Role of CYP1A1 haplotypes in modulating susceptibility to coronary artery disease

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To investigate the role of cytochrome P450 1A1 (CYP1A1) haplotypes in modulating susceptibility to coronary artery disease (CAD), a case-control study was conducted by enrolling 352 CAD cases and 282 healthy controls. PCR-RFLP, multiplex PCR, competitive ELISA techniques were employed for the analysis of CYP1A1 [m1 (T→C), m2 (A→G) and m4 (C→A)] haplotypes, glutathione-S-transferase (GST) T1/GSTM1 null variants and plasma 8-oxo-2’ deoxyguanosine (8-oxodG) respectively. Two CYP1A1 haplotypes, i.e. CAC and TGC showed independent association with CAD risk, while all-wild CYP1A1 haplotype i.e. TAC showed reduced risk for CAD. All the three variants showed mild linkage disequilibrium (D’: 0.05 to 0.17). GSTT1 null variant also exerted independent association with CAD risk (OR: 2.53, 95% CI 1.55–4.12). Among the conventional risk factors, smoking showed synergetic interaction with CAC haplotype of CYP1A1 and GSTT1 null genotype in inflating CAD risk. High risk alleles of this pathway showed dose-dependent association with percentage of stenosis and number of vessels affected. Elevated 8-oxodG levels were observed in subjects with CYP1A1 CAC haplotype and GSTT1 null variant. Multiple linear regression model of these xenobiotic variants explained 36% variability in 8-oxodG levels. This study demonstrated the association of CYP1A1 haplotypes and GSTT1 null variant with CAD risk and this association was attributed to increased oxidative DNA damage.

Keywords: Coronary artery disease, One-carbon metabolism, Xenobiotic metabolism, 8-Oxo-2’deoxyguanosine, CYP1A1 haplotypes
derivatives. These derivatives oxidize to form highly reactive species, such as semiquinone and quinone with significant generation of reactive oxygen species (ROS). These highly reactive species form adducts with DNA triggering the formation of 8-oxo-dG lesions in DNA. This lesion being the most abundant oxidative lesion in mammalian DNA induces mutagenicity, leading to G:C→A:T transversion mutations.

There are many subclasses of cytochrome P450 and among them CYP1A1 is involved in phase I xenobiotic metabolism. CYP1A1 m1, m2 and m4 variations are the most common variations that have been explored in different diseases. CYP1 A1 m1 is a T→C transition creating MspI restriction site in the 3' non-coding region; m2, an I462V polymorphism in the heme-binding domain of exon 7; m4, a T461N polymorphism in the same exon. Among these three polymorphisms, CYP1A1 m1 is reported to be associated with the smoking-related CAD. This polymorphism is shown to be associated with high-inducible phenotype, thus resulting in elevated polycyclic aromatic hydrocarbon (PAH)-adducts. Although there are limited studies on CYP1A1 m2 and m4 polymorphisms in various diseases, but their association with CAD has not been explored. COMT H108L polymorphism induces thermolabile form of COMT, which has shorter half-life compared to wild COMT. Despite the crucial role of this genetic variant in xenobiotic metabolism, no studies have explored its association with CAD till date. Among the phase II genetic variants, GSTT1 null and GSTM1 null have been reported to be risk factors for CAD.

One-carbon metabolism plays a crucial role in phase II of xenobiotic metabolism by supplying (i) methyl group for O-methylation of catechol derivatives, and (ii) glutathione for GST-mediated conjugation step. Any perturbation in this pathway might influence phase II reactions. Our recent study on one-carbon metabolism has shown glutamate carboxypeptidase II (GCPII) C1561T, methylene tetrahydrofolate reductase (MTHFR) C677T and methionine synthase reductase (MTRR) A66G as risk factors and cytosolic serine hydroxyl methyltransferase (cSHMT) C1420T and thymidylate synthase (TYMS) 5'-UTR 28 bp tandem repeat as protective factors for CAD. We also demonstrated that this association is attributed to alteration in oxidative stress. Since, S-adenosyl methionine (SAM) levels might be altered due to defective one-carbon metabolism; there is likelihood of altered gene expression, specifically of anti-oxidant defense enzymes that scavenge free radicals.

