# Analysis of Homocysteine Metabolism Enzyme Gene Polymorphisms in Non-Syndromic Congenital Heart Disease Patients among Malaysians

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**Abstract:** Congenital heart disease (CHD) mainly is caused by the incomplete development of the heart during the first 6 weeks of pregnancy. Chromosomal and genetic abnormalities in the child and high levels of homocysteine in the blood are some of the risk factors related to CHD. Several studies in various populations have been done to determine the candidate genes in the predisposition to CHD with contradictory results, but there have been no studies that had been found in Malaysian CHD patients on homocysteine gene polymorphisms. Hence, this study was conducted to determine the allelic and genotypic analysis of the polymorphisms in candidate genes of the homocysteine enzymes; *Methylenetetrahydrofolate Reductase* (MTHFR), *Cystathionine-b-synthase* (CBS), *Methionine Synthase* (MTR) and *Methionine Synthase Reductase* (MTRR) genes. Based on the inclusion and exclusion criteria, buccal or blood samples were collected from 150 Malaysian non-syndromic CHD patients and 150 samples from healthy subjects as controls with no matching of age, genders and race between cases and controls. Genomic DNA was extracted from the samples using commercially available kits and the genotyping analysis for C677T MTHFR, A1298C MTHFR, A66G MTRR, A2756G MTR and 844ins68 CBS gene polymorphisms were analyzed using PCR-RFLP analysis. There was a significant difference observed in MTHFR A1298C gene polymorphism between cases and controls (P=0.008). However, there was no significant difference was observed for MTHFR C677T, MTRR A66G, MTR A2756G and CBS 844ins68 gene polymorphism. The association of MTHFR A1298C with the development of CHD in this study emphasis the role of MTHFR gene in the pathogenesis of non-syndromic CHD in Malaysian subjects.

## 1. Introduction

Congenital heart disease (CHD) is a common disorder or defect among newborn infants and children (Goldmuntz, 2001) where it can be caused by environmental or genetic factors. The genetic factors may include chromosomal or genetic abnormalities of the infant or single gene defect. Maternal viral infection, certain medication taken during pregnancy and drug abuse are also some of the risk factors in the development of CHD (Deeparani *et al.*, 2009). A few types of CHD are induced by single gene mutation and chromosomal aberration but most of CHDs are polygenic diseases caused by both genetic and environmental factors (Zhu *et al.*, 2004). CHD has been featured in many genetic syndromes, while the genetic associations of non-syndromic CHD are starting to be increasingly recognized and being studied by many researchers. Both genetic causes of syndromic and non-syndromic CHD are important as it will provide an opportunity to develop new diagnostic and therapeutic strategies and decrease the economic burden, mortality and morbidity of CHD (Hinton *et al.*, 2005). Moreover, it has been reported from a study that 26.6% of infant deaths are due to congenital abnormalities with total number of death for infants less than 1 year of age was 86% (Curtis & Stuart, 2005).

Homocysteine is an amino acid present in the blood and an elevated level of homocysteine known as hyperhomocysteinemia has been extensively studied in its relation to the development of CHD (Deeparani *et al.*, 2009). Elevated level of homocysteine may impair endothelial vasomotor function or damage coronary arteries causing

abnormal blood flows. The understanding of the etiology of CHD is rapidly progressing starting from recognition of embryologic origins to insights into the genetic basis of this disease. Recent studies have discovered the genetic basis of some common types of CHD and this provides a better understanding into how the heart develops and a defect in the heart development could lead to CHD (Bruneau, 2008).

The common enzymes involved in homocysteine or folate metabolism are the Methylenetetrahydrofolate Reductase (MTHFR), the Methionine Synthase Reductase (MTRR), Methionine Synthase (MTR) and Cystathionine-b-synthase (CBS) enzymes. Deficiencies or polymorphism in any of these enzymes are suspected to lead to an inborn error in the metabolism of homocysteine and also causing homocystinuria. There is an evidence that this deficiency could alter the susceptibility to CHD (Garcia-Fragoso *et al.*, 2010).

Previous studies in various populations have been done in identifying the association of the homocysteine enzyme gene polymorphisms and CHD with contradictory results (Junker *et al.*, 2001; van Driel *et al.*, 2008, Zhu *et al.*, 2004, Song *et al.*, 2006; van Beynum *et al.*, 2007; Li *et al.*, 2005). This initiated us to determine the association of the genetic polymorphisms of homocysteine genes (C677T and A1298C polymorphism of MTHFR; A2756G polymorphism of MTR; A66G polymorphism of the MTRR and 844ins68 polymorphism of CBS) with the development of non-syndromic CHD in Malaysian subjects.

