

Genetic Polymorphism of ITGA2 Gene and the Risk of Heart Attack and Stroke in Al-Anbar Population/Iraq

Reem M. Al-Qaisi¹, Louay Al-Ani¹, Mohammed M.F. Al-Halbosiy²

¹ Department of Biology-College of Science-University of Anbar

² Biotechnology Research Center-University of Al-Nahrin, Baghdad

Abstract

Objectives:

The study was aimed to determine the association of mutant patterns TT/AA of both SNPs C807T and G873A of ITGA2 gene with two types of cardiovascular diseases, Myocardial Infarction (MI) and Cerebrovascular Accident (CVA), in Al-Anbar population.

Methods:

A case-control study involved 120 individuals divided into two groups (80 patients and 40 controls), dealt with two types of cardiovascular diseases: (MI 40%) and (CVA 27%) for both genders. Blood samples were collected in Al-Ramadi Teaching Hospital within the period from first of July, 2017 to mid-August, 2017. Genotyping of DNA samples was accomplished by AS-PCR technique in Biotechnology Research Center at Al-Nahrin University and statistical analysis was done by SPSS version: 21.0.

Results:

Results showed that mutant patterns TT/AA are completely present in patients and in about half of controls with genotype frequency (100%) in patients and (53%) in controls. While frequency of wild patterns CC/GG was 0 in patients and (47%) in controls, the mutant patterns of both SNPs showed association with hyperlipidaemia with X^2 (5.013) and significant P value (0.04) which reflects a strong relation between SNPs and high levels of lipids. SNPs C807T and G873A are at high linked disequilibrium with D value (0.1) and D' (0.99) at P value (0.0001) refers to high LD between two SNPs.

Conclusion:

The mutant pattern of both SNPs, 807TT/873AA, is the main cause and risk for MI and CVA by their association with hyperlipidaemia in Al-Anbar population.

Key words: AS-PCR, Collagen receptor, CVA, ITGA2 SNPs, MI.

INTRODUCTION:

Glycoprotein Ia/IIa (GPIa/IIa), also called integrin $\alpha 2\beta 1$, is a glycoprotein on the membrane of platelets which is a collagen receptor [Ref.]. In spite of the presence of several supposed platelet collagen receptor, GPIa/IIa is theorized to be essential receptor contained in platelet adhesion and activation [1]. Different types of cells have the GPIa/IIa which is expressed on them including platelets, megakaryocytes, endothelial cell, epithelial cell and fibroblasts [2]. The expression level of this glycoprotein on the surface of platelet is related with the allelic differences in the ITGA2 gene which located on fifth chromosome5q11.2.

Many of nucleotide polymorphism of GPIa gene has been substantive. Two linked dimorphism in the coding region of the GPIa gene at the site of 807 and 873 have been recognized which have C-T polymorphism nucleotide at 807 nucleotide and G-A polymorphism nucleotide at 873 nucleotide. These two SNPs described as silent dimorphism because they doesn't change the amino acid sequence of the GPIa gene. The expression level of the GPIa/IIa on the surface of platelet has a significant correlation with these two dimorphisms [3]. There are two silent bi-allelic polymorphisms on subunit $\alpha 2$ of the GPIa have been recognized; C807T locates on the exon7 and G873A locates on the exon8. These two mutations are in complete linkage disequilibrium, having known to be related with the density of GP Ia/IIa expression [4]. These two SNPs C807T (phe224) and G873A (Thr246) within ITGA2 when it is related with low receptor densities, it is in homozygous genotype for 807C/873G allele while platelet with high receptor densities is related with T807/A873 alleles which are homozygous mutant alleles [5]. Consequently, related with cardiovascular diseases such as myocardial infraction (MI) which considered the major reason of morbidity and mortality in the western world [6]. Also, Cerebrovascular Accident (CVA), the essential type of stroke is ischemic stroke, which causes high morbidity and mortality [7]. These diseases have been theorized to be associated with genes, environment and interaction between them. There are many risk factors, which are recognized to be etiological in the progression of myocardial infraction (heart attack) and cerebrovascular

accident (stroke), like hypertension, smoking, diabetes, hyperlipidaemia, obesity, thrombotic diseases and so on [8, 9, 10].