Despite the biochemical evidence of cross-link between the one-carbon and xenobiotic metabolic pathways, no studies have been conducted to explore the possible cross-talk between these two metabolic pathways in relation to CAD pathophysiology. Thus, in this study, we aimed to explore the association of xenobiotic genetic variations with CAD risk and also the gene-gene and gene-environment interactions between the xenobiotic and one-carbon metabolic pathways.

Materials and Methods

Recruitment of subjects
The study population comprised 634 subjects (352 cases with documented CAD and 282 healthy controls) enrolled during the period of January 2009 to February 2011 at Nizam's Institute of Medical Sciences, Hyderabad, India. Subjects with angiographically documented CAD having at least 50% stenosis or history of coronary angioplasty/surgical revascularization and age, sex and ethnicity-matched healthy controls were included in this study group. Subjects having chronic inflammatory disease, immunological diseases and cancer were excluded from the study.

Demographic data related to the subjects such as age, gender, body mass index (BMI), diabetes, hypertension, hyperlipidemia, smoking and alcohol intake was obtained. The distribution of well-established risk factors in cases and controls was as follows: BMI (24.10 ± 3.42 vs. 24.36 ± 3.81 kg/m^2), smoking (37.78% vs. 28.87%), diabetes (32.67% vs. 23.81%), hypertension (39.21% vs. 14.08%) and hyperlipidemia (15.10% vs. 6.00%). The study protocol was approved by the Institutional Ethical Committee of the Nizam's Institute of Medical Sciences, Hyderabad, India (EC/NIMS/896(a)/2010). Informed consent was obtained from all the subjects.

Sample collection
Whole blood samples were collected from the subjects (cases and controls) in EDTA vacutainers prior to the surgical revascularization or treatment. Plasma was separated by centrifugation at 4000 rpm and stored at -70°C until use. Genomic DNA was isolated from WBC pellet by using the standard protocols.
Estimation of biochemical parameters

Plasma 8-oxo-2’-deoxyguanosine (8-oxodG) estimation was done using competitive ELISA kit (Northwest Life Sciences specialties, USA) as per the manufacturer’s instructions.

Genetic analysis of one-carbon metabolism

PCR-RFLP technique was used for the analysis of GCPII C1561T, reduced folate carrier 1 (RFC1) G80A, cSHMT C1420T, MTHFR C677T, methionine synthase (MTR) A2756G and MTRR A66G polymorphisms. PCR-AFLP technique was used for the analysis of TYMS 5’-UTR 28bp tandem repeat polymorphism.

Genetic analysis of xenobiotic metabolism

PCR-RFLP technique was used for the analysis of COMT H108L, CYP1A1 m1, m2 and m4 polymorphisms. Multiplex-PCR was used for the analysis of deletions in GSTT1 and GSTM1 loci.

Statistical analysis

The genotype data were computed as 0, 1 and 2 based on number of variant alleles at each locus. For CYP1A1 haplotype construction, web-based computational platform SHEsis was used. Linkage disequilibrium calculations were also performed using the same statistical tool. The univariate analysis of each polymorphism was done using multiple logistic regression to adjust for confounding effects, such as age, body mass index, gender, diabetes, hypertension, hyperlipidemia and family history of CAD. This analysis suggested independent association of CAC (OR: 1.61, 95% CI: 1.08-2.42, P = 0.02) and TGC (OR: 2.31, 95% CI: 1.39-3.85, P = 0.0007) haplotypes of CYP1A1 with CAD after adjusting for all these risk factors. On the other hand, all-wild TAC haplotype exerted reduced risk for CAD. All the three variants of CYP1A1 namely, m1, m2 and m4 showed mild linkage disequilibrium with one another (D’: 0.05-0.17) (Table 1).

As shown in Table 2, among the other variants tested, GSTT1 null [OR: 2.53, 95% CI (1.55-4.12)] exerted independent association with CAD risk, while no association was observed with GSTM1 null and COMT H108L variants (Table 2).

