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SCN1A AND CASR ASSOCIATED TO INHERITED EPILEPSY IN BABYLON PROVINCE –IRAQ

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Abstract:

Background: SCN1A mutations are the most common Voltage Gated Sodium Channel mutations in epilepsy, with approximately 1,250 harmful variations causing a variety of epilepsies. SCN1A mutations are, in fact, the most implicated of all epilepsy genes. The parathyroid gland was the first to identify the calcium sensing receptor (CaSR), and it has been shown to play a crucial role in maintaining systemic calcium homeostasis by controlling the production of parathyroid hormone (PTH). Researchers have also looked at the receptor in cells and tissues that aren't directly involved in calcium homeostasis, such as the nervous system (NS), where it plays crucial roles in early neural development for the differentiation of neurons and glial cells, as well as synaptic transmission and plasticity in the adult nervous system. **This study aims** to detect the role of Scn1a and CasR genes in epilepsy in Babylon province Iraq. **Material and methods**: The samples were obtained from Babil Maternity and Children's Hospital visitors and Out patience Pediatric Clinics in AL-Hillah, over the period of 12/2/2020 – 30/12/2020, genetic association of Scn1a rs2298771 single Strand Conformation polymorphism and Sequencing technique was used in this study and *CaSR* rs1801726 and for PCR-RFLP technology for studying *CaSR* rs1801725. **Results** Comparing the genotypes of the *scn1a* gene rs2298771 did not show a significant difference, as the frequency of the patterns AA, AG, and GG in epilepsy patients was (33.3%, 48.5%, and 18.2%, respectively) and in the control group, the percentage frequency of the three patterns was (33.3%, 48.5%). , 18.2%, respectively).Although genotypes (GG, TG, TT) appeared in the SNP (rs1801725) and genotypes (CC, CG, GG) for the SNP (rs1801726) of the *Casr* gene, None of the (SNPs) were shown to be related to the emergence of the disease in the city of Hilla, despite being one of the important genes that cause epilepsy.

Keywords: Epilepsy, pediatrics, Scn1a, CasR

INTRODUCTION

SCN1A mutations are the most common Voltage Gated Sodium Channel mutations in epilepsy, with approximately 1,250 harmful variations causing a variety of epilepsies[1]. SCN1A mutations are, in fact, the most implicated of all epilepsy genes. SCN1A mutations are found in the majority of Dravet syndrome (DS) and Generalized Epilepsy with Febrile Seizures Plus (GEFS+) patients. Both hereditary and de novo mutations in the SCN1A gene are known to cause epilepsy and epileptic encephalopathies. For example, SCN1A gene mutations account for almost three-quarters of DS cases, but SCN1A mutations responsible for GEFS+ are often inherited. De novo SCN1A mutations that cause Down syndrome are loss-of-function mutations in inhibitory interneurons, although this is just one example. [2]. The activity of GABAergic inhibitory interneurons is reduced by these alterations. SCN1A mutations in seizure disorders may have several forms, including missense, nonsense, protein truncating variations, and so on. For example, missense mutations account for approximately half of all DS cases, while nonsense mutations, deletions, frame-shifts, and splice-site variations account for the rest. Although DS and GEFS+ may be utilized as models for studying SCN1A alterations, similar mutations are also seen in other epilepsy subtypes[3,4].

Seizure and neuro-behavioral changes seen in various epilepsy depend on the type of mutations discovered in sodium channels. Therefore, in epilepsy treatment, VGSC gene screening is essential. Total mutations in SCN1A loss-off function lead to greater problems[5].Meanwhile, amino acid replacement point mutations that alter sodium channels lead to less severe epileptic diseases. This is shown in DS that results from the SCN1A gene loss-of-function mutation, an EPD greater than the GEFS+[6,7].

Zhang et al. [8] demonstrated the connection between various kinds of seizures and the incidence of SCN1A mutations among clinical presentations of 13 children with SMEI. 10 with mutations in SCN1A were found out of 13 cases studied. It consists of 1 shift and nine non-synonymous mutations (seven missenses and two non-sense). The authors stated that a definite SCN1A genetic diagnostic method must be implemented for the appropriate treatment of patients, while a well-known electro-clinical pattern has been identified in these individuals. Zucca et al[9] identified 12 SCN1A mutations in a further analysis of 58 Italian and 1 Spanish patients who had cryptogenic epilepsy. Muzzo-nonsense and frameshift mutations in individuals with DS were shown in the mutational screening of SCR1A in 13 children. Only 15 per cent have aberrant brain imagery, while most patients have normal electromagnetic activity (EEG). Despite the lack of clear EEG findings,consideration of genetic diagnosis in epileptic individuals should not be dissipated[7].