## 2. Material and Methods

Upon approval from the Ethical Committee, the case samples were recruited from the Pediatrics Ward at National Heart Institute (IJN), Kuala Lumpur, from September 2010 until June 2011. A total of 160 CHD patients' was approached and 150 samples have been recruited based on an inclusion and exclusion criteria. The diagnosis of non-syndromic CHD and the classification of the type of the cardiac defect had been done by an experienced consultant pediatric cardiologist based on the clinical and the echocardiography findings with or without the diagnostic cardiac catheterization findings and surgical notes. An informed consent and a questionnaire had been obtained either from the parents or guardian of the selected patients. A total of 150 unrelated healthy individuals (age >21 years old) was recruited from screening programs that were conducted at Seri Kembangan, Pusat Kesihatan UPM, Bangi and Seremban. Similar to the collection

of the case samples, questionnaires were also given to these individuals and consents were obtained.

Blood samples from the patients were taken by a pediatric cardiology specialist doctor or by a phlebotomist in the pediatric ward at IJN. Blood sampling for both the CHD patients and controls have been done by a phlebotomist. Meanwhile, buccal samples were also collected from both CHD patients and controls were taken by scraping the cheeks using a cytobrush which was then stored in a 1.5 ml tube containing saline water until the DNA extraction process was done.

In this study, the PCR-RFLP technique was used to amplify the desired gene mutation and the amplification process was done using G-Storm Thermal Cycler (GRI, UK). Each gene polymorphisms were carried out using PCR by their respective primers, restriction enzymes, PCR reaction mixtures and reaction conditions (Table 1).

The PCR amplified products and the restricted fragments were all visualized under UV light and the image was captured using the Alpha Imager (Alpha Innotech, San Leandro, CA). The size of the PCR and RE products was determined by comparing it with a 100bp/1kb DNA ladder which was used as a DNA marker.

*Statistical Analysis*

All data and statistical and analysis was done using SPSS (release 20.0, SPSS Inc., Chicago, USA). Allelic frequencies were calculated by gene-counting method. Alleles and genotypes distribution was tested for deviation from the Hardy-Weinberg by chi-squared test. Testing for the association was also done by chi-squared and Fisher's Exact test. In order to confirm absence of genotyping errors, Hardy–Weinberg equilibrium was applied to the case group and testing for deviation for the Hardy–Weinberg equilibrium of observed genotypes was done by using Pearson's chi square between the observed and expected genotypes.

## 3. Results

In this study, 150 case subjects and 150 controls were recruited. The majority of the case subjects were males (53.3%) compared to females (46.7%), whereas in the control subjects, females are greater than males 73.3% and 26.7% respectively. The age range for CHD subjects was between two months and 45 years old with a mean of 5.82 year ± 5.1 years. The majority of the cases were aged between one and 10 years old. While for the control subjects, the age range is between 19-70 years old with a mean of 39.3 ± 14.53. The phenotypes of CHD cases those are included in this study are explained in table 2.

Table 1: Primers, PCR reaction conditions, PCR product, RE product for each type of gene polymorphism