Results

The distribution of all the genetic variations was in accordance with Hardy-Weinberg equilibrium. As shown in Table 1, among the different haplotypes of CYP1A1, CAC and TGC haplotypes showed independent association with CAD risk. Since age, gender, body mass index (BMI), diabetes, hypertension and hyperlipidemia are known contributing factors for CAD, we used multiple logistic regression analysis to control for these confounding effects. This analysis suggested independent association of CAC (OR: 1.61, 95% CI: 1.08-2.42, P = 0.02) and TGC (OR: 2.31, 95% CI: 1.39-3.85, P = 0.0007) haplotypes of CYP1A1 with CAD after adjusting for all these risk factors. On the other hand, all-wild TAC haplotype exerted reduced risk for CAD. All the three variants of CYP1A1 namely, m1, m2 and m4 showed mild linkage disequilibrium with one another (D’: 0.05-0.17) (Table 1).

As shown in Table 2, among the other variants tested, GSTT1 null [OR: 2.53, 95% CI (1.55-4.12)] exerted independent association with CAD risk, while no association was observed with GSTM1 null and COMT H108L variants (Table 2).

Table 1—Association of CYP1A1 haplotypes in coronary artery disease

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>CYP1A1m1/m2/m4</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>C/A/C</td>
<td>127</td>
<td>69</td>
<td>1.72(1.25-2.36)</td>
<td>0.0008*</td>
</tr>
<tr>
<td>H2</td>
<td>C/G/A</td>
<td>1</td>
<td>0.94</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>H3</td>
<td>C/G/C</td>
<td>17</td>
<td>25</td>
<td>0.56(0.30-1.04)</td>
<td>0.06</td>
</tr>
<tr>
<td>H4</td>
<td>T/A/A</td>
<td>3</td>
<td>1</td>
<td>-----</td>
<td>---</td>
</tr>
<tr>
<td>H5</td>
<td>T/A/C</td>
<td>409</td>
<td>417</td>
<td>0.56(0.44-0.72)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>H6</td>
<td>T/G/C</td>
<td>99</td>
<td>45</td>
<td>2.05(1.41-2.98)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>H7</td>
<td>C/A/A</td>
<td>0.19</td>
<td>-</td>
<td>-----</td>
<td>---</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: confidence interval, *: P value statistically significant
Table 2—Genotype and allele frequency distribution of other xenobiotic genetic variants in cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes 0</th>
<th>Genotypes 1</th>
<th>Genotypes 2</th>
<th>Variant allele frequency</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT H108L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>236</td>
<td>102</td>
<td>11</td>
<td>124/698 (17.77%)</td>
<td>0.82 (0.58-1.17)</td>
<td>0.28</td>
</tr>
<tr>
<td>Controls</td>
<td>176</td>
<td>93</td>
<td>8</td>
<td>109/554 (19.67%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST T1 null</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>271</td>
<td>81</td>
<td>-</td>
<td>81/704 (11.51%)</td>
<td>2.53 (1.55-4.12)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Controls</td>
<td>243</td>
<td>39</td>
<td>-</td>
<td>39/564 (6.91%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0, 1 and 2 Number of variant allele at each locus; Adjusted OR odds ratio adjusted for age, body mass index, gender, diabetes, and family history of coronary artery disease using logistic regression analysis; CI confidence interval; * statistically significant.

Note: GST T1 and GSTM1 '0' represents presence of allele while '1' represents null genotype.

