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A population frequency analysis of the FABP2 gene polymorphism in the Egyptian population

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ABSTRACT

Background: Intestinal fatty acid-binding protein (IFAPB) is expressed only in intestinal enterocytes. It may participate in the uptake, intracellular metabolism and/or transport of long chain fatty acids. A polymorphism at codon 54 in exon 2 of the FABP2 gene, which encodes for the IFAPB, exchanges an alanine (Ala) for threonine (Thr). The goal of this study was to determine the frequency of the Ala54Thr FABP2 polymorphism in the Egyptian population.

Patients and Methods: Genotyping was carried out in 180 unrelated Egyptian subjects. DNA was extracted from blood samples for genotype analysis. A PCR-RFLP assay was applied for the determination of Ala54Thr FABP2 polymorphism. Allele frequencies were calculated by direct counting. Hardy Weinberg Equilibrium was evaluated using a Chi-square goodness of fit test.

Results: Showed that 102 (56.7%) of the studied Egyptian subjects were homozygous for the Ala54/Ala54 genotype, 60 (33.3%) were heterozygous for the Ala54/Thr54 genotype and 18 (10.0%) were homozygous for the Thr54/Thr54 genotype. The frequencies of the allele Ala54 and the allele Thr54 of the FABP2 Gene were found to be 0.733 and 0.267, respectively. The results revealed a similar population polymorphism frequency as in previous European studies.

Conclusion: This is the first study to look at the population frequency of the Thr54 allele in Egypt.

Key Words:

FABP2 gene polymorphism, Egyptians, human population genetics.

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BACKGROUND

Fatty acid – binding proteins (FABPs) are intracellular proteins found in many tissues. They are involved in fatty acid transfer and metabolism[1]. The FABPs are a family of 15-kDa proteins which are abundantly found in cytosol, representing up to 6% of protein depending on cell type[2,3]. Approximately 10 separate mammalian FABP have been identified[4]. FABP2 are encoded by a family of different genes. The FABP2 gene encodes intestinal FABP (I-FABP), which is expressed only in intestinal enterocytes[5]. The FABP2 gene consists of approximately 3.4 kilobases located in chromosomal region 4q28-4q31[6]. It me-

diates the absorption and transport of fatty acids inside the enterocyte and has a high affinity for both saturated and unsaturated long chain fatty acids[7]. The G to A polymorphism at codon 54 in exon 2 of the human FABP2 gene exchanges an Alanine (Ala) in the small helical region of the protein for Threonine (Thr). Thr-containing protein has been shown to have 2-fold greater affinity for long-chain fatty acids than does Ala-containing protein[8]. This greater affinity has been suggested to cause increased absorption and processing of fatty acids[8]. The Ala54Thr polymorphism is the most extensively studied FABP2 variant, as this variant seems the most likely candidate to alter the protein's function. Different studies suggest that the Ala-to-Thr substitution is in fact a functional mutation[8,9]. If the FABP2 gene polymorphism in any way modifies the absorption of fatty acids, it could in turn affect the lipid metabolism and/or correlate with cardiovascular disease risk. Earlier studies have shown that the IFABP Thr54 allele is significantly associated with higher total cholesterol, with stroke incidence[10], elevation of fasting and postprandial triglyceride[11], higher nonesterified fatty acid concentrations[12] and insulin resistance in the Pima Indians[8], Native Canadians[13] and Mexican-Americans[14]. Also, the Thr allele has been associated with visceral fat in Japanese subjects[15], triglycerides in Finnish hyperlipidemic males[16] and Body Mass Index and percent body fat in Aboriginal Canadians[13]. There are many contradicting studies, however, that have found non-significant association with these parameters[17-20].

The frequencies of the FABP2 alleles have been studied in many ethnic groups and demonstrated interethnic variation in their distribution. The frequency of the wild-type alanine allele was highest in Tongans (Polynesians)[21] and lowest in the Japanese[22]. However, no information is available for Egyptian population. In the light of the potential physiological role of the FABP2 polymorphism, the goal of this study was to determine the frequency of the Thr54 allele in the Egyptian population.

PATIENTS AND METHODS

Subjects:

The blood samples studied were collected from 180 unrelated individuals from the city of Ismailia, Egypt. A written informed consent from all participants included in this study was done under institutionally approved internal review board protocols. This study was carried out in the Arabian Gulf University, Bahrain.

