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Association of Angiotensin Converting Enzyme (ACE) Gene insertion/deletion (I/D) Polymorphism with Obesity and Obesity Related Phenotypes in Malay Subjects

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Abstract

Angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism has been identified as a potential candidate gene for obesity. The aim of this study was to identify the genotypic and allelic frequencies of ACE gene I/D polymorphism and its association with anthropometric parameters, lipid profiles and the susceptibility for obesity in Malay subjects. This cross-sectional, comparative study involved 219 subjects; 94 obese and 123 non-obese as controls. Anthropometric and lipid profiles were measured according to the standard method, alongside with genotyping analysis by polymerase chain reaction (PCR). Anthropometric and lipid profiles were compared between groups, and the association of this polymorphism with obesity was evaluated. Genotypic frequencies of II (47.9%), DD (42.7%) and DD genotypes (9.6%) in normal group were compared with genotypic frequencies of II (54.5%), DD (36.6%) and DD genotypes (8.9%) in obese group (P=0.620). The D allele distribution was 31.0% in normal comparing with 27.0% in obese group (P=0.410). Anthropometric parameters and lipid profiles did not differ significantly between the genotypes. However, D allele carriers exhibit consistently higher triglycerides, total cholesterol and LDL-cholesterol levels than the non-carriers without statistical significance. The *ACE* gene I/D polymorphism is not associated with obesity and obesity related phenotypes in Malay subjects; a weak interaction effect between the D allele with lipid profiles is seen.

Keywords: ACE gene I/D polymorphism, obesity, Malay, anthropometric parameters, lipid profile

1. Introduction

Obesity develops from a sustained positive energy balance that involves the interaction between genetic, environmental and behavioural factors (Yang et al., 2007; Galgani and Ravussin, 2008). The renin-angiotensin system (RAS) is an important regulator of blood pressure, body fluid homeostasis and other metabolic pathways associated with it (Grobe et al., 2013). Angiotensin II is the main effector of this system, produced from angiotensin I (Ang I) and angiotensinogen (AGT) via the effect of renin and angiotensin converting enzyme (ACE)(Frigolet et al., 2013).

In addition to some vital organs and tissues, adipose tissue also hosts a local renin-angiotensin system (Schling et al., 1999; Engeli et al., 2000). Adipose tissue RAS has been implicated in the regulation of visceral adipose tissue accumulation (Achard et al., 2007) and lipid metabolism (Jones et al., 1997), and thus may contribute towards the development of obesity and obesity-related metabolic disorders (Yang et al., 2013).

ACE contributes toward effective functioning of the RAS (Lemes et al., 2013). The gene encoding ACE maps to chromosome 17q23, spans 21 kilo bases (kb) long and comprises 26 exons and 25 introns (Sayed-Tabatabaei et al., 2006). A common insertion (I) /deletion (D) polymorphism in intron 16 of the ACE gene has been associated with differences in circulating ACE levels, with DD genotype contributing to the highest, followed by the ID and II genotypes for the intermediate and lowest levels, respectively (Rigat et al., 1990).

The ACE gene I/D polymorphism has been extensively studied for the distribution of ACE genotypes and alleles in different ethnic populations (Jayapalan et al., 2008) and its association with numerous disease conditions including hypertension (Heidari et al., 2014), coronary artery disease (Seckin et al., 2006), insulin resistance (Celik et al., 2010) and obesity (Strazzullo et al., 2003; Yang et al., 2013). However, the mechanism through which this

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* **Abbreviations:** Hypoxia-inducible factor-2, ACE; Angiotensin I-converting enzyme, BDNF: Brain-derived neurotropic factor, BLAST: Basic Local Alignment Search Tool, BMI: Body mass index

polymorphism affects obesity and its related phenotypes remains unclear. Therefore, we determine the genotypic and allelic frequencies of ACE gene I/D polymorphism and its association with anthropometric measurements, lipid profile and the risk for obesity in Malay subjects.