The effectiveness, dose and toxicity of various AEDs are the main genes involved in the encoding of Nav channels, namely SCN1a and SCN2A. The common single-nucleotide polymorphisms (SNPs) of SCN1A rs2298771, SCN1A rs10188577, SCN2A rs17183814, and SCN2A rs2304016 are these genes and they have been extensively studied to relate them to resistance to AED. The findings, however, remain inconsistent. For example, in Chinese children with generalized epilepsy (P =0,035, for example, [10]. showed that SCN1A rs10188577 polymorphism was associated with valpreic acid (VPA) resistance. However, another research shows that the link between polymorphism SCN 1A rs.10188577 and the SCB-response for sodium channel blockers was only marginally significant in epileptic Caucasans (p = 0.049), including phenytoin, carbamazepine, and oxcarbazepine, lamotrigine, topiramate, and valproic acid. Moreover, this connection was not supported by many additional research. The SCN1A rs2298771, SCN2A rs17183814 and SCN2A rs2304016 polymorphisms have a similar situation. In 2013, no connection between SCN1A rs2298771 and SCN2A rs17183814 polymorphisms, and the resistance to AEDs was found for [11] meta-analysis. Another metaphor has shown that SCN-AEDs were substantially impacted by the A allelel of SCN1A rs2298771, and the AA genotype, in particular[12]

The Ca^{+2} -sensing receptor (*CASR*) in transfected cells increases inositol 1,4,5-trisphosphate preferentially and inhibits the production of cAMP. Several *CASR* mutations that cause disease have been shown to alter this preferential signaling by stabilizing receptor conformations that couple intracellular signaling pathways differently [e.g., arrestin-biased signaling [13,14]

Human CaSR mutations are a known cause of epilepsy. Serum [Ca+2] change owing to PTH level damage may contribute to epilepsy by downregulating inhibitory neurons, upregulating excitatory neurons, or having a more systemic influence on certain brain circuits. CaSR mutations have been linked to epilepsy in a surprising number of cases, despite the fact that these mutations do not change systemic PTH or blood Ca+2 levels. The impact of these changes on cognitive performance remains unknown. Cang et al.[15] show that the mutations in CaSR interfere with the protein's capacity to monitor the Na+-leak channel NALCN in neurons.

Previous studies have shown that decreasing [Ca+2] stimulates neurons in cultured hippocampal neurons and midbrain dopaminergic neurons via increasing NALCN-mediated Na+ leak channel current (INALCN) [16]. CaSR agonists, such as spermidine and neomycin, inhibit Ca+2 sensitivity of NALCN, suggesting that this Ca+2e NALCN control is G-protein dependent and involves CaSR. NALCN is regulated in part by other Gi/o-coupled receptors. Whether or whether CaSR-mediated regulation of NALCN also happens in other CNS neurons and peripheral neurons is still unclear. The structural requirements for coupling the Ca+2e receptor to the channel, known as CaSR, are still unknown. Using shRNA knockdown, Cang et al. demonstrated that CaSR is required in neurons for Ca+2e-induced modulation of NALCN. Ca+2 -sensing and an unusual C-terminal region that is not required for the receptor's capacity to perceive and link [Ca+2]e with other conventional objectives both contribute to CaSR's ability to regulate NALCN. Last but not least, epilepsy-implied mutations in this location impair the receptor's capacity to modulate NALCN, revealing a unique mechanism by which the mutations induce epilepsy despite not affecting systemic [Ca+2] levels[16].

MATERIALS AND METHODS

Sample: The samples were obtained from Babil Maternity and Children's Hospital visitors and Outpatience Pediatric Clinics in AL-Hillah, over the period of 12/2/2020 – 30/12/2020. After comprehending the project goal and testing, a formal agreement was signed by the parents of all participants. Children with epilepsy 121 and healthy children 58, both of whom were between 2 months and 15 years old. The 5 ml disposable syringe emptied into the EDTA Tube for DNA Extraction yielded 2 milliliters of venous blood.

Genetic association: genetic association of Scn1a rs2298771 single Strand Conformation polymorphism and Sequencing technique was used in this study, DNA was extracted according to Hashim, and Al-Shuhaib, 2020. The selection of genotyping technique according to [17] .The design of PCR primers according to the protocol of [18.

Table (1) designed primer of *Scn1a* rs2298771; *CasR* rs1801726 and *CasR* rs1801725

Gene	Direction	Sequence (5'->3')	Product size	Annealing temp.
Scn1a	forward	AACACTGCTGCCAGTTCCTA	317	58°C

rs2298771	reverse	TTCTGGCCTTGCTTCTGAGC		
CasR rs1801726	F	GGTCACCTTCTCACTGAGCTT	256	55°C
	R	GGACACTACAAGTGCTGGGG		
CasR rs1801725	F	GAGCTTTGATGAGCCTCAGAAGtAC	142	55°C
	R	ATCTAAGTCCGTTTCCCCGC		

RESULTS AND DISCUSSION

Scn1a rs2298771

The homozygote pattern (AA) were more frequent in both patients with epilepsy and control (33.3%), the heterozygote (AG) pattern frequency in control was (48.1%) and n patients was (48.5%), and the homozygote (GG) pattern was in patients (18.2%) and in control group (18.5%) as in table (4-6) ; The figures (4-2) and(4-1) explains the results of electrophoresis in agarose gel for PCR products, the electrophoresis by using polyacrylamide gel for the SSCP technique results, this image indicates the presence of three different genotypes for this genetic site. When a scan of the sequence of nitrogen bases for these products was obtained the results in the table(2) ;These results combined did not show a significant difference for the three types between the control groups and patients with epilepsy. . Based on the results obtained in the current study, the *Scn1a* gene is not considered one of the genes with a direct relationship to the heritability of epilepsy, as there is no significant difference between control and patients in the community of Babylon province.