| Gene Polymorphism | Primers | PCR Reaction Conditions | PCR Product | RE Product | Reference |
|---|---|---|---|---|---|
| MTHFR C677T | Forward: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'<br><br>Reverse: 5'-AGGACGGTGCGGTGAGAGTG-3' | Initial denaturation: 94℃, 8 min<br>Denaturation: 94℃, 1 min<br>Annealing: 63℃, 1 min<br>Extension: 72℃, 1 min<br>(40 cycles)<br>Final extension: 72℃, 7 min | 198 bp | *HinfI*<br>MT: 175, 23bp<br>HT: 198, 175, 23bp<br>WT: 198bp | (Deeparani *et al.,* 2009) |
| MTHFR A1298C | Forward: 5'-CTTT GGGGAGCTGAA GGACTACTAC-3'<br><br>Reverse: 5'-CACTTTGTGACCATTCCG GTTTG-3' | | 163 bp | *MboII*<br>WT: 56, 31, 30, 28 and 18 bp<br>HT: 84, 56, 31, 30, 28, 18 bp<br>MT: 84, 31, 30, 18 bp | (Rahimi *et al.,* 2010) |
| MTR A2756G | Forward: 5'-TGTTCCCAGCTGTTAGATGAAAATC-3<br><br>Reverse: 5'-GATCCAAAGCCTTTTACACTCCTC-3' | Initial denaturation: 94℃, 10 min<br>Denaturation: 95℃, 30 sec<br>Annealing: 60℃, 30 sec<br>Extension: 72℃, 30 sec<br>(40 cycles)<br>Final extension: 72℃, 5 min | 211 bp | *HaeIII*<br>WT: 211bp<br>HT: 211, 131, 80 bp<br>MT:131 & 80bp | (Vinukonda *et al.,* 2009) |
| MTRR A66G | Forward: 5'-CAGGCAAAGGCCATCGCAGAAGAC AT-3'<br><br>Reverse: 5'-CACTTCCCAACCAAAATTCTTCAAA G-3' | Initial denaturation: 95℃, 6 min<br>Denaturation: 94℃, 40 sec<br>Annealing: 53℃, 40 sec<br>Extension: 72℃, 1 min<br>(35 cycles)<br>Final extension: 72℃, 10 min | 152 bp | *NdeI*<br>WT: 124 & 27bp<br>HT: 151, 124, 27 bp<br>MT: 151bp | (O'Leary *et al.,* 2002) |
| CBS 844ins68 | Forward: 5' -GCAGTTGTTAACGGCGGTAT-3'<br><br>Reverse: 5'-GATGAAGTCGTAGCCGATCC-3' | Initial denaturation: 95℃, 5 min<br>Denaturation: 94℃, 50 sec<br>Annealing: 61℃, 50 sec<br>Extension: 72℃, 1 min<br>(30 cycles)<br>Final extension: 72℃, 7 min | NN: 184bp<br>IN: 184, 252 bp<br>II: 252bp | - | (Goldmuntz *et al.,* 2008) |

Note: WT- wild-type; HT- heterozygous type; MT- mutant type; sec- seconds; min- minutes

Table 2: Phenotypes of CHD subjects included in the study cohort (n=150)

| Diagnosis | n | % |
|---|---|---|
| VSD | 52 | 34.7 |
| TOF* | 36 | 24.0 |
| ASD | 16 | 10.7 |
| TGA | 12 | 8.0 |
| AVSD** | 8 | 5.3 |
| Others (CCTGA, PDA, TAPVD, DILV, PS, PAIVS, DORV, CoA, Ebstein anomaly, bicuspid aortic valve AS, TA&PA , single ventricle&PA, TR, severe congenital MR, third degree AV block, atrial tachycardia) | 26 | 17.3 |
| Total | 150 | 100 |

Note: AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CCTGA, congenitaly corrected transposition of the great arteries ; CoA, coarctation of aorta ; DCSA, Doubly committed subarterial ventricular septal defects DILV, double inlet left ventricle;DORV, double outlet right ventricle; MS, mitral stenosis; MR,mitral valve regurgitation; PA, pulmonary valve atrasia; PAPVD, partial anomalous pulmonary venous drainage; PDA, patent ductus arteriosus; PM, perimemewnouse PS, pulmonary artery stenosis;TA, tricuspid atresia; TAPVD, total anomalous pulmonary venous draiage; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect. *Associated PDA (1), ** Associated with single ventricle& PS (1).

Table 3 shows the genotypic and allelic frequency of homocysteine gene polymorphisms in the subjects along with odds ratio and 95% confidence interval. There was significant association found only in A1298C of MTHFR gene among CHD subjects compared to controls (p<0.05). The distribution of the MTHFR C677T gene polymorphism genotypes of CC and CT among CHD subjects were 118 (78.67%) and 37 (21.33%) respectively, compared to 131 (87.33%) and 19

(12.67%) among control subjects. There was absence of the TT genotype for both groups. No significant difference was observed in both genotypic and allelic distributions (*P*>0.05). The distribution of the MTHFR A1298C gene polymorphism genotypes among CHD subjects for the AC genotype was higher compared to the AA and CC genotype with a frequency of 62.7%, while in controls the AA genotype has the highest frequency with frequency of 48.7%. The allele frequency for the A allele was higher in control groups whereas the allele C was higher in the CHD groups. A significant difference was observed only in the genotype distribution between CHD and control groups with a *P* value of 0.008. There was no significant association was observed among the groups in both genotypic and allelic distributions in MTR A2756G, MTRR A66G and CBS 844ins68 gene polymorphisms (*P*>0.05). Both CHD and control groups had a high frequency of the NN genotypes whereas the II genotype of CBS 844ins68 polymorphism was not observed in both subjects. No significant difference was observed in both genotype and allele frequencies (*P*>0.05).