2. Materials and Methods

2.1. Subjects

This was a cross-sectional, comparative study. A total of 217 Malay adult subjects, aged between 18-60 years were recruited by a convenience volunteer sampling from educational establishments around Kuala Terengganu, Terengganu, Malaysia. They were 94 obese and 123 non-obese as control, selected after primary obesity screening using body mass index (BMI) which classifies them as obese (BMI $\geq$ 30kg/m^2) and non-obese (BMI 18.50 – 24.99kg/m^2) according to the World Health Organization criteria (WHO, 2000). Those who were eligible but with cardiovascular and respiratory disease, diabetic patients, underweight (BMI < 18.5kg/m^2) and overweight (25.0kg/m^2 $\leq$ BMI $\leq$ 29.99kg/m^2) were excluded from the study. Study approval was obtained from the Institutional Review Board of the Universiti Sultan Zainal Abidin (UniSZA), the Unit of Planning and Education Policy Research, Ministry of Education, Malaysia and Terengganu State Education Department before conducting the study. All subjects agree to participate and have signed their informed consent.

2.2. Anthropometric measurements

Weight (in kg) and height (in m) were measured to calculate the BMI by dividing the weight by the square of the body height. Waist circumference (WC) was measured between the final rib and the iliac crest at the end of the normal expiration, hip circumference was measured around pelvis at midpoint of maximum protrusion over buttocks by Ergonomic circumference measuring tape (Seca 201). Waist and hip circumferences were obtained to calculate waist-hip ratio (WHR). Waist-height ratio (WHtR) was calculated by dividing the measurement of waist circumference to that of the height. The assessment of body fat percentage (BF%) was done using a Slim Manager N40 (AIIA communications Inc, South Korea). Body adiposity index was determined using the formula: BAI = [hip (cm) / height (m)$^{1.5}$] – 18 (Bergman et al., 2011). Abdominal volume index was calculated using waist and waist-hip ratio (AVI= [2cm (waist) ^{2} + 0.7cm (waist-hip) ^{2}] / 1000 (Guerrero-Romero and Rodriguez-Moran, 2003). Conicity index was determined based on the previously established formula (Valdez, 1991).

2.3. Blood collection and biochemical assays

Blood samples were drawn after an overnight fast into plain and EDTA-coated tubes (Beckton Dickinson, Franklin Lakes, NJ). After serum separation, total cholesterol, triglycerides and HDL-cholesterol were analysed using an Olympus-AU400 chemistry analyser (Olympus, Tokyo, Japan) by enzymatic calorimetric method. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald's formula.

2.4. Blood extraction and Genotyping

The blood extraction procedure was based on manufacturer's protocol (Vivantis, CA, USA).

Genotyping was carried out by polymerase chain reaction (PCR) using the primers described from the previous studies (Rigat et al., 1992; Nikzamir et al., 2008). PCR was carried out

in a total volume of 20 µL containing 1X PCR buffer, 1.7mM $MgCl_2$, 0.34mM dNTPs, 0.8 µM of each primer and 1U *Taq* polymerase (Promega, Madison, WI). The amplified products at 190bp, 490bp and both 190bp and 490bp for II, ID and DD genotypes, respectively, and 335bp for confirmatory analysis of ID genotype were resolved on 2% agarose gel and visualized by ethidium bromide staining as shown in Figure 2.

2.5. Statistical analysis

All calculations were carried out using SPSS version 20.0 (IBM Corporation, Armonk, NY). Data was expressed as mean ± standard deviation (SD) or median (interquartile range). Genotypic and allelic frequencies were determined by manual counting and compared using the chi-square test. Significant differences between groups were evaluated using a one-way ANOVA, Kruskal-Wallis, independent sample *t*-test or Mann-Whitney U test where necessary. A binary logistic regression was used to identify the significant risk factors for obesity, considering their odds ratio (OR), 95% confidence intervals (CI) and the corresponding P-values. A *P*-value of less than 0.05 was considered as significant.

3. Results

3.1. Characterictics of the study subjects

The anthropometric data and lipid profiles of the study subjects by ACE I/D genotypes are presented in Table 1. All parameters for anthropometric characterization and lipid profiles did not differ significantly between the genotypes, however, total cholesterol and LDL-cholesterol levels were consistently higher in ID and DD subjects than the II subjects without significant differences.

Table 1. Anthropometric measurements and lipid profiles in all subjects according to ACE I/D genotypes.

