, double-vessel, or triple-vessel disease.

The control (non-CAD) population consisted of 220 subjects (173 males and 47 females) (mean age years 54.94±8.12,) with no clinical evidence of CAD or LV dysfunction (by echocardiography) and also without positive family history of CAD or myocardial infarction (MI). In addition, the inclusion criteria for controls were absence of prior history of high systolic blood pressure, abnormal lipid profile, diabetes mellitus and obesity. Both patient and control were frequency-matched to age, gender and ethnicity. After obtaining informed consent, all the individuals were personally interviewed for information on food habits, occupation and tobacco usage.

2.2 Data collection

The clinical data was obtained by reviewing the patient’s medical records. Left ventricle ejection fraction (LVEF) was calculated quantitatively by echocardiography, just before angiography procedure, using the Simpson’s method.[28] Echocardiography was repeated in 10% of patients and results were totally concordant. Hypertension was defined as systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg or patients using antihypertensive drugs. Smoking was classified as smokers (ex-smoker and current smokers) and non-smokers. Similarly, diabetes mellitus was defined as patients with fasting plasma glucose >6.9 mmol/L or patients using anti-diabetic medication. All laboratory parameters, as stated in the medical record, were determined in fasting patients. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured by standard enzymatic methods. LDL cholesterol concentrations were calculated using the Friedewald’s formula.[29]

2.3 Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method. [30] The polymorphism was genotyped using the polymerase chain reaction method. As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. Laboratory personnel were blinded to the case–control status of the subjects and genotyping was done using a pair of primers [20] flanking the 25 bp deletion region in MYBPC3. PCR products (Wild Type [WW]: 403 bp and mutant [DW]: 378 bp) were analysed on 6% polyacrylamide gel. Ten percent of samples from patients and controls were sequenced to evaluate the quality of genotyping, which showed 100% concordance.

2.4 Statistical analysis

Descriptive statistics were presented as mean and standard deviation (SD) for continuous measures while absolute value and percentages were used for categorical measures. The chi-square goodness of fit test was used for any deviation from Hardy Weinberg Equilibrium in controls and Fisher exact test was applied as given by Emigh [31] Differences in genotype and allele frequencies between study groups were estimated by chi-square test. The ORs were adjusted for confounding factors such as age and gender. In addition, the association between MYBPC3 25 bp deletion and significant risk factors of CAD were analyzed using binary logistic regression. Student t test was applied to find the significance for plasma cholesterol. Differences between groups stratified on the basis of genotypes were assessed using z proportional test. Overall we performed meta analysis by Stouffers method to combine the results of two cohorts (primary and replication) to calculate the overall z and p values. A two-tailed p-value of less than 0.05 was considered a statistical significant result. All statistical analyses were performed using SPSS software version 16.0 (SPSS, Chicago, IL, USA).

Author Contributions

Conceived and designed the experiments: BM NG AS. Performed the experiments: AS. Analyzed the data: AS SG. Contributed reagents/materials/analysis tools: AS RK PKS TM. Wrote the paper: AS BM NG.

