IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 3 Ser. I (May – June 2021), PP 54-61
www.Iosrjournals.Org

Associations of the Cannabinoid Receptor -1 Polymorphisms with the Susceptibility to Major Depressive Disorder and the Response to the Antidepressant Escitalopram in a Sample of Egyptian Patients

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Abstract
The endocannabinoid system, specially the cannabinoid receptor-1 (CNR-1) is associated with depression and antidepressant treatment. Some polymorphisms of CNR1 gene of the cannabinoid receptor-1 are associated with depression and clinical response to antidepressants.
This study investigated the effect of the polymorphisms 4895A/G and 1359 G/A of the CNR1 gene on the etiology of depression, and on response to treatment withEscitalopram.
CNR1 polymorphisms 4895A/G and 1359 G/A of cases and controls were determined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique. Diagnosis of cases was determined by Diagnostic and Statistical Manual of Mental Disorders-5th edition, and then they were treated with Escitalopram for six weeks. Drug response was determined by Hamilton Rating Scale of Depression.
The association between both polymorphisms and Major Depression was not statistically significant. While there was a statistically significant difference between the genotypes (AG and GG) of the polymorphism 4895A/G of responders and non-responders, especially in males ($p<0.001$). The polymorphism 1359G/A showed no significant difference between the genotypes of responders and non-responders.
The polymorphism 4895A/G is not associated with Major Depression, but is associated with treatment response to Escitalopram, especially in males. While the polymorphism 1359G/A showed no association with Major Depression or treatment response in Egyptian population.

Keywords: *Major Depressive Disorder; Endocannabinoid system; CNR1 gene; 4895A/G; 1359 G/A; rs806368; rs1049353; Escitalopram; Egyptian population*

Date of Submission: 30-05-2021 Date of Acceptance: 13-06-2021

I. Introduction

Major depressive disorder (MDD), is characterized by at least two weeks of low mood present across most situations. It is often accompanied by low self-esteem, loss of interest/pleasure in normally enjoyable activities and low energy (American Psychiatric Association, 2013). It is a serious disorder because it was found to be the major cause of premature mortality (Ferrari, Charlson, Norman, Flaxman, et al., 2013), and it represented a high risk for committing suicide (World Health Organization, 2017). MDD was estimated to be the second leading cause of years lived with disability (YLDs) worldwide in 2010, according to the Global Burden of Diseases (Ferrari, Charlson, Norman, Patten, et al., 2013). The number of people affected by MDD worldwide approximately represents 4.4% of the global population (World Health Organization, 2017). Although MDD is very common in Arabic countries, few studies examined the disorder in this part of the world (Beshai et al., 2012; Karam et al., 2006). It was found that MDD had the first rank as the cause of YLDs in the years 1990, 2005 and 2010 in the Arab world (Mokdad et al., 2014). In 2009, a national survey was made in Egypt, to detect the prevalence of mental disorders in the Egyptian population, the overall prevalence of mental disorders was 16.9%, among which MDD was the most common disorder among all the mental disorders, with the highest prevalence rate of 2.7%(Ghanem et al., 2009).

The endocannabinoid system is consisted of two receptors (CB1 and CB2), it was found to have a role in the etiology of depression (Bifulco, 2009; Vinod & Hungund, 2006). The CB1 receptor is found mainly in CNS and it's the most abundant G-protein coupled receptor found in the limbic system responsible for depression(Hill et al., 2009; Mackie, 2005).

There is a strong association between CB1 receptor and depression as it has been shown that the absence the CB_1 receptor (knock-out mice) can cause depression(Martin et al., 2002). Whileadministration of CB1 receptor agonists or endogenous cannabinoid uptake inhibitors, resulted in antidepressant-like effects (Hill & Gorzalka, 2005; Hill et al., 2009; Sartim et al., 2016).

There is also an association between the endocannabinoid system and antidepressant treatment. It was found that endocannabinoid system is regulated by antidepressant treatment regimens as shown by pharmacological studies. For instance, it was found that the antidepressants imipramine, desipramine, escitalopram and tianeptine up-regulate the CB_1 receptor in the hippocampus, hypothalamus and amygdala, which are regions involved in depression (Hill, Carrier, et al., 2008; Hill & Gorzalka, 2009; Hill et al., 2006; Smaga et al., 2017). Serotonin has a role in the ability of the CB1 receptor to couple to its G-protein second messenger system to launch a cellular response at the end(Devlin & Christopoulos, 2002; Hill & Gorzalka, 2005). Prolonged treatment with the SSRI fluoxetine was found to enhance the signaling of CB_1 receptor, thus antidepressants act to increase the endocannabinoid system activity (Mato et al., 2010; Smaga et al., 2017). Furthermore, it resulted in increased CB_1 receptor mRNA, protein and signaling in rodents(Hill, Ho, et al., 2008; Mato et al., 2010).