	ACE genotypes (n=217)			*P*-value
	II (n=112)	ID (n=85)	DD (n=20)	
Age (year)[t]	36.31 ± 11.27	36.64 ± 9.76	31.70 ± 10.08	0.160
Weight (kg)[t]	66.77 ± 16.93	69.76 ± 18.69	67.29 ± 15.79	0.488
Height (m)[t]	1.59 ± 0.08	1.59 ± 0.08	1.60 ± 0.06	0.786
BMI(kg/m^2)[t]	26.37 ± 5.75	27.40 ± 6.15	26.27 ± 6.16	0.450
WC (cm)[t]	80.60 ± 14.46	83.80 ± 15.12	82.55 ± 16.30	0.326
HC (cm)[t]	98.86 ± 12.93	99.98 ± 10.40	99.98 ± 10.61	0.782
WHR[t]	0.82 ± 0.09	0.83 ± 0.10	0.82 ± 0.10	0.407
WHtR[t]	0.51 ± 0.08	0.53 ± 0.09	0.52 ± 0.10	0.322
CI[t]	1.14 ± 0.10	1.17 ± 0.10	1.17 ± 0.12	0.263
AVI[t]	13.70 ± 4.87	14.74 ± 5.15	14.42 ± 5.68	0.358
BAI[t]	31.53 ± 6.68	31.97 ± 5.30	31.51 ± 6.26	0.872
BF (%)[t]	28.25 ± 8.35	28.76 ± 7.52	26.60 ± 10.80	0.575
TG (mmol/L)[l]	1.00 (0.30-14.30)	1.00 (0.40-7.90)	0.85 (0.50-4.90)	0.677
TC (mmol/L)[t]	5.44 ± 1.39	5.68 ± 1.39	5.55 ± 1.38	0.499
HDL-C (mmol/L)[t]	1.27 ± 0.32	1.36 ± 0.32	1.30 ± 0.38	0.159
LDL-C (mmol/L)[t]	3.85 ± 1.18	3.99 ± 1.25	3.98 ± 1.06	0.720

[t]One-way ANOVA test; [l] Kruskal-Wallis non-parametric test. Data expressed as mean ± SD, or as median (minimum – maximum) for the skewed data.

Abbreviations: n, number of subjects; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; WHtR, waist height ratio; CI, conicity index; AVI, abdominal volume index; BAI, body adiposity index; BF (%), body fat percentage; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol

3.2. Genotypic and allelic frequencies of ACE gene I/D polymorphism

The ACE gene II, ID and DD genotype distributions were 47.9%, 42.7% and 9.6% in obese subjects and 54.5%, 36.6% and 8.9% in non-obese subjects, respectively, Figure 1 shows the vasiulization of the ACE alleles and genotypes on the gell picture. There was no significant difference observed between groups (P=0.620). The I allele frequency in obese and non-obese subjects was 69.0% and 73.0%, and the D allele frequency was 31.0% and 27.0%, respectively. The difference in allelic distribution between obese and non-obese was also not significant (*P*=0.410) (Figure 2).

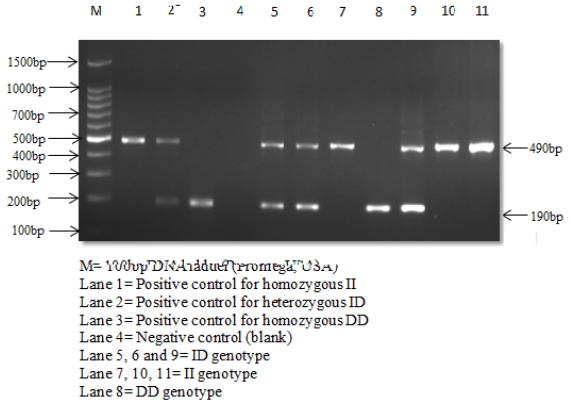

Figure 1. Determination of ACE gene I/D alleles by PCR (separated on a 2% ethidium bromide stained agarose gel)