The genotypic distribution of homocysteine gene polymorphisms among the various types of CHD has been tabulated in Table 4. A significant difference was found when compared between VSD (p-0.036) and ASD (p-0.018) with control groups for MTHFR C677T gene polymorphism. Whereas for MTHFR A1298C gene polymorphism, CHD subjects with TGA only have a significant association when compared to control groups (P<0.05). There was a

significant difference (*P*<0.05) observed for the MTR A2756G gene polymorphism in the other groups; a combination of all the minor groups of CHD. There was no significant association found for the other gene polymorphisms MTRR A66G, CBS 844ins68 gene polymorphism compared within different diagnosis of CHD (*P*>0.05).

## 4. Discussions

Congenital heart disease is a common disorder that causes mortality and morbidity among infants. The mortality rate of children under five years old was 7.2 per thousand still births with congenital malformations, deformations and chromosomal abnormalities being the most common causes of death with a percentage of 25.1% (Ministry of Health 2006, Malaysia). In Malaysia, 100 infants are born with an abnormal heart and it is estimated that at least 5000 children could be at risk of suffering from CHD with two-thirds will require surgical intervention (Lan and Ismail, 2006).

Genetic factors are one of the risk factors for the development of CHD. Several studies had published on the association of gene polymorphisms of homocysteine metabolism with CHD (van Beynum *et al.,* 2007). To our knowledge, there is a lack of information available on the association of the homocysteine enzyme gene polymorphisms with CHD in Malaysia. This prompted us to determine the between homocysteine enzyme gene polymorphisms and CHD among Malaysian subjects.

Table 3: Genotypic and allelic frequency of homocysteine gene polymorphisms in the subjects

| Gene Polymorphism | Genotypes (%) | | | *P* value | Alleles (%) | | *P* value | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|---|---|
| MTHFR C677T Cases Controls | CC 118(78.67) 131(87.33) | CT 32 (21.33) 19(12.67) | TT 0 (0) 0 (0) | 0.064 | C 268 (89.33) 281 (93.67) | T 32 (10.67) 19 (6.33) | 0.057 | 0.566 (1.023-0.313) |
| MTHFR A1298C Cases Controls | AA 49 (32.7) 73 (48.7) | AC 94 (62.7) 67 (44.7) | CC 7 (4.7) 10 (6.7 ) | **0.008** | A 192 (64.0) 213 (71.0) | C 108 (36.0) 87 (29.0) | 0.067 | 0.726 (1.023-0.515) |
| MTR A2756G Cases Controls | AA 106 (48.1) 114 (51.9) | AG 41 (53.2) 36 (46.8) | GG 3 (100) 0 (0) | 0.164 | A 253 (48.9) 264 (51.1) | G 47 (56.6) 36 (43.4) | 0.193 | 0.734 (1.171-0.460) |
| MTRR A66G Cases Controls | AA 26 (17.33) 26 (17.33) | AG 99 (66.0) 104 (69.33) | GG 25 (16.67) 20 (13.34) | 0.712 | A 151 (50.33) 156 (52.0) | G 149 (49.67) 144 (48.0) | 0.683 | 0.935 (1.289-0.679) |
| CBS 844ins68 Cases Controls | NN 145 (96.67) 149 (99.33) | IN 5 (3.33) 1 (0.67) | II 0 (0) 0 (0) | 0.214 | N 295 (98.33) 299 (99.67) | I 5 (1.67) 1 (0.33) | 0.216 | 0.197 (1.699-0.023) |