The gene responsible for the formation of the CB_1 receptor is CNR1 (Hoehe et al., 1991). Studies proved that some polymorphisms of CNR1 gene were associated with the etiology of MDD (Monteleone et al., 2010) and clinical response to antidepressants (Domschke et al., 2008; Mitjans et al., 2012; Mitjans et al., 2013). The SNP rs806368 (4895A/G) is believed to be associated with increased risk of MDD. Also, it was found that this SNP showed a significant effect on remission after treatment with Citalopram at the 12th week (Mitjans *et al*., 2013). As for the polymorphism rs1049353 (1359G/A), it has been shown in many studies its association with MDD (Chakrabarti et al., 2006; Domschke et al., 2008; Monteleone et al., 2010).This polymorphism was also found to be associated with the clinical response to antidepressants (Domschke et al., 2008; Mitjans et al., 2012).

Pharmacogenetic research suggests that Single Nucleotide Polymorphisms (SNPs) can be used to determine the contribution of genetic variance in drug response (Drago et al., 2009; Serretti et al., 2005). In order to minimize the disorder duration, adverse drug reactions and to increase the patient's compliance, it would be useful to be able to predict the pharmacological intervention likely to be effective and tolerable for each patient according to the patient's specific genetic makeup (Crisafulli et al., 2011; Porcelli et al., 2012). Therefore, the aim of this study is to investigate the effect of both polymorphisms rs806368 and rs1049353 on the occurrence of MDD, and on response to the antidepressant Escitalopram.

II. Method

Participants

The study was conducted in 2018 on fifty adult cases (30 males and 20 females) of MDD from the outpatients unit of New Nozha Psychiatric Hospital after a psychiatric evaluation using the Structured Clinical Interview for The Diagnostic and Statistical Manual of Mental Disorders-5th edition (SCID-5)(First, 2015), and after applying the inclusion and exclusion criteria.

Inclusion criteria of the patient group:

- Patients newly diagnosed with MDD only.
- Adult patients > 18 years old.
- Patients with Egyptian nationality only.
- Patients who can read and write.

Exclusion criteria of the patient group:

- Cases of other major psychiatric disorders (Bipolar disorder, Schizophrenia and psychosis).
- Patients with substance use disorders or using any illicit mood altering substances.
- Patients that were previously treated with antidepressants or on poly-antidepressants.

Fifty healthy controls (34 males and 16 females) without current or past psychiatric diagnoses were selected from patients' friends and family memberswere included as a control group and fulfilled the inclusion criteria.

Inclusion criteria of the control group:

- No current or past psychiatric disorders as assessed by SCID-non-patient edition.(First et al., 1996)
- No current substance use disorder's or even using any illicit mood-altering substances.

- Age of the selected group > 18 years old.
- Controls with Egyptian nationality only.
- Controls who can read and write.

The study was performed after the approval of the Ethics Committee of the Institute where the study was carried. All study participants were asked to volunteer to the study and provided a signed informed consent.

All patients and controls were subjected to a questionnaire to assess the demographic data such as: age, gender and ethnicity.

Hamilton Rating Scale of Depression (HRSD) was used to assess baseline depression severity upon admission to the hospital, after 2 weeks of treatment for follow-up and after 6 weeks of treatment to determine the drug response.(Hamilton, 1967)

Treatment of patients was conducted using Escitalopram with a dose of 20 mg/day for six weeks. Clinical response was determined by comparing the baseline score of HRSD with the score after 6 weeks of treatment according to the guidelines of World Federation of Societies of Biological Psychiatry. Patients were classified as responders with a 50% or greater decrease in the score of HRSD, compared with the baseline score. And non-responders with a 25% or less decrease in the score of HRSD, compared with the baseline score(Bauer et al., 2013).