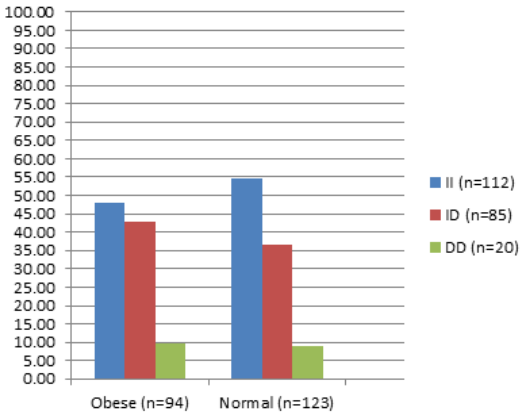

Figure 2. Genotypic and allelic frequencies of ACE I/D gene polymorphism with obesity

3.3. Anthropometric measurements and lipid profiles by ACE I/D genotypes in obese and non-obese subjects

The comparison of anthropometric measurements and lipid profiles in obese and non-obese subjects with their respective ACE I/D genotypes are shown in Table 3 and Table 4. Anthropometric parameters and lipid profiles in obese group did not differ among the genotypes, however, triglycerides, total cholesterol and LDL-cholesterol levels were consistently higher in ID and DD subjects as compared to the II subjects without significant differences (Table 2). In non-obese group, LDL-cholesterol was slightly higher in II subjects than the DD and ID subjects without statistical significance (Table 3).

Table 2. Comparison of anthropometric parameters and lipid profiles in obese subjects grouped by ACE genotypes

	ACE genotypes			*P*-value[a]	*P*-value[b]
	II (n=45)	ID (n=40)	DD (n=9)		
Age (year)[t]	40.13 ± 11.11	39.18 ± 8.51	34.44 ± 9.66	0.298	0.378
Weight (kg) [t]	83.74 ± 12.23	85.70 ± 13.89	82.70 ± 7.92	0.706	0.591
Height (m)[t]	1.60 ± 0.08	1.60 ± 0.09	1.59 ± 0.06	0.937	0.879
BMI(kg/m^2)[t]	32.64 ± 3.37	33.20 ± 3.51	32.52 ± 2.42	0.706	0.528
WC (cm)[t]	94.05 ± 10.86	96.10 ± 11.04	97.17 ± 12.82	0.601	0.328
HC (cm)[t]	109.98 ± 12.45	107.80 ± 9.06	109.72 ± 6.55	0.632	0.408
WHR [t]	0.87 ± 0.08	0.89 ± 0.08	0.89 ± 0.12	0.366	0.156
WHtR [t]	0.59 ± 0.06	0.59 ± 0.06	0.61 ± 0.08	0.511	0.293
CI [t]	1.19 ± 0.09	1.21 ± 0.09	1.24 ± 0.14	0.456	0.327
AVI [t]	18.21 ± 4.15	18.86 ± 4.26	19.39 ± 4.89	0.663	0.399
BAI [t]	36.51 ± 6.74	35.19 ± 4.61	36.63 ± 4.50	0.534	0.372
BF (%)[t]	33.87 ± 6.23	33.41 ± 6.01	34.22 ± 8.80	0.918	0.817
TG (mmol/L)[l]	1.20 (0.50 – 14.30)	1.35 (0.60 – 3.60)	2.10 (0.90 – 4.90)	0.403	0.233
TC (mmol/L)[t]	5.48 ± 1.45	5.96 ± 1.43	5.92 ± 1.59	0.294	0.117
HDL-C (mmol/L)[t]	1.16 ± 0.32	1.27 ± 0.25	1.18 ± 0.38	0.247	0.146
LDL-C (mmol/L)[t]	3.89 ± 1.16	4.35 ± 1.28	4.28 ± 1.19	0.216	0.081

[t]One-way ANOVA test; [l] Kruskal-Wallis non-parametric test. Data expressed as mean ± SD, or as median (minimum – maximum) for the skewed data

[a] Comparison by one-way ANOVA test (II genotype vs. ID genotype vs. DD genotype) for all variables except for TG by Kruskal-Wallis non-parametric test

[b] Comparison by *t*-test (II genotype vs. ID + DD genotypes) for all variables except for TG by Mann-Whitney U test

n, number of subjects; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; WHtR, waist height ratio; CI, conicity index; AVI, abdominal volume index; BAI, body adiposity index; BF (%), body fat percentage; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol

Table 3. Comparison of anthropometric parameters and lipid profiles in non-obese subjects grouped by ACE genotypes