Table 3—Interaction of CYP1A1 haplotypes with cSHMT C1420T variant in CAD

<table>
<thead>
<tr>
<th>CYP1A1 Haplotypes</th>
<th>cSHMT 1420 CC</th>
<th>cSHMT 1420 CT</th>
<th>cSHMT 1420 TT</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>H1 (CAC)</td>
<td>28</td>
<td>4</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (ref)</td>
<td>0.30 (0.076-1.096)</td>
<td>0.11 (0.028-0.42)</td>
<td></td>
</tr>
<tr>
<td>H3 (CGC)</td>
<td>15</td>
<td>7</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (ref)</td>
<td>0.31 (0.077-1.21)</td>
<td>0.37 (0.056-2.35)</td>
<td></td>
</tr>
<tr>
<td>H5 (TAC)</td>
<td>51</td>
<td>21</td>
<td>42</td>
<td>72</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (ref)</td>
<td>0.24 (0.12-0.47)</td>
<td>0.24 (0.12-0.51)</td>
<td></td>
</tr>
<tr>
<td>H6 (TGC)</td>
<td>18</td>
<td>4</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00 (ref)</td>
<td>0.26 (0.06-1.01)</td>
<td>0.53 (0.11-2.45)</td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval, *: P value statistically significant, H: CYP1A1 haplotype

Elevated 8-oxodG levels were observed in CYP1A1 CAC haplotype (8.92 ± 3.07 vs. 6.59 ± 2.14 ng/ml, P = 0.01) and GSTT1 null variant (9.25 ± 3.36 vs. 6.85 ± 2.01 ng/ml, P = 0.02), while no association was observed with other haplotypes or variants. Multiple linear regression model of xenobiotic variants explained 36% variability in 8-oxo-dG levels.

Using CYP1A1 all-wild haplotype in non-smokers as the reference, inflated risk for CAD was observed in smokers harboring CAC (OR: 2.95, 95% CI: 1.34-6.55, P = 0.005), TGC (OR: 11.64, 95% CI: 2.45-75.69, P < 0.0001) and CGC (OR: Inf, 95% CI: 2.50-Inf, P = 0.001) haplotypes.

Since one-carbon metabolism shows biochemical evidence of cross-link with xenobiotic metabolism through COMT mediated catalysis, we explored the possible cross-talk between the one-carbon and xenobiotic metabolic pathways using MDR analysis. Only one polymorphism of one-carbon metabolic pathway i.e. cSHMT C1420T showed significant interaction with xenobiotic variants. As shown in Table 3, there was a significant interaction of cSHMT C1420T variant with CYP1A1 CAC haplotype in reducing the CAD risk by 9-folds. The probable protective role of cSHMT C1420T variant might be due to its association with elevated plasma folate (CC: 4.1 ± 1.0 ng/ml; CT: 5.55 ± 2.12 ng/ml; TT: 5.90 ± 1.39 ng/ml, $P_{\text{trend}} < 0.05$) and elevated plasma glutathione (CC: 416.92 ± 174 µmol/L; CT: 446.85 ± 97.2 µmol/L; TT: 569.00 ± 144 µmol/L, $P_{\text{trend}} = 0.02$).

The high-risk alleles of xenobiotic were correlated with markers of disease severity i.e. extent of stenosis and number of vessels affected. Xenobiotic high-risk alleles, which included CYP1A1 m1, CYP1A1 m2 and GSTT1 null were found to be positively associated with extent of stenosis (0 variant alleles = 67.96±14.4%, 1 variant alleles = 70.71 ± 14.0%, ≥2 variant alleles = 77.71 ± 10.7%, $P_{\text{trend}} = 0.03$) and number of vessels affected (0 variant alleles = 1.27 ± 0.53, 1 variant alleles = 1.61 ± 0.70, ≥2 variant alleles = 1.77 ± 0.82, P = 0.03) (Fig. 1).
Figs. 1—Correlation of xenobiotic high risk alleles (CYP1A1 m1, CYP1A1 m2 and GSTT1 null) with the markers of disease severity i.e. extent of stenosis and number of vessels affected [(A) Xenobiotic high-risk alleles were found to be positively associated with extent of stenosis (P trend = 0.03*). 0, 1, 2 represents the number of risk alleles in xenobiotic pathway. Y-axis indicates % of stenosis; and (B) Xenobiotic high risk alleles were found to be positively associated with the number of vessels affected (P=0.03*). 0, 1, 2 in Y-axis indicates (1: single vessel disease; 2: Double vessel disease), 0, 1, 2 on X-axis represent number of risk alleles in xenobiotic pathway.