Molecular study

Genotyping of the two CNR1 polymorphisms; rs806368 (4895A/G) and rs1049353 (1359 G/A) was performed using Polymerase Chain Reaction - restriction fragment length polymorphism (PCR-RFLP). The oligonucleotides used for amplification of rs806368 (4895A/G) were: 5'-GAGACCACCCATATCATGCACACA -3'(Forward) and 5'-AACTCTGATCCCCAGTAGGCCTAG -3' (Reverse) according to the method described by Russo et al. (2007)(Russo et al., 2007). While for rs1049353 (1359 G/A): 5'- GAAAGCTGCATCAAGAGCCC-3' (Forward) and 5'- TTTTCCTGTGCTGCCAGGG-3'(Reverse) oligonucleotides were used as described by Gadzicki et al. (1999)(Gadzicki et al., 1999). Then, the amplicons were digested using restriction endonucleases FokI for rs806368 (4895A/G) (Russo et al., 2007) and MspI for rs1049353 (1359 G/A)(Baransel Isir et al., 2015) .The produced restriction fragment sizes were 346 for the AA genotype, 271 and 346 bp for the AG genotype and 271 bp for the GG genotype, as described by Russo et al. (2007)(Russo et al., 2007). While the produced restriction fragments sizes were 111 bp for the GG genotype, 111 and 92 bp for the AG genotype and 92 bp for the AA genotype(Baransel Isir et al., 2015).The digested fragments of both polymorphisms were separated by electrophoresis on a 3% agarose gel stained with Ethidium bromide, then observed by a UV transilluminator.

Statistical analysis

Allele frequencies were estimated by direct gene counting. Chi square goodness-of-fit test was used to assess statistical significance of the difference in genotype frequencies between cases and controls and to investigate the associations between genotypes and response to treatment.

A P-value ≤ 0.05 was considered statistically significant, and a p-value ≤ 0.001 was considered statistically highly significant.

III. Results

Molecular results

In the studied samples, PCR-RFLP of CNR1 polymorphism rs806368 (4895 A/G) revealed two alleles (i.e. allele A and allele G) and three genotypes (i.e. homozygotes GG, AA and heterozygote AG); as shown in Figure 1. For the polymorphism rs1049353 (1359 G/A), alleles G and A, and genotypes GG, GA and AA were present (Figure 2).

The distribution of genotypes and alleles of both CNR 1 polymorphisms identified in both MDD patients and control group is shown in Table 1.

Regarding the polymorphism rs806368 (4895 A/G), the frequency of the G allele and the GG genotype were higher among patients with MDD compared to the control group. However, both differences were found statistically insignificant (p = 0.395 for alleles and 0.429 for genotypes), thereby defying (excluding, declining) any association between CNR1 rs806368 polymorphism and susceptibility to MDD.

Likewise**,** in rs1049353 (1359 G/A) no significant difference was observed in the distribution of alleles and genotypes among patients compared to controls (p = 0.174 for alleles and 0.271 for genotypes).

After stratification of patients and controls by gender, still no significant difference was detected in the distribution of both polymorphisms (rs806368: p = 0.538for males and 0.831for females; rs1049353: p = 0.288 for males and 0.737 for females) (Table1).

Response to treatment

After treating patients for 6 weeks, 43 patients responded, while 7 patients did not respond. In order to investigate a putative association of the response to Escitalopram with variants of rs806368 (4895 A/G) and rs1049353 (1359G/A), the genotype and allele frequencies for each polymorphism were calculated separately for responders compared to non-responders.

Concerning the polymorphism rs806368 (4895 A/G),there was a statistically significant difference between the genotypes (AG and GG genotypes) of responders and non-responders in total patient group (p = 0.015). The same statistically significant difference was found between the genotypes (AG and GG genotypes) of male responders and non-responders (p <0.001), while no significant difference was detected in the distribution of genotypes of female patients (P = 0.435) (Table 2)

As for the polymorphism rs1049353 (1359G/A), no significant difference was detected between the genotypes of responders and non-responders(p = 0.865). Again, the three genotypes were not associated with response to treatment after stratification by gender (p = 0.690 for males and p=0.619 for females) (Table 2).

IV. Discussion

The gene responsible for the formation of the CB_1 receptor is CNR1 (Hoehe *et al*., 1991). Many SNPs have been associated with major depression (Hillard et al., 2012).The first SNP investigated in the current study is the SNP rs806368 (4895 A/G), which is found in exon 4 in the 3' Untranslated region (Zhang *et al*., 2004). Although the genotype GG was foundmore among patients than controls, we couldn't' find asignificant association between MDD and the genotypes of the polymorphism rs806368 (4895 A/G). These results are in line with Murphy et al. (2011) study, where the SNP rs806368 was not associated with predisposition for attempting suicide (Murphy *et al*., 2011), that is considered to occur as a result ofMDD (Malone et al., 1995; Vahia et al., 2000).