	ACE genotypes			*P*-value[a]	*P*-value[b]
	II (n=67)	ID (n=45)	DD (n=11)		
Age (year)[t]	33.75 ± 10.71	34.38 ± 10.33	29.45 ± 10.29	0.378	0.861
Weight (kg) [t]	55.38 ± 7.36	55.60 ± 7.72	54.68 ± 5.93	0.934	0.974
Height (m)[t]	1.58 ± 0.08	1.58 ± 0.08	1.61 ± 0.06	0.531	0.790
BMI(kg/m^2)[t]	22.17 ± 1.82	22.25 ± 1.89	21.25 ± 1.85	0.198	0.697
WC (cm)[t]	71.56 ± 8.16	72.86 ± 8.27	70.59 ± 4.86	0.590	0.556
HC (cm)[t]	91.39 ± 6.07	93.03 ± 5.36	92.00 ± 4.92	0.333	0.166
WHR [t]	0.78 ± 0.07	0.78 ± 0.08	0.77 ± 0.05	0.791	0.809
WHtR [t]	0.45 ± 0.05	0.46 ± 0.05	0.44 ± 0.04	0.361	0.626
CI [t]	1.11 ± 0.09	1.13 ± 0.10	1.11 ± 0.07	0.570	0.353
AVI [t]	10.68 ± 2.30	11.08 ± 2.31	10.34 ± 1.32	0.517	0.533
BAI [t]	28.18 ± 4.04	29.11 ± 4.13	27.33 ± 3.94	0.316	0.433
BF (%)[t]	24.48 ± 7.43	24.62 ± 6.23	20.35 ± 8.04	0.361	0.593
TG (mmol/L)[l]	0.90 (0.30-4.90)	0.80 (0.40-7.90)	0.70 (0.50-1.20)	0.181	0.416
TC (mmol/L)[t]	5.42 ± 1.35	5.43 ± 1.32	5.25 ± 1.17	0.914	0.917
HDL-C (mmol/L)[t]	1.33 ± 0.30	1.43 ± 0.36	1.39 ± 0.38	0.309	0.136
LDL-C (mmol/L)[t]	3.82 ± 1.21	3.66 ± 1.15	3.73 ± 0.92	0.775	0.485

[t]One-way ANOVA test; [l] Kruskal-Wallis non-parametric test. Data expressed as mean ± SD, or as median (minimum – maximum) for the skewed data

[a] Comparison by one-way ANOVA test (II genotype vs. ID genotype vs. DD genotype) for all variables except for TG by Kruskal-Wallis non-parametric test

[b] Comparison by *t*-test (II genotype vs. ID + DD genotypes) for all variables except for TG by Mann-Whitney U test

n, number of subjects; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; WHtR, waist height ratio; CI, conicity index; AVI, abdominal volume index; BAI, body adiposity index; BF (%), body fat percentage; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

3.4. sociation of ACE gene I/D polymorphism and other predictor variables with obesity

In a logistic regression analysis, the ACE I/D genotypes were analyzed as categorical variables while age, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were included as continuous variables (Table 4). There was no evidence that the ACE I/D genotypes can be regarded as an independent risk factor for obesity.

Table 4. Logistic regression analysis for the association of ACE genotypes and other predictor variables with obesity

		OR	95% CI	*P*-value
Age		1.05	1.03 – 1.08	< 0.001
ACE genotypes				
	II	1.00 (ref.)		
	ID	1.32	0.75 – 2.34	0.335
	DD	1.22	0.47 – 3.18	0.687
	II	1.00 (ref.)		
	ID + DD	1.30	0.76 – 2.23	0.335
TG (mmol/L)		1.81	1.24 – 2.65	0.002
TC (mmol/L)		1.18	0.97 – 1.44	0.096
HDL-C (mmol/L)		0.19	0.08 – 0.47	<0.001
LDL-C (mmol/L)		1.31	1.03 – 1.65	0.026

Age, TG, TC, HDL-C and LDL-C are included as continuous variables; ACE genotype (II, ID and DD genotypes; II and ID + DD genotypes) are included as continuous variables

TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; OR, odds ratio; CI, confidence intervals.