A study conducted by Moshfegh et al. [5] showed that alleles 807T/873A, which are related with high density of platelet membrane receptors, are associated with increasing risk of myocardial infraction. Another study [11] had revealed a relation of the 807T/873A allele with non fatal myocardial infraction in younger patients. Also, some studies showed that the GPIa/IIa polymorphism were completely critical foreteller of the danger of ischemic stroke [12]. According to these findings, we did a case-control study aimed to study the association between MI/CVA and these two SNPs in Al-Anbar population.

MATERIALS & METHODS

Population

The study was a case-control study involved 120 individuals divided into two group (80 patients and 40 controls). The study was planned to deal with two types of cardiovascular diseases (myocardial infraction MI and cerebrovascular accident CVA) in both genders. The mean age of participants was 60.24 years. Patients with myocardial infraction (46 individuals) included 14 females and 32 males. On the other hand, patients with cerebrovascular accident (34 individuals) included 20 females and 13 males. Blood samples were collected in Al-Ramadi Teaching Hospital/ Recovery room at morning time for the period from first of July till mid of August, 2017.

Risk factors

Questions were directed to patients include risk factor of MI and CVA that involve gender, age, hight, weight, hypertension, diabetes, smoking, hyperlipidaemia and family history of MI and CVA. Body Mass Index (BMI) was calculated for each participant as follows: $BMI = \text{Weight (kg)} / [\text{Height(m)}]^2$.

Genotyping

By using standard procedure, DNA was isolated from leukocytes. The sequences of two SNPs, C807T/G873A, have been extracted from Gene Bank (Accession No. AF035968) [2]. Genotyping of GPIa was achieved by (PASA) polymerase amplification of specific allele, a technique which is also known as (AS-PCR)

allele specific polymerase chain reaction that depends on specifically designed primers; two forward primers to each SNP (one to the wild type allele and the other for mutant allele) and reverse primer for both of them. Table (1)

Table (1): Primers sequence

Primer	Sequence
Forward 1	5'ATGGTGGGGACCTCACAAACACATAT'3
Forward 2	5'GGTGGGCGACGAAGTGCTAGG'3
Reverse 1	5'GATTTAACTTCCAGCTGCCTTC'3
Forward 3	5'GTGGGGACCTCACAAACACATGC'3
Forward 4	5'GGTGGGCGACGAAGTGCTAGA'3
Reverse 2	5'CTCAGTATATTGTCATGGTTGCATTG'3

So that, the two SNPs, 807T and 873G, were amplified in first reaction whereas 807C and 873A were multiplexed in the second reaction [13]. PCR reactions were equipped in 25µl volume as follows: reaction1 (12.5µl master mix, 0.6µl forward 1 primer, 0.7µl forward 2 primer, 0.6µl reverse 1 primer, 2µl DNA, 8.6 distilled water D.W) and reaction2 (12.5µl master mix, 0.5µl forward 3 primer, 0.7µl forward 4 primer, 0.7µl reverse 2 primer, 2µl DNA, 8.6µl distilled water D.W). PCR programs were conducted as initial denaturation 94°C for 2minutes and(94°C denaturation- 1minutes, 55°C annealing- 1minute, extension 72°C- 1minute) for 35 cycles to the first reaction. while second reaction program: 94°C for 2minutes as initial denaturation and (denaturation 94°C for 1minute, annealing 62°C for 1minute, extension 72°C for 1minute). 5µl of each sample from PCR product were loaded into 2% agarose gel and stained with red safe. Determination of genotype was proceeded as clarified in [14].