Results of this study also agree with the study of Mitjans et al. (2013), in which it was found that the genotype frequencies did not differ significantly between MDD patients and the controls (Mitjans *et al*., 2013). Juhasz et al. (2009)found that the G allele was associated withsusceptibility to develop MDD (Juhasz *et al*., 2009), but we couldn't find a significant association although the G allele ofthe polymorphism rs806368 (4895 A/G) was slightly higher in patients, than in controls.

Concerning treatment response, in the current study there was a significant association of AG and GG genotypes with response to treatment. These results are consistent with Mitjans et al. (2013) who found that G carriers presented a better response to treatment than AA homozygotes (Mitjans *etal*., 2013). In addition, in the present study, males with AG and GG genotypes were found to be responders to treatment, while there wasn't a significant association between the genotypes of females and treatment response. These results agreed with the finding in Mitjans et al. (2013) study that G carrier males had a better response to treatment than AA homozygous males or females (Mitjans *et al*., 2013). The results of the present study thus confirm the role of G allele in achieving response to Citalopram and Escitalopram.

The second SNP investigated in the current study was rs1049353 (1359 G/A). Our results showed that the polymorphism rs1049353 had no effect on the occurrence of MDD, which agrees with the study conducted by Mitjans et al. (2013) (Mitjans *et al*., 2013), and also with the meta-analysis of Kong et al.(2019). (Kong *et al*., 2019)

Likewise, the genotypes of rs1049353 had no effect on treatment response to Escitalopram. The same result was found when patients were stratified by gender. These results contradict with the studies conducted by Domschke et al. (2008) and Mitjans et al. (2012).

Concerning the effect of genotypes of rs1049353 in MDD patients on response to Escitalopram, both studies reported positive, albeit opposite results.Domschke et al. (2008) found that the G allele was associated with worse response to treatment to a variety of antidepressant medications, compared to the homozygous AA group, especially in female patients with G allele when stratification for gender was applied. This effect could not be found in the male patients (Domschke *et al*., 2008). On the contrary, Mitjans et al. (2012) found that GG genotype presented better response to Citalopram than A allele carriers. This effect was stronger when the patients were stratified by gender, where GG homozygous men showed better response than A carrier men or all women (Mitjans et al., 2012). It seems that the SNP rs1049353 is involved in antidepressant response, however, the contradictory results between the current study and the other two studies will require more research to be conducted on the SNP rs1049353, to make sure if the results of this study are due to difference in populations (Egyptians) and to determine which antidepressants are affected with this polymorphism and which are not.

In conclusion, the important findings of this study are: the polymorphism rs806368 of the CNR1 gene in patients had no impact on susceptibility to MDD, but the genotypes AG and GG of the polymorphism rs806368 were linked to response to treatment with Escitalopram, especially in male patients. The polymorphism rs1049353 (1359 G/A) of the CNR1 gene had neither an impact on susceptibility to MDD, nor an effect on treatment response to Escitalopram.

Among the strengths of this study, it's the first one to be conducted in Egypt that links both SNPs of the CNR1 gene with depression and to drug response. It can be considered as a preliminary study, because of the limited literature investigating these two polymorphisms, specially the polymorphism rs806368 (4895 A/G). It's also an important step in the way of establishing a personalized medicine for MDD, rather than wasting the patient's time in trial and error for the best suited treatment, which will lead to prolonged disorder duration and reduced patient's compliance.

As for the limitation, there is the small sample size, therefore further studies with larger sample size and other antidepressant medications are required, to find which antidepressants are affected by these polymorphisms, in order to make it easier in the future to treat MDD patients based on their genetic makeup.A truly personalized medicine approach for MDD will only be achieved through the identification of genetic variants responsible for the susceptibility to depression and response to antidepressants.