References

1. Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801–809.
2. Takai E, Akita H, Kanazawa K, Suga N, Terashima M, et al. (2002) Association between aldosterone synthase (CYP11B2) gene polymorphism and left ventricular volume in patients with dilated cardiomyopathy. Heart 80: 649–650.
3. Murphy RT, Mogensen J, Shaw A, Kubo T, Hughes S, et al. (2004) Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. Lancet 363: 371–372.
4. Simonson TN, Zhang Y, Hoff CD, Xing J, Watkins WS, et al. (2010) Limited distribution of a cardiomyopathy-associated variant in India Ann Hum Genet 74: 104–108.
5. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, et al. (2004) SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arthrythmia. Circulation 110: 2163–2167.
6. Biengengerle M, Olsen TM, Selivanov VA, Kasthmann EC, O’Cochlin F, et al. (2004) ABC3 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. Nat Genet 36: 382–387.
7. Kimura A (2010) Molecular basis of hereditary cardiomyopathy: abnormalities in calcium sensitivity, stretch response, stress response and beyond. J Hum Genet 55: 81–90.
8. Flavigny J, Robert P, Camelin JC, Schwartz K, Carrier L, et al. (2003) Biomolecular interactions between human recombinant beta-MHC and cMyBP-Cs implicated in familial hypertrophic cardiomyopathy. Cardiovasc Res 60: 385–396.
9. Flavigny J, Seuchet M, Schillén B, Berzelius-Bertrand I, Hainque B, et al. (1999) COOH-terminal truncated cardiac myosin-binding protein C mutants resulting from familial hypertrophic cardiomyopathy mutations exhibit altered expression and/or incorporation in fetal rat cardiomyocytes. J Mol Biol 294: 443–456.
10. Gruen M, Gaetel M (1999) Mutations in beta-myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin-binding protein-C. J Mol Biol 286(3): 933–949.
11. Freiburg A, Gaetel M (1996) A molecular map of the interactions between titin and myosin-binding protein C: Implications for sarcomeric assembly in familial hypertrophic cardiomyopathy. Eur J Biochem 235(1–2): 317–326.
12. Squire JM, Luther PK, Knapp C (2003) Structural evidence for the interaction of C-protein (MyBP-C) with actin and sequence identification of a possible actin-binding domain. J Mol Biol 331: 713–724.
13. Sadayappan S, Klevtsoy R, Lorenz JN, Sargent M, Molkenstj JD et al (2006) Cardiac myosin binding protein-C phosphorylation is cardioprotective. Proc Natl Acad Sci U S A 45: 16918–16923.
14. McClellan G, Kulikovskaya I, Winegrad S (2001) Changes in cardiac
contractility related to calcium-mediated changes in phosphorylation
of myosin-binding protein C. Biophys J 81: 1083–1092.
15. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ (2005)
Sarcomeric genotyping in hypertrophic cardiomyopathy. Mayo Clin Proc
80: 463–469.
16. Niimura H, Patton KK, McKenna WJ, Soultis J, Maron BJ, et al. (2002)
Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the
elderly. Circulation 105: 446–451.
17. Hitomi N, Kubo T, Kitaoaka H, Hirosa T, Hamada T, et al. (2010) A frameshift
deletion mutation in the cardiac myosin-binding protein C gene associated with
dilated phase of hypertrophic cardiomyopathy and dilated cardiomyopathy.
J Cardiol.
18. Tanjore RR, Rangaraju A, Kerkar PG, Calambur N, Nallari P (2008) MYBPC3
gene variations in hypertrophic cardiomyopathy patients in India. Can J Cardiol
24: 127–130.
19. Waldmüller S, Sukhiyel S, Saadi AV, Seliguen C, Rakesh PG, et al. (2003)
Novel deletions in MYH7 and MYBPC3 identified in Indian families with
familial hypertrophic cardiomyopathy. J Mol Cell Cardiol 35: 623–636.
20. Dhandapani PS, Sadayappan S, Yue X, Powell GT, Rani DS, et al. (2009) A
common MYBPC3 (cardiac myosin binding protein C) variant associated with
cardiomyopathies in South Asia. Nat Genet 41: 107–191.
21. Galasko GI, Senor R, A. Lahini (2005) Ethnic differences in the prevalence and
aetiology of left ventricular systolic dysfunction in the community: the Harrow
heart failure watch. Heart 91: 595–600.
22. Chahal NS, Lim TK, Jain P, Chambers JC, Kooner JS, et al. (2010) Ethnicity-
related differences in left ventricular function, structure and geometry: a
population study of UK Indian Asian and European white subjects. Heart 96: 466–471.
23. Yang Q, Osánka H, Klevitsky R, Robbins J (2001) Phenotypic deficits in mice
expressing a myosin binding protein C lacking the titin and myosin binding
domains. J Mol Cell Cardiol 33: 1649–1650.
24. Särkäs An, Carrierb L, Schenkka C, Dolb A, Flavigny J, et al. (2005)
Impairment of the ubiquitin-proteasome system by truncated cardiac myosin
binding protein C mutants. Cardiovasc Res 66: 33–44.
25. Buteau AL, Seveda L, Fritjut B (2002) Age-dependent declines in proteasome
activity in the heart. Arch Biochem Biophys 397: 296–304.
26. Okada K, Wanggoengtrakul C, Osawa T, Toyokuni S, Tanaka K, et al. (1999)
4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during
oxidative stress. Identification of proteasomes as target molecules. J Biol Chem
274: 23787–23793.
27. Delleface LM, Pytel P, Mewborn S, Mora B, Guris DL, et al. (2009) Sarcomere
mutations in cardiomyopathy with left ventricular hypertabeculation. Circ
Cardiovasc Genet 2: 442–449.
28. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, et al. (1989)
Recommendations for quantitation of the left ventricle by two-dimensional
echocardiography. American Society of Echocardiography Committee on
Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms.
J Am Soc Echocardiogr 2: 358–367.
29. Johnson R, McNutt P, MacMahon S, Robson R (1997) Use of the Friedewald
Formula to Estimate LDL-Cholesterol in Patients with Chronic Renal Failure on
Dialysis. Clin Chem 43: 2183–2184.
30. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for
extracting DNA from human nucleated cells. Nucl Acids Res 16: 1215.
31. Emigh JF (1988) Pharmacy’s glaucoma alert. Am Pharm 11: 35–6.