Genotype analysis:

DNA extracted from blood samples by standard methods[23]. The DNA samples were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[24]. A 180-bp fragment was amplified. DNA was amplified in a total volume of 25 µl, with the upstream primer: 5'-ACAG-GTGTTAATATAGTGAAAAG-3' and the downstream primer: 5'-TACCCT-GAGTTCAGTTCCGTC-3'[9]. The PCR program consisted of an initial denaturation step at 94°C for 5 min followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30s and extension at 72°C for 30s. The final extension step was performed at 72°C for 10 min. For Restriction Fragment length Polymorphism (RFLP) analysis, 5 µl of PCR

product were incubated with 0.4 µl of enzyme HhaI (GCG/C) (10 U/µl, New England Biolabs) in a final volume of 10 µl overnight at 37°C. The products were run on 3% agarose gel containing 1mg/mL ethidium bromide, electrophoresed for 40 min at 120 volts and photographed under ultraviolet light. The PCR products that lack the HhaI site migrate as one 180-bp fragment (those carrying the Thr-54), but PCR products containing the HhaI site are cleaved to two fragments (a 99-bp and an 81-bp).

Statistical analysis:

Genotype and Allele frequencies were calculated by direct counting. Hardy Weinberg Equilibrium was evaluated using a Chi square goodness of fit test. The confidence limits were calculated by the online statistic tool.[25]

RESULTS

The genotype and allele frequencies of Ala54/ Thr54 mutation in the Egyptian population have been shown in Table 1. The study population was found to be in Hardy–Weinberg equilibrium. The data in Table 1 show that the Ala54/Ala54 genotype is the most prevalent (56.67%) followed by Ala54/Thr54 (33.33%) and Thr54/Thr54 (10.00%). In this Egyptian population sample, the frequencies of allele Ala54 and allele Thr54 were 0.733 (95% CI, 0.684-0.778) and 0.267 (95% CI, 0.222-0.316), respectively. Figure 1 shows the electrophoresis patterns for FABP2 genotypes by PCR-RFLP based assay.

Table 1: Distribution of the various FABP2 genotype and allele frequencies among the Egyptian population.

Egyptians (n) = 180	FABP2 Genotype	Percentage Observed (and Expected)	FABP2 Allele Frequency		Heterozygosity	
			Ala 54	Thr 54	Observed	Expected
	Ala54/Ala54	56.7 (53.9)				
	Ala54/Thr54	33.3 (38.9)	0.733	0.267	0.333	0.392
	Thr54/Thr54	10 (7.2)				

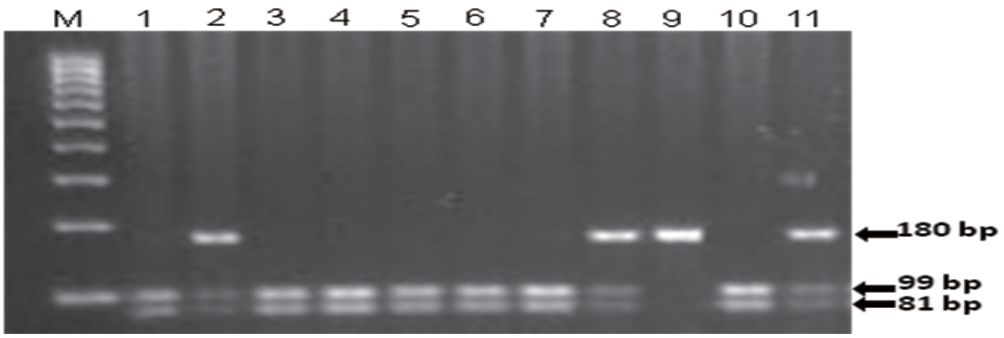

Fig. 1: Electrophoresis patterns for FABP2 genotypes by PCR-RFLP based assay, persons with homozygous genotype (Ala54/Ala54) (lanes 1, 3-7 and 10), homozygous genotype (Thr54/Thr54) (lane 9) or heterozygous genotype (Ala54/Thr54) (lanes 2, 8 and 11), are shown. M is 100 bp DNA marker.