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Tables

Table (1):Allelic and genotypic frequencies of CNR1 polymorphisms; 4895 A/G (rs806368) and 1359G/A (rs1049353) among Egyptian patients with MDD compared to healthy controls

Alleles / Patients & controls		Patients (n = 50) N (%)	Controls (n= 50) N (%)	P-value	Male Patients (n = 30) N (%)	Male Controls (n = 34) N (%)	P-value	Female Patients (n = 20) N (%)	Female Controls (n =16) N (%)	P-value
rs806368 (4895 A/G)	Allele A	44 (44%)	50 (50%)	X^2=0.72 p= 0.395						
	Allele G	56 (56%)	50 (50%)							
	Genotype AA	3 (6%)	5 (10%)	X^2= 1.69 p= 0.429	2 (6.7%)	4 (11.8%)	X^2 = 1.24 p= 0.538	1 (5%)	1 (6.2%)	X^2 = 0.37 p= 0.831
	Genotype AG	38 (76%)	40 (80%)		23 (76.7%)	27 (79.4%)		15 (75%)	13 (81.2%)	
	Genotype GG	9 (18%)	5 (10%)		5 (16.7%)	3 (8.8%)		4 (20%)	2 (12.5%)	
rs1049353 (1359 G/A)	Allele G'	63 (63%)	72 (72%)	X^2= 1.84 p=0.174						
	Allele A'	37 (37%)	28 (28%)							
	Genotype G'G'	18 (36%)	26 (52%)	X^2 = 2.6 p=0.271	10 (33.3%)	18 (52.9%)	X^2 = 2.4 p=0.288	8 (40%)	8 (50%)	X^2= 0.36 ^{FE}p = 0.737
	Genotype G'A'	27 (54%)	20 (40%)		15 (50%)	12 (35.3%)		12 (60%)	8 (50%)	
	Genotype A'A'	5 (10%)	4 (8%)		5 (16.7%)	4 (11.8%)		0 (0%)	0 (0%)	

N: number

χ^2, p: χ^2 and $p$are values for Chi square test for comparing between the two groups

^{FE}p**:** p-value forFisher Exact for Chi square test for comparing between the two groups

Table (2): Distribution of genotypes of CNR1 4895 A/G (rs806368) and 1359 G/A (rs1049353) polymorphisms among Egyptian patients with MDD according the response to Escitalopram

Treatment / Patients	Responders (n = 43) N (%)	Non-Responders (n = 7) N (%)	p-Value	Responders (n = 43) Males (n = 27) N (%)	Responders (n = 43) Females (n = 16) N (%)	Non-Responders (n = 7) Males (n = 3) N (%)	Non-Responders (n = 7) Females (n = 4) N (%)	p-Value Males p-value	p-Value Females p-value
Total Patients (n;50)									
rs806368 (4895 A/G)									
Genotype AA	1 (2%)	2 (29%)	X^2 = 8.3 P= 0.015*	0 (0%)	1 (6%)	2 (67%)	0 (0%)	X^2= 19.37 p<0.001**	X^2 = 1.66 P= 0.435
Genotype AG	33 (77%)	5 (71%)		22 (81%)	11 (69%)	1 (33%)	4(100%)		
Genotype GG	9 (21%)	0 (0%)		5 (19%)	4 (25%)	0 (0%)	0 (0%)		
rs1049353 (1359G/A)									
Genotype G'G'	16 (37%)	2 (29%)	X^2= 0.289 df= 2 p=0.865	9 (33%)	7 (44%)	1 (33%)	1 (25%)	X^2= 0.741 df=2 p= 0.690	X^2= 0.469 df= 1 ^{FE}p = 0.619
Genotype G'A'	23 (53%)	4 (57%)		14 (52%)	9 (56%)	1 (33%)	3 (75%)		
Genotype A'A'	4 (9%)	1 (14%)		4 (15%)	0 (0%)	1 (33%)	0 (0%)		

N: number

χ^2, p: values for Chi square test for comparing between the two groups

^{FE}p**:** p value forFisher Exact for Chi square test for comparing between the two groups