Statistical analysis

Statistical analysis was performed using the SPSS software (Version 21.0; SPSS Inc., Chicago, IL, USA). The X² test was used to compare the distributions of GPIa C807T/G873A genotypes, allele frequencies and qualitative risk factors between patients and controls. Odd ratios were calculated as estimates of the relative risk of CVD associated with carrier ship of the GPIa 807T/873A alleles, with 95% confidence limits determined. Adjustment was made for the dichotomized risk factors: gender (male/female), history of cardiovascular disease (yes/no), hypertension (yes/no), hyperlipidaemia (yes/no), diabetes (yes/no), smoking (yes/no), and for the continuous risk factors; age and BMI. Statistical significance was assumed for P values <0.05. Linkage disequilibrium analysis was calculated by ICO qualitat: calidesa. www.bioinfo.iconcologia.net/SNP stats (2018).

RESULTS AND DISCUSSION:

Allele and genotype frequencies for 807 and 873 SNPs

120 samples were studied to detect their possible relation of ITGA2 polymorphisms C807T/G873A with Myocardial Infarction (MI) and Cerebrovascular Accident (CVA). There was complete presence of the mutant patterns of both SNPs TT/AA in patients samples (Figure 1) with allele frequencies (1 807T/873A, 0 C807/G873 vs 0.53 807T/873A, 0.47 C807/G873), and genotype (Table 2, 3).

In order to know the genetic diversity of our population according to the Hardy-Weinberg equilibrium, the statistical analysis for SNP 807 which includes two types of alleles (polymorphism) C and T, the frequency of T mutant allele in patients was 1.0 compared with healthy controls where is the frequency of mutant allele T was 0.53. on the other hand, the wild allele C in patients was with 0.0 frequency compared to healthy controls where it was frequent (0.47). Study of Park et al [15] showed that the frequency of C allele was 0.68 while T allele was 0.32 in patients with MI in the Korean population in

comparison with their controls where the frequencies were 0.65 and 0.35 for C and T, consequently. Therefore, it can be concluded that there is no association between T807 allele and MI. Study on Germany population, by Santoso et al. [16] confirmed the prospective importance of inherited differences in ITGA2 in healthy and patients together. Allele frequency of patients with MI was 0.59 for C allele and 0.41 for T allele in comparison with healthy individuals where allele frequency of C allele was 0.60 and 0.40 for T allele.

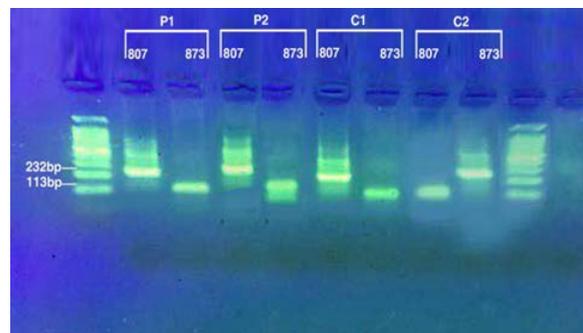


Figure (1): AS-PCR products of ITGA2 analyzed on 2% agarose and stained with red safe with 100bp ladder standard size. P1 and P2 both are homozygous mutant 807TT/873AA sample, C1 homozygous mutant type 807TT/873AA, C2 homozygous wild type 807CC/873GG.

Table (2): Distribution of C807T/ G873A allele frequencies

SNPs.807 and 873 allele frequencies (n=62)						
	All subjects		Status=control		Status=patient	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
T/A	106	0.85	20	0.53	86	1
C/G	18	0.15	18	0.47	0	0

Table (3): Distribution of CC807TT/ GG873AA genotype frequency

SNPs.807 and 873 genotype frequencies (n=120)						
	All subjects		Status=control		Status=patient	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
CC/GG	9	0.15	9	0.47	0	0
TT/AA	53	0.85	10	0.53	43	1
NA	58	---	21	---	37	---

Table (3) shows that G873 was with no frequency in patients i.e. 0.0% while A allele frequency was completely 1.0 in comparison with 0.47 frequency for allele G and 0.53 for allele A in control group, explaining the expression power of mutant allele A in patients compared to wild allele G. The study conducted by Moshfegh et al. [5], showed that mutant allele related with increased risk of MI with allele frequency 0.68 for G allele and 0.32 for A allele in white Swiss patients. Polat et al. [17] did a study on patients with venous thrombosis and reported that G/A also C/T SNPs (because they showed two SNPs always at high linked) were related with increased pathogenesis with allele frequency G 0.57 and A 0.42 in patients in comparison to 0.68 and 0.31 for G and A, respectively, in controls.