Discussion

Earlier, we have demonstrated that among one-carbon variants, GCPII C1561T, MTHFR C677T and MTRR A66G are risk factors and cSHMT C1420T and TYMS 5'-UTR tandem repeat are protective factors for CAD. We further explored the role of these genetic variations as potential modulators of oxidative DNA damage in CAD. In the current study, we investigated the independent as well as synergetic effects of xenobiotic genetic variants either among themselves or with the one-carbon variants. We observed independent association of CYP1A1 CAC and TGC haplotypes, and GSTT1 null variations with CAD risk, which was influenced by the elevated 8-oxodG levels. It is not known whether elevated 8-oxodG is a cause or effect, however, elevated 8-oxodG levels have been reported in atherosclerotic plaques compared to normal arteries.

The current study provided evidence for gene-environment interaction i.e., CYP1A1 CAC haplotype × smoking that inflated CAD risk. MDR analysis gave evidence for counteracting interactions of cSHMT C1420T in modulating CYP1A1 CAC haplotype-mediated risk.

The association of CYP1A1 CAC haplotype with CAD risk as observed in the current study was consistent with Wang et al, who demonstrated that this variant increases the risk for triple vessel disease. However, other studies have shown no direct association of this variant with CAD risk. Taspinar et al have reported the synergetic effect of CYP1A1 m1 and GSTT1 null on CAD risk. It has also been shown that in smokers CYP1A1 m1 exerts increased risk for CAD.

Studies have shown an independent association of GSTT1 and GSTM1 null variants with CAD. Maciel et al have attributed the risk associated with GSTT1 and GSTM1 null variants to dyslipidemia. Another study has demonstrated independent association of GSTT1 null variant with CAD and synergetic effect with GSTM1 null on disease severity. The combined genotype of GSTT1 null/GSTM1 null is also reported to be associated with disease severity in smokers. However, another study has shown that the impact of GSTT1 and GSTM1 null genotypes is independent of smoking. Increased DNA damage has been observed in smokers carrying GSTT1 and GSTM1 null alleles compared to smokers having wild genotype at these loci. Studies on GSTT1 and GSTM1 have demonstrated the protective role of null variants, however their results are underpowered due to limited sample size. Results of the current study were consistent with the majority of the studies in highlighting the role of gene-gene and gene-environmental interactions in modulating disease susceptibility associated with these variants. Our results were consistent with Wang et al in demonstrating association of xenobiotic variants with disease severity.

The protective role of cSHMT variant against CYP1A1 CAC haplotype further explains the disparities among the different association studies. The C1420T variation of cSHMT helps in maintaining folate homeostasis, which is further supported by our earlier study, thus facilitating the synthesis of glutathione essential for phase II-mediated conjugation step (Scheme I).

We observed no association of COMT H108L either independently or in synergy with one-carbon variants. Although there are no studies focused on association of this variant in CAD, however, in one study, increased risk for acute coronary events has been reported with this variant.
Scheme I—Xenobiotic mediated oxidative DNA damage and CAD risk [Polycyclic aromatic hydrocarbons (PAH) derived from environmental toxins, such as smoking are hydroxylated in the presence of cytochrome P450 (CYP)1A1 m1 and m2 variants to form catechol derivatives. Catechol derivates may be converted to highly reactive intermediates i.e. semiquinones and quinines. In the presence of GSTT1 null variant, conjugation of semiquinones/quinines with low levels of glutathione resulting in increased generation of free radicals, leading to 8-oxo-dG formation resulting in oxidative DNA damage]

The strengths of this study were: i) the incorporation of MDR analysis to explore gene-gene and gene-environment interactions with specific focus on one-carbon and xenobiotic metabolic pathways, and ii) This study explained the variations in multi-locus investigations and with the different environmental exposures. Future studies are warranted to explore the interactions of different environmental agents with xenobiotic genetic variants to understand the molecular pathophysiology of CAD.

To conclude, CYP1A1 CAC and TGC haplotypes and GSTT1 null are independent risk factors for CAD in our population. Gene-gene and gene-environment interactions play a vital role in inflating CAD risk.

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References