**Supplementary Data 1**

**Materials and methods**

**Genotyping of antioxidant gene polymorphisms**

 The primer sequences for *GSTT1* genotyping were followed Sprenger et al (Sprenger et al. 2000). Genotyping was performed in a final 25 μL PCR reaction buffer containing 100 ng genomic DNA, 1.0 µM of each primer, 2X PCR enhancer (Invitrogen), 1.5 mM MgCl2, 0.20 mM of each dNTP, and 0.5 unit of Taq DNA polymerase (Invitrogen). PCR reaction was performed with a 94°C initial denaturation step for 4.30 minutes, then followed by 30 cycles of [94°C denaturation (30 second), 65°C annealing (30 second), and 72°C extension reaction (90 second)], and a final step of 72°C for 7 minutes. The amplified products were size-separated on 2.0% agarose gel electrophoresis. Genotyping was based on size-specific PCR bands. The PCR product of 1,460 bps was interpreted as *GSTT1* null (-) allele, whereas the 466 bps product indicated *GSTT1* presented (+) allele.

The PCR primers for *GPX3* c.87+1494A>G (rs3828599) were 5’- TCCCCAACTCA GAAGGCATTTTCCA-3’ (outer forward primer), 5’-GGCATGCCCAGGCTTTCATTAGC-3’ (outer reverse primer), 5’-AGTCAGTCCCAACCTTCAGTTTTGGTAG-3’ (allele specific forward primer), and 5’-GCCCAATTGTATCTTCTTTGATCT-3’ (allele specific reverse primer). PCR was performed in a total 25 μL reaction buffer, consisting of 100 ng genomic DNA, 0.6 µM of each primer, 2.00 mM MgCl2, 0.20 mM of each dNTP, and 0.5 unit of Taq DNA polymerase (Invitrogen). The reaction cycle was 94°C initial denaturation step for 4.30 minutes, followed by 30 cycles of [94°C denaturation (30 second), 52°C annealing (30 second), and 72°C extension reaction (30 second)], and a final step of 72°C for 5 minutes. Genotyping was also based on the size-specific PCR bands as: AA (347 and 143 bps), AG (347, 143 and 255 bps), and GG (347 and 255 bps).

The primers for *SOD2* c.47C>T (rs4880) genotyping were 5’-GTGTGCGGG TGAGAAGAAAGG-3’ (outer forward primer), 5’-ACTTCTCCTCGGTGACGTTCAGGT-3’ (outer reverse primer), 5’-AGCAGGCAGCTGGCTCCAGT-3’ (allele specific forward primer), and 5’-TGGAGCCCAGATACCCCATAG-3’ (allele specific reverse primer). The PCR reaction was carried out as described above, except for using 1X PCR enhancer and 1.50 mM MgCl2, with 58oC annealing temperature. The genotypes were described according to size-specific PCR bands as: TT (471 and 178 bps), TC (471, 333 and 178 bps), and CC (471 and 333 bps).

 The primers for *SOD3* c.172G>A (rs2536512) genotyping were 5’-TCCCGCA GGTGCCCGACTCCAG-3’ (outer forward primer), 5’-GGCGCAAGCTGCCGGAAGAGGA-3’ (outer reverse primer), 5’-CAGCGGCGGGACGACGACGACG-3’ (allele specific forward primer), and 5’- CTGGCAGGCGGCGTGGAGAGT-3’ (allele specific reverse primer). The PCR reaction was carried out as described above, except for using 1.0 µM of each primer, 1X PCR enhancer, and 1.75 mM MgCl2, with 63oC annealing temperature. The genotypes were described according to size-specific PCR bands as: GG (290 and 116 bps), GA (290, 116 and 216 bps), and AA (290 and 216 bps).

The primers for *SOD3* g.9892T>C (rs2855262) genotyping were 5’-GACGGCA GCCTCTGGAGGTA-3’ (outer forward primer), 5’-TCGGTACAAATGGAGGCCTTCAGA-3’ (outer reverse primer), 5’- TCCACTCTGAGGTCTCACCTTCGCGTT-3’ (allele specific forward primer), and 5’- AGGGCTGCGGGGAGACTTCAGGAG-3’ (allele specific reverse primer). The PCR reaction was carried out as described above except for 0.5 µM of each primer, 1X PCR enhancer, and 1.5 mM MgCl2, with 60oC annealing temperature and 55 second for 72°C extension reaction. The combination of size-specific PCR products indicating genotypes were 511 and 161 bps for TT; 511, 161 and 400 bps for TC; and 511 and 400 bps for CC.

**Sample size calculation**

 Sample size calculation was performed before study completion, using statistical power of 80% and a significance level alpha of 0.05. Calculation was determined at 46.0% and 28.0% expected prevalence of *GSTT1*−/− in CAD cases and controls, respectively, from previous report (Charan and Biswas 2013; Manfredi et al. 2009) and found to consist of at least 121 subjects per group. Estimation of statistical power was performed using CaTS power calculator: (<http://www.sph.umich.edu/csg/abecasis/CaTS>) (as shown in Table S2, supplementary data 3).