The observed genotype results did not differ significantly from the expected genotypes for a population in Hardy Weinberg equilibrium (Table 1) ($p > 0.05$, Chi squared goodness of fit). Comparison of reported population frequencies for the FABP2 genotypes and alleles are given in (Table 2).

Table 2: Genotype percentages and allele frequencies of FABP2 polymorphism in various ethnic groups.

Study Group (n)	Genotype %			Allele frequency		P (vs. Egyptians)	Reference
	Ala/Ala	Ala/Thr	Thr/Thr	Ala	Thr		
Egyptians (180)	56.7	33.3	10	0.733	0.267		This study
Finnish (170)	49	45	5	0.725	0.275	0.7290	(19)
Sweden (59)	52.5	35.6	11.9	0.703	0.297	0.1492	(10)
United Kingdom (69)	62.3	31.9	5.8	0.783	0.217	0.0105	(26)
Middle Europe (102)	51.0	43.1	5.9	0.726	0.275	0.7401	(26)
South Europe (90)	56.7	36.7	6.7	0.750	0.250	0.4131	(26)
Japanese (258)	45	41	14	0.665	0.345	0.0003	(22)
Indians (899)	53.6	39.3	7.1	0.732	0.268	1	(27)
Argentineans (202)	52.0	40.6	7.4	0.723	0.277	0.6547	(24)
Native Canadians (188)	0.74	0.24	0.02	0.860	0.140	0.0001	(13)
African Americans (1831)	60	35	5	0.775	0.225	0.0333	(28)
Tongans (1022)	76	23	1	0.876	0.124	0.0001	(21)

DISCUSSION

The present study investigated for the first time the frequency of the Ala54Thr SNP in FABP2 gene among 180 unrelated Egyptians and compared the results with data reported for other ethnic groups (Table 2).

The observed frequency of the Thr54 allele (0.267) in Egyptian subjects is similar to that reported in different European countries including, North Europe: Finland[19] and Sweden[10]; Middle Europe[26]: Denmark, Germany, Belgium and Switzerland; southern Europe[26]: Portugal, Italy, Spain and Greece. Also, it is similar to that reported in Argentineans, who are mostly of Europeans descent[24]. It is significantly different from that of British.[26]

The frequency of the wild Ala54 allele among Egyptians is significantly different from that reported for the Japanese[22]. It is also different from the African-Americans[27], Native Canadians[13] and Tongans (Polynesians)[21]. Interestingly, the frequency of the wild Ala54 allele in Egyptians subjects did not differ from that reported in Indians[28]. There were no available data from literature in other Arab population for comparison.

It is well known that the single nucleotide polymorphism (SNP) at codon 54 of the FABP2 gene is a missence variant that has a definite effect on the pri-

mary structure of the protein and affects its fatty acid binding properties. It is not known, however, whether this change can affect the lipid metabolism of carriers[29]. Many earlier studies have reported associations between this polymorphism and insulin resistance, BMI, dyslipidemia, stroke, metabolic syndromes and hypertriglyceridemia[5,8, 10-13,27,30]. The Thr polymorphism has been associated with diabetes related variables in Mexican Americans[14], Pima Indians[8,31], Japanese men[22], Indian migrants[32] and various Caucasian populations[33-35]. In contrast, other studies report no association with the same parameters[16,35-37]. Part of the discrepancy among the studies may come from the different dietary habits of the analyzed populations.[38]

Thr-containing protein has been shown to have 2-fold greater affinity for long-chain fatty acids than does Ala-containing protein[8]. This greater affinity has been suggested to cause increased absorption and processing of fatty acids[8]. In the present study, about 10% of Egyptian subjects were homozygous for the Thr54 allele and 27% of them have at least one Thr allele, with their Thr-containing proteins may have greater affinity to long-chain fatty acids which may leads to increased absorption and processing of fatty acids in those subjects. This group of population may be liable to different types of diseases associated with increase absorption of the long-chain fatty acid including, cardiovascular diseases, stroke, obesity, diabetes, etc. Association studies between Thr54 allele of FABP2 gene and these diseases among Egyptians are needed.

CONCLUSION

It should be noted that this was the first description of Thr54 allele of FABP2 gene in Egyptian population which does not exclude the possibility of the presence of new SNPs unique to the Egyptian population. The population frequency of the Thr54 allele in Egypt does not differ from previously reported frequencies in European populations.

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