Table 4: Genotypic distribution of homocysteine gene polymorphisms among the various types of CHD

| Gene polymorphism | VSD | TOF | ASD | TGA | AVSD | Others | Total | Controls |
|---|---|---|---|---|---|---|---|---|
| MTHFR C677T CC | 39 (75) | 30(83.3) | 10(62.5) | 11(91.7) | 7(87.5) | 21(80.8) | 118 | 131 |
| CT | 13 (25) | 6 (16.7) | 6 (37.5) | 1 (8.3) | 1(12.5) | 5 (19.2) | 32 | 19 |
| TT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total no. of subjects | 52(34.7) | 36(24.0) | 16(10.7) | 12 (8.0) | 8 (5.3) | 26(17.3) | 150 | 150 |
| P value[a] | **0.036** | 0.586[b] | **0.018** | 1.000[b] | 1.000[b] | 0.368 | | |
| MTHFR A1298C AA | 18(34.6) | 11(30.5) | 8(50.0) | 0(0) | (0) | 15(57.7) | 52 | 55 |
| AC | 32(61.5) | 24(66.7) | 8(50.0) | 11(91.7) | 7(87.5) | 9(34.6) | 91 | 85 |
| CC | 2(3.8) | 1(2.7) | 0(0) | 1(8.3) | 1(12.5) | 2(7.7) | 7 | 10 |
| Total no. of subjects | 52(34.7) | 36(24.0) | 16(10.7) | 12(8.0) | 8(5.3) | 26(17.3) | 150 | 150 |
| P value[a] | 0.698 | 0.462 | 0.395 | 0.034 | 0.103 | 0.105 | | |
| MTR A2756G AA | 37(71.2) | 27(75.0) | 14(87.5) | 10(83.3) | 6(75.0) | 12(46.2) | 106 | 114 |
| AG | 14(26.9) | 8(22.2) | 2(12.5) | 2(16.7) | 2(25.0) | 12(50.0) | 41 | 36 |
| GG | 1(1.9) | 1(2.8) | 0(0) | 0(0) | 0(0) | 1(3.8) | 3 | 0 |
| Total no. of subjects | 52 | 36 | 16 | 12 | 8 | 26 | 150 | 150 |
| P value[a] | 0.208 | 0.122 | 0.530[b] | 0.734[b] | 1.000[b] | **0.001** | | |
| MTRR A66G AA | 11(21.2) | 6(16.7) | 3(18.8) | 1(8.3) | 0(0) | 5(19.2) | 26 | 26 |
| AG | 32(61.5) | 24(66.7) | 10(62.5) | 11(91.7) | 6(75.0) | 16(61.5) | 99 | 104 |
| GG | 9(17.3) | 6(16.7) | 3(18.8) | 0(0) | 2(25.0) | 5(19.2) | 25 | 20 |
| Total no. of subjects | 52 | 36 | 16 | 12 | 8 | 26 | 150 | 150 |
| P value[a] | 0.582 | 0.874 | 0.810 | 0.232 | 0.339 | 0.675 | | |
| CBS 844ins68 NN | 50(96.2) | 35(97.2) | 16(100) | 12(1000 | 8(100) | 24(92.3) | 145 | 149 |
| IN | 2(3.8) | 1(2.8) | 0(0) | 0(0) | 0(0) | 2(7.7) | 5 | 1 |
| II | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total no. of subjects | 52 | 36 | 16 | 12 | 8 | 26 | 150 | 150 |
| P value[a] | 0.163 | 0.350 | 1.000 | 1.000 | 1.000 | 0.06 | | |

[a]Data were evaluated by Pearson chi square test, P<0.05. [b]Data were evaluated by Fisher's Exact Test, *P*<0.05. Data in parentheses represent percentage frequency

In this study, a total of 300 samples of cases and controls was recruited with 150 of the samples are non-syndromic CHD patients with various phenotypes and compared with the other 150 samples as control subjects. The MTHFR gene is located on the short arm of chromosome 1 at locus 1p36.3, with the polymorphism being studied is a substitution of cytosine to a thymine at nucleotide position 677 which converts an alanine amino acid to valine (Frosst *et al.*, 1995). It has been known that the MTHFR C677T polymorphism might likely effect in the production of methionine and S-adenosylmethionine which plays an important role in DNA methylation, protein and lipid reactions (Wenstrom *et al.*, 2001). However, there was no significant association (P>0.05) between MTHFR C677T gene polymorphism with the development of CHD patients in Malaysia. Our findings are well supported with the other studies done in Austria (Wintner *et al.*, 2007), Brazil (Pereira *et al.*, 2005), Taiwan (Lee *et al.*, 2005) and China (Zhu *et al.*, 2006). In our study, we have found that, there was a significant difference between MTHFR A1298C gene polymorphism with CHD (p<0.05). There has been