Genotype frequencies results for 807 SNP showed that the mutant genotype TT frequency was 1.0 in patients in comparison with controls 0.53 while the wild genotype CC was with no frequency in patients (0.0%) compared to 0.47 in controls. These data refers to the possibility that TT formed an etiological factor to diseases.

Moreover, Lu et al. [18] declared that mutant allele T of 807 SNP is related with ischemic stroke with genotype frequency 42.3 for CC, 18.3 for TT and 40.7 for CT. In Chinese patients

with ischemic stroke compared to controls, frequencies of 46.3, 8.3 and 40.0 were reported for CC, TT and CT, respectively. Furthermore, Zhang et al. [19] showed that CC, TT, and TC were not statistically different between patients and controls, but these data have not been confirmed by previous studies. The latter study reported frequencies of 46.5 and 12.8 for CC and TT, respectively, in patients with stroke, and frequencies of 50.0 and 10.0 for CC and TT, respectively, in healthy control.

Genotype frequency for GG873AA results showed that wild genotype GG was with no (0.0%) frequency in patients while mutant genotype AA was complete (1.0%) in patients compared to controls (GG was 0.47 while AA was 0.53). It appears that the results of both SNPs are the same in all individuals which led us to the conclusion that these two SNPs are linked tightly together with high linked disequilibrium which is compatible with many previous studies. These data will be discussed in light of available literature.

Moshfegh et al. [5] demonstrated that GG frequency was 83.6 while AA frequency was 16.4 by a study on white Swiss MI patients in comparison with controls, GG frequency was 94.4 and AA frequency was 65.6 which indicates significantly higher prevalence of MI patients with homozygous T/A rather than controls. Casorelli et al. [20] declared that increased risk of ischemic stroke is associated with mutant pattern in Italian population with a frequency for GG of 38.2 and 12.7 for AA in patients with ischemic stroke versus controls (GG frequency 49.7 and AA frequency 4.8).

It should be noted that the heterozygous genotype for both SNPs C807T and G873A never appeared within the total of our samples. This absolutely doesn't mean that our population doesn't contain this gene structure in the concerned gene ITGA2, but this result represents only the collection of samples that included in this study where the genetic and environmental conditions were the greatest helpers in the appearance of mutant genotype that dominated the rest of genotypes. These results indicated the dominance of the mutant alleles T/A for both SNPs in patients group and about half of the healthy group in Al-Anbar population.

Table (4): Population characteristics according to GPIa C807T/G873A genotypes

Independent Variables	Cases of study				X ²	P value
	AA	GG	CC	TT		
History	9	0	0	9	1.788	0.333
	44	9	9	44		
Gender	32	4	4	32	0.802	0.589
	21	5	5	21		
Hypertension	31	3	3	31	1.966	0.277
	22	6	6	22		
Diabetes	14	2	2	14	0.071	1.00
	39	7	7	39		
Hyperlipidaemia	20	0	0	20	5.013	0.047
	33	9	9	33		
Smoking	11	1	1	11	0.458	0.675
	42	8	8	42		

Association between SNPs and risk factors

Results of the possible association between SNPs and risk factors for MI and CVA in the study population are presented in Table 4. The results showed that, except for hyperlipidaemia, there weren't significant associations between SNPs and the independent variables history, gender, hypertension, diabetes, and smoking with P values of 0.33, 0.58, 0.27, 1, 0.67, respectively. These data indicated that both mutant and wild patterns didn't have effect on increasing the risk of MI and CVA.