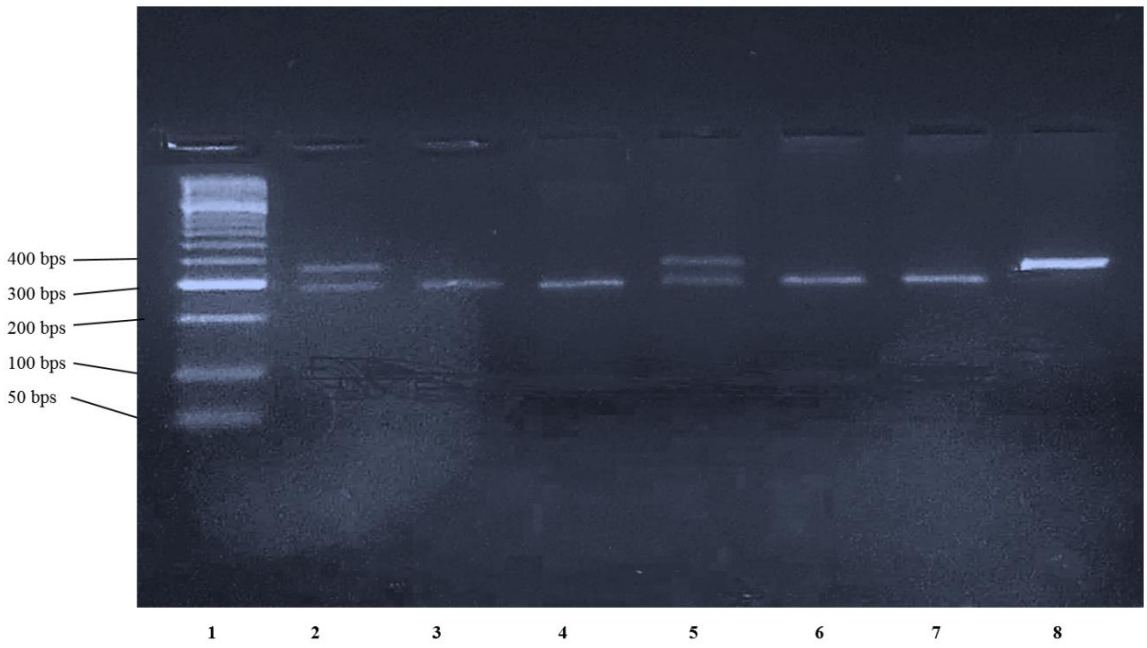
* Significant value at $p \leq 0.05$

**Highly significant value at $p \leq 0.001$

Figures:

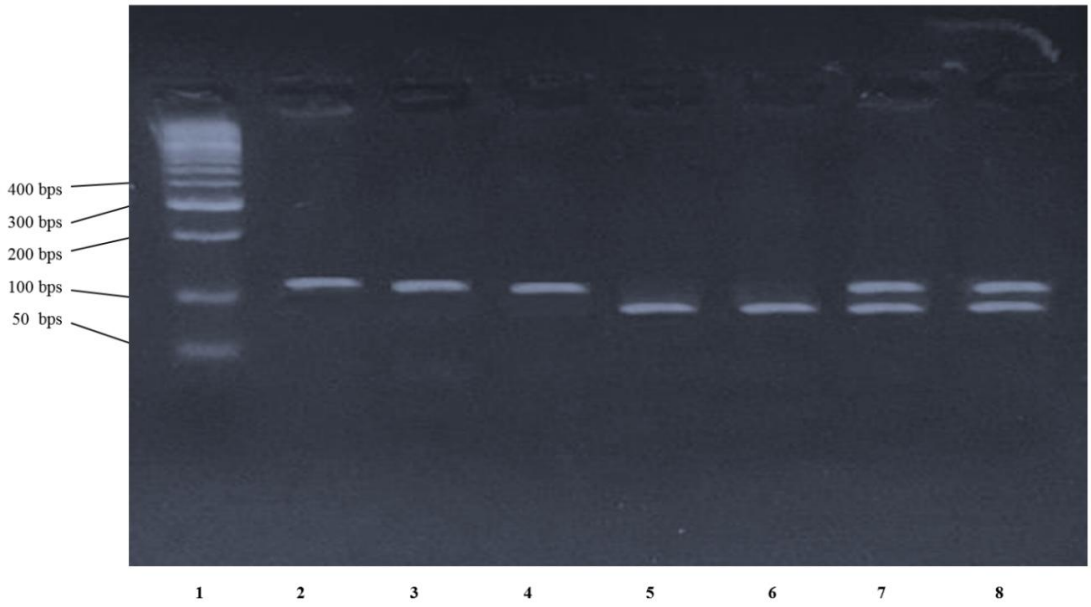
Figure (1): RFLP product of 4895 A/G after digestion with Fok1

RFLP Product of 4895 A/G After Digestion With Fok1



*Note.*Lane 1: 100bp DNA ladder, lanes 2and 5: patients with genotype 4895 AG of 271bp and 346 bp bands, lanes 3, 4, 6 and 7: patients with genotype 4895 GG of 271 bp band, lane 8: patient with genotype AA of 346 band

Figure (2): RFLP product of 1359 G/A after digestion with MSPI



Lane 1: 100bp DNA ladder; lanes 2, 3, 4 patients with genotype 1359 G'G'of 111 bp bands; lanes 5 and 6: patients with genotype 1359 A'A' of 92 bp bands; lanes 7 and 8: patients with genotype 1359 G'A' of 92bp and 111 bp bands.