no infant case-control study found being conducted in other populations. Instead, most of the studies involving the association of MTHFR A1298C gene polymorphism with CHD are done only in case-control family studies (Lupo *et al.*, 2010). Our results showed that 1298CC genotypes and C allele significantly increased the risk of CHD in Malaysian children. These results were in accordance (Van Driel *et al.,* 2008; Kotby *et al.,* 2012) and contradictory (Galdieri *et al.,* 2007; Li *et al.*, 2005) with the other studies. The A2756G gene polymorphism of MTR gene plays a role in susceptibility to congenital anomalies in fetus (Li *et al.*, 2005) and may lead to the development of CHD (Garcia-Fragoso *et al.*, 2010). In contradictory to those studies, our study failed to show a significant difference between A2756G gene polymorphism and CHD subjects (P>0.05) but is well supported in Zhu *et al.,* 2004. The MTRR gene is located on chromosome 5 at 5p15.3 to p15.2 and A66G gene polymorphism; a common variant has been associated in the devlopment of CHD (Apitz *et al.*, 2009; Hanchard, 2005; Garcia- Fragoso *et al.*, 2010).
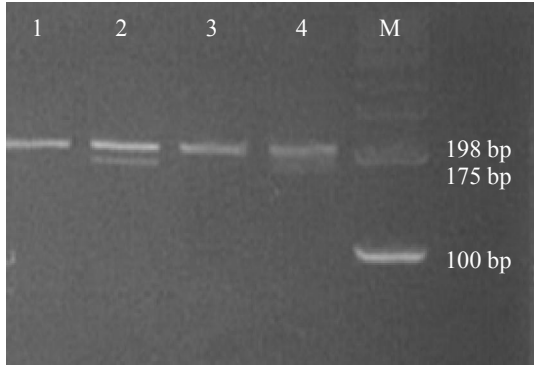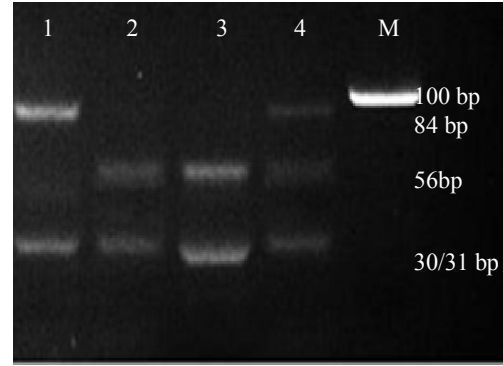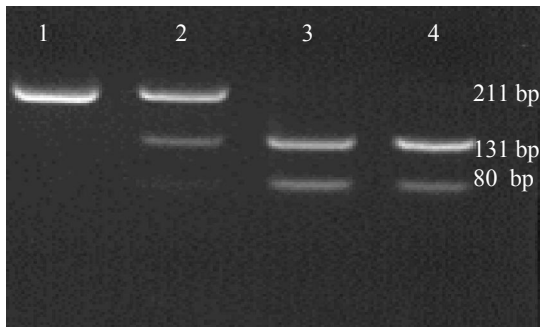
**Figure 1a**



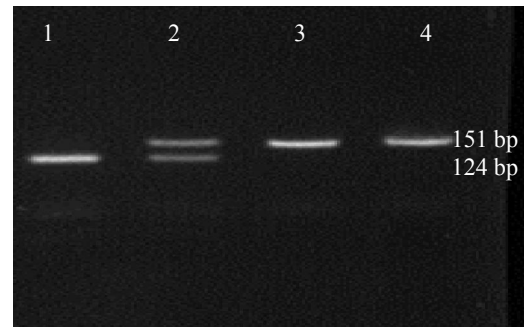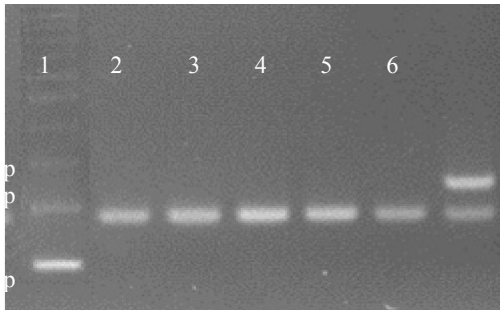**Figure 1b**