Our case-controlled study concluded that GPIa C807T/G873A genotypes are not related with increasing risk of MI and CVA except hyperlipidaemia where P value was 0.04.

Therefore we concluded that the mutant genotypes of both SNPs TT/AA are associated just with increasing lipid levels in blood and consequently increasing the risk of myocardial infarction and cerebrovascular accident. Thus, it is possible to say that excessive levels of lipids in blood vessels alone, have a major effect on blood vessel wall and increased risk of thrombus formation rather than when it acts in combination with other risk factors.

The results of current study are partially consisted with those reported by Komurcu et al. [14] on Turkish population which showed that there were an association between hyperlipidaemia with mutant genotype TT/AA in patients with MI with a P value of 0.05. Also, our results are in agreement with the study of Lu et al. [18] on Chinese population which demonstrated that polymorphism of ITGA2 especially 807T which acts as hereditary factor for cerebral stroke by its relation with increase total cholesterol levels. The polymorphism was related as though with individuals having hyperlipidaemia with consequent increased risk of cardiovascular death or in individuals with mutant alleles T/A who have signs of endothelial damage. It gives a raised risk just when the endothelium is submitted to detrimental stress. Clot formation may result from the interaction between environmental and genetic factors where a complete role returns to homeostasis.

Linkage disequilibrium analysis

Non-random association of alleles at two or more loci is defined as linkage disequilibrium (LD) [Ref]. It is now known that LD is not only present between SNPs in close physical proximity along the genome, but it also often presents between widely spaced markers to form haplotype blocks [21, 22]. Contrary to normal linkage due to a physical connection of neighboring loci on the same chromosome, LD can even occur between loci on different chromosome [23] (Table 5).

Table (5): Linkage disequilibrium analysis

SNPs	D'	D	R	P value
807 873	0.999	0.124	0.9995	0.0001

The largest possible level of LD for this combination of allele frequencies would thus be at D = 0.12. To describe the extent of LD relative to the range of possible values of D' which is guaranteed to a range from 0.0 for no LD to 1.0 for maximum levels of LD, a useful property of D' is that we can directly see if one haplotype is missing.

At D' = 0.999, D must be at its maximum for one combination and consequently one of the haplotype frequencies must be 0.0 or non-significant as with haplotype CG. It turns out that immediately after a mutation creates a novel allele, D'=0.999 between that locus and any other polymorphic locus on the chromosome five. This is another indication for the D value with high significance (P <0.0001). So, it can be concluded, based on our calculations, that there is a significant LD between loci and it is 50% of the theoretical maximum. Also, we noted that two SNPs are in complete LD (not separated by recombination) when D' = 1 or r²=1 [24]. All these proofs have boosted our hypothesis that these SNPs are the main cause of heart attack and stroke in our population. This result is partially consistent with that of study conducted by Di Paola et al. [25] who studied LD between 807 and 1648 SNPs. The authors found that there is a strong correlation with D value 0.05 and 0.0 in Israel and Brazil population, respectively. Also, the study of Casorelli et al. [20] on Italian population, showed that in all cases and controls the

807T genotype was associated with the 873A genotype confirming the complete linkage disequilibrium between 807C/873G and 807T/873A. The results of this study are in agreement with [5, 17, 26] which showed complete linkage disequilibrium between 807 and 873.

CONCLUSION:

The mutant pattern of both SNPs, 807TT/873AA is completely present in patients with MI, CVA and in about half of healthy individuals of Al-Anbar population. Gender, age and other risk factors of concerned diseases are also used to covariant in disease occurrence while these factors in association with SNPs, they are improbable to significantly modify the genetic risk except in case of hyperlipidaemia. In addition, the mutant pattern of 807TT/873AA demonstrated an association with high lipid levels. Also, 807 and 873 SNPs are at high linked disequilibrium, consequently, that enables them to have the tendency of transition together by one parent.

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