**Table S1** Comparison of malondialdehyde (MDA) among genotypes in each polymorphism [243 pilot community-based subjects (aged >50 years) were recruited from Khon Kaen, Thailand]

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | ***SOD3*****rs2536512** | ***SOD3*****rs2855262** | ***GPX3*****rs3828599** | ***GSTT1* gene deletion polymorphism** | ***SOD2*****rs4880** |
| **Total subjects (n=243)** |  |  |  |  |  |
| MDA (μmol/L) | GG (n=85) | 5.37±1.89 | TT (n=71) | 5.11±1.48 | GG (n=93) | 5.34±1.66 | +/+ (n=46) | 5.30±1.54 | TT (n=131) | 5.49±2.05 |
| GA (n=130) | 5.59±2.13 | TC (n=122) | 5.57±2.16 | GA (n=104) | 5.58±2.22 | +/- (n=116) | 5.62±2.27 | TC (n=96) | 5.56±1.96 |
| AA (n=28) | 5.51±1.80 | CC (n=50) | 5.91±2.20 | AA (n=46) | 5.66±2.15 | -/- (n=81) | 5.46±1.85 | CC (n=16) | 5.26±2.03 |
| *p*-value | 0.681 | *p*-value | 0.067 | *p*-value | 0.686 | *p*-value | 0.745 | *p*-value | 0.765 |
| *p* for trend | 0.624 | *p* for trend | **0.022** | *p* for trend | 0.412 | *p* for trend | 0.766 | *p* for trend | 0.564 |
| **Non-MetS subjects (n=149)** |  |  |  |  |  |
| MDA (μmol/L) | GG (n=47) | 4.98±1.54 | TT (n=39) | 4.89±1.56 | GG (n=54) | 4.95±1.50 | +/+ (n=21) | 4.50±0.95 | TT (n=85) | 5.21±2.09 |
| GA (n=84) | 5.28±2.18 | TC (n=81) | 5.29±2.23 | GA (n=66) | 5.19±2.29 | +/- (n=77) | 5.40±2.25 | TC (n=55) | 5.02±1.52 |
| AA (n=18) | 4.81±0.92 | CC (n=29) | 4.99±1.04 | AA (n=29) | 5.30±1.47 | -/- (n=51) | 4.97±1.45 | CC (n=9) | 4.98±1.89 |
| *p*-value | 0.626 | *p*-value | 0.576 | *p*-value | 0.599 | *p*-value | 0.101 | *p*-value | 0.829 |
| *p* for trend | 0.894 | *p* for trend | 0.558 | *p* for trend | 0.313 | *p* for trend | 0.274 | *p* for trend | 0.628 |
| **MetS subjects (n=94)** |  |  |  |  |  |
| MDA (μmol/L) | GG (n=38) | 5.84±2.17 | TT (n=32) | 5.37±1.36a | GG (n=39) | 5.88±1.73 | +/+ (n=25) | 5.96±1.63 | TT (n=46) | 6.02±1.88 |
| GA (n=46) | 6.18±1.93 | TC (n=41) | 6.13±1.93 | GA (n=38) | 6.26±1.95 | +/- (n=39) | 6.06±2.27 | TC (n=41) | 6.29±2.24 |
| AA (n=10) | 6.79±2.30 | CC (n=21) | 7.17±2.72a | AA (n=17) | 6.29±2.93 | -/- (n=30) | 6.28±2.16 | CC (n=7) | 5.63±2.29 |
| *p*-value | 0.350 | *p*-value | **0.009** | *p*-value | 0.704 | *p*-value | 0.875 | *p*-value | 0.663 |
| *p* for trend | 0.179 | *p* for trend | **0.002** | *p* for trend | 0.736 | *p* for trend | 0.705 | *p* for trend | 0.557 |

**Note:** Thiobarbituric acid (TBA) assay was used for the measurement of serum malondialdehyde (MDA) as an end product of lipid peroxidation (Nielsen et al. 1997; Wong et al. 1987). a *p*-value=0.010

**References**

**Charan J, Biswas T**. **2013**. How to calculate sample size for different study designs in medical research? *Indian journal of psychological medicine* 35:121-126. doi:10.4103/0253-7176.116232

**Manfredi S, Calvi D, del Fiandra M, Botto N, Biagini A, Andreassi MG**. **2009**. Glutathione S-transferase T1- and M1-null genotypes and coronary artery disease risk in patients with Type 2 diabetes mellitus. *Pharmacogenomics* 10:29-34. doi:10.2217/14622416.10.1.29

**Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P**. **1997**. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry* 43:1209-1214

**Sprenger R, Schlagenhaufer R, Kerb R, Bruhn C, Brockmoller J, Roots I, Brinkmann U**. **2000**. Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. *Pharmacogenetics* 10:557-565

**Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN, Jr., Sunderman FW, Jr. 1987**. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry* 33:214-220