4. Discussion

In this cross-sectional, comparative study, the ACE gene I/D polymorphism was determined for its association with lipid profile, anthropometric measurements and the risk for obesity in Malay adult subjects. The D allele frequency in our study obese group (31.0%) was similar to that range established in other Asian populations between 30.6% - 42.4% for the Indonesian (Sinorita et al., 2010), Korean (Um et al., 2003; Kim, 2009; Yang et al., 2013) and Japanese (Uemura et al., 2000), except for the higher frequency among the Turkish (Bitigen et al., 2007; Akin et al., 2010), Asian Indian (Poornima et al., 2014), Pakistan (Javaid et al., 2011) and Arabs (El-Hazmi and Warsy, 2003). Asia is known for its ethnically diverse populations and therefore could have a diverse patterns of D allele. Comparative studies for obese Africans (Cooper et al., 1997; Mehri et al., 2012) and Caucasians (Alvarez-Aguilar et al., 2007; Bell et al., 2007; Wacker et al., 2008; Fiatal et al., 2011) from different countries confirm their phylogenetic similarities despite being from different geographical areas.

This study found that the ACE gene I/D polymorphism was not a predisposing factor for obesity in Malay, demonstrated by similar D allele distribution in both obese and non-obese groups. Furthermore, logistic regression analysis showed that this polymorphism is unlikely to increase the risk for obesity in this ethnicity. Also, no significant differences between the ACE genotypes in all parameters for anthropometric characterization indicates that this polymorphism was not involved in the regulation of body mass and adipose tissue. Previous studies among the Pakistani (Javaid et al., 2011), Mediterranean population in Southern Europe (Riera-Fortuny et al., 2005), Turkish (Bitigen et al., 2007), Greeks (Moran et al., 2005) Italian (Strazzullo et al., 2003) and Korean (Yang et al., 2013) populations provide considerable support for the association of this polymorphism with obesity and its associated phenotypes. However, other studies failed to demonstrate any association at all (Nagi et al., 1998; Um et al., 2003; Wacker et al., 2008). Differences in study design (Um et al., 2003; Riera-Fortuny et al., 2005; Javaid et al., 2011), effects of gender and age (Strazzullo et al., 2003; Wacker et al., 2008), racial differences in D allele distributions, different criteria used to define obesity (Uemura et al., 2000; Um et al., 2003; Riera-Fortuny et al., 2005; Kim, 2009) and nutrition profile (Yang et al., 2013) could be the reason for inconsistencies between these studies.

This study also found no significant interaction effect between the ACE gene I/D polymorphism with lipid profiles in the entire subjects, obese and non-obese groups. However, carriers for D allele exhibit consistently higher triglycerides, total cholesterol and LDL-cholesterol without significant differences. The association between this polymorphism and lipid profiles was still at the matter of controversy. Carriers for D alelle have been associated with altered lipid values in Japanese (Suzuki et al., 1996), Israeli (Oren et al., 1999), Korean (Kim, 2009) and Mexican (Alvarez-Aguilar et al., 2007) populations. However, results from some other reports were contradictory (Nagi et al., 1998; Uemura et al., 2000; Um et al., 2003). The mechanism by which ACE gene variants may influence lipid levels could be attributable to the specific localization of the renin-angiotensin system in adipose tissue (Schling et al., 1999), which suggests that it may also involved in lipid metabolism. Differences in ethnic background (Mao and Huang, 2013) and dietary patterns (Yang et al., 2013) may also become significant contributors.

Literature regarding the association of this genetic polymorphism with obesity among Malay subjects is scarce. Although the ACE gene I/D polymorphism was not associated with obesity and its related phenotypes in Malay, the risk imparted by this polymorphism still merits attention. With regard to obese individuals potentially developing favorable metabolic profile (Seo and Rhee, 2014), adipose RAS overexpression is also likely to occur in certain obese individuals (Kalupahana and Moustaid-Moussa, 2012), which could indirectly suggest that further studies are needed to undermine the impact of this genetic polymorphism in obesity among this ethnicity.

5. Conclusion

In conclusion, we have successfully identified the genotypic and allelic frequencies of ACE gene I/D polymorphism in Malay subjects. Although this polymorphism is not associated with obesity in this ethnicity, triglycerides, total cholesterol and LDL-cholesterol levels showed a consistently higher trend in D allele carriers as compared to the non-carriers.

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