**Figure 1c**



**Figure 1d**



**Figure 1e**

Figure 1a: RFLP product for MTHFR C677T gene polymorphism in 3.0% agarose gel electrophoresis. Lane 1 and 3 shows the wild-type fragments (198 bp) and Lane 2 and 4 show the heterozygous-type fragments (23 bp, 175 bp, 198 bp). The 23 bp band does not retain on the gel. 1b: RFLP product for MTHFR A1298C gene polymorphism in 3.0% agarose gel electrophoresis. Lane 2 and 3 shows the wild-type fragments (56, 31, 30, 28 and 18 bp), Lane 4 shows the heterozygous-type fragment (84, 56, 31, 30, 28 and 18 bp) and Lane 1 shows the mutant-type fragment (84, 31, 30 and 18 bp). The 18 bp band does not retain on the gel. 1c: RFLP product for MTR A2756G gene polymorphism in 3.0% agarose gel electrophoresis. Lane 1 shows the wild-type fragment (211 bp), Lane 2 shows the heterozygous-type fragment (211, 131 and 80 bp) and

Lane 3 and 4 shows the mutant-type fragment (131 and 80 bp). 1d: RFLP product for MTRR A66G gene polymorphism in 3.0% agarose gel electrophoresis. Lane 2 shows the wild-type fragment (124 and 27 bp), Lane 2 shows the heterozygous-type fragment (151, 124 and 27 bp) and Lane 3 and 4 shows the mutant-type fragment (151 bp). The 27 bp band does not retain on the gel. 1e: PCR product for CBS 844ins68 gene polymorphism in 2.0% agarose gel electrophoresis. Lane 1 to 5 shows the NN fragment (184 bp). Lane 6 shows the IN fragment (184 and 252 bp). M is the genetic marker.

Our present study did not find any significant association (P>0.05) between MTRR A66G gene polymorphism and the development of CHD. Similarly, a study on family-based transmission disequilibrium analysis among case-control of 169 CHD subjects and 213 child controls

reported that there were no significant association (P=0.086) of the fetal 66G allele with the development of CHD (Barron *et al.*, 2009).

The CBS enzyme is coded by the CBS gene located on the chromosome 21q22.3 chromosome and consists of 23 exons ranging from 42 to 209 bp (Cherry *et al.*, 2009). It has been reported that a deficiency of this enzyme could lead to homocystinuria resulting in an imbalance between the transsulfuration and methylation pathways in homocysteine metabolism (Malinowska & Chmurzynska, 2009; Zigelman & Edelstein, 2009).

The insertion-carrying allele of the 844ins68 polymorphism does not change the enzyme activity, but is poorly transcribed and causing an abnormal elevation of homocysteine and methionine in plasma and urine (Malinowska & Chmurzynska, 2009; Bi *et al.*, 2010). Li *et al.,* 2005, reported the heterozygosity of CBS 844ins68 was more prevalent in case compared to controls and yielded an odds ratio of 4.7 (95% CI 1.34-25.15) in the children's.

The result of our study showed that there was no significant difference between CBS 844ins68 gene polymorphism in both CHD and control groups (P>0.05). This was similar to a study done among Brazilian population between CBS 844ins68 gene polymorphism and CHD (Galdieri *et al.*, 2007). As the table 4 shows, there was no significant differences were observed between the genotypes of homocysteine gene polymorphisms and the various types of CHD subjects. The contradictory findings from our study with the other populations are might be due the ethnic differences of different populations that can result in different genetic predispositions. Another factor is the different sample size being used compared to other studies (Zhu *et al*., 2006). The present study was not focused on the protein expression of mRNA for the homocysteine enzyme genes and the samples were recruited from the three ethnic races: Malays, Chinese, and Indians was also a limiting factor for this study as most of the studies had a homogenous group of populations.

## Conclusion

The A1298C gene polymorphism of MTHFR gene showed a significant difference between CHD and controls when comparing between genotypes and it might be considred as a risk factor in CHD among Malaysian subjects. However, there was no significant association was found in the other gene polymorphisms, MTHFR C677T, MTRR A66G, MTR A2756G and CBS 844ins68 with development of CHD. However, replication studies with the larger number of samples and the other polymorphisms of the homocysteine enzyme genes are strongly recommended to confirm the association for the development of CHD among Malaysians.

## Authors' contributions

NFM contributed to the conceptualizing of the paper, data entry and writing of the manuscript while RV contributed in data analysis, drafting and writing of the manuscript. PI, NIJ, AE, AFAA, NFKAs, MA has contributed equally in drafting the manuscript. All the authors read and approved the final manuscript.

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