Table (2) Allele frequency and allelic association of rs2298771.

	Control		Patients		Odd ratio	*CI* 95%	*p-value*
Allele	No.	%	No.	%			
A	62	0.57	76	0.58	1.007	(0.602-1.684)	0.97906
G	46	0.43	56	0.42	0.993	(0.594-1.661)	

Table (3): rs2298771 genotypes frequency and the P-values of exact test for the deviation from Hardy-Weinberg (HW) equilibrium.

Genotype	Control	Patients	Odd Ratio	*CI* 95%	*P-value*
AA	18 (33.3%)	22 (33.3%)	1.00		1
AG	26 (48.1%)	32 (48.5%)	1.01	(0.45-2.26)	1
GG	10 (18.5%)	12 (18.2%)	0.98	(0.35-2.79)	1

Table (4): Association of rs2298771 genotypes with epilepsy under different models of inheritance.

Model	Genotype	Control	Patients	OR (95% CI)	P-value
Codominant	G/G	32 (58.2%)	48 (71.6%)	1.00	0.16*
	T/G	19 (34.5%)	18 (26.9%)	0.63 (0.29-1.38)	
	T/T	4 (7.3%)	1 (1.5%)	0.17 (0.02-1.56)	
Dominant	G/G	32 (58.2%)	48 (71.6%)	1.00	0.12
	T/G-T/T	23 (41.8%)	19 (28.4%)	0.55 (0.26-1.17)	
Recessive	G/G-T/G	51 (92.7%)	66 (98.5%)	1.00	0.17*
	T/T	4 (7.3%)	1 (1.5%)	0.19 (0.02-1.78)	
Overdominant	G/G-T/T	36 (65.5%)	49 (73.1%)	1.00	0.36
	T/G	19 (34.5%)	18 (26.9%)	0.70 (0.32-1.51)	

*Two tailed p- value of Fisher's Test

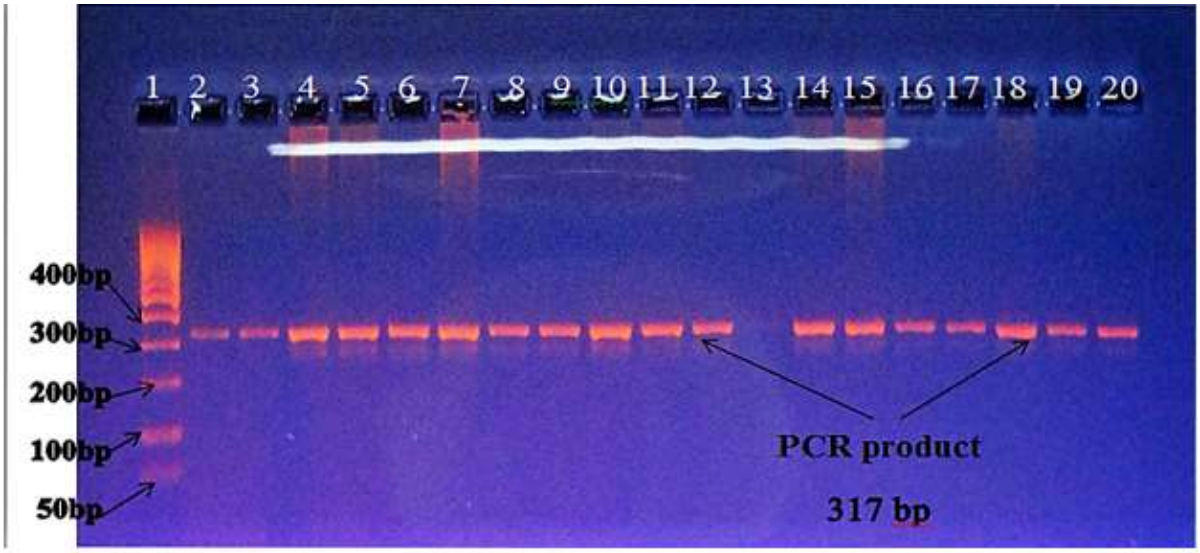

Figure (1) The pattern of PCR products' electrophoresis for *Scn1A* gene (rs2298771), 0.8% agarose, 75 V, 20mA for 60min. Lane 1 DNA ladder, lane 13 control sample, lane 2-12 and 14-10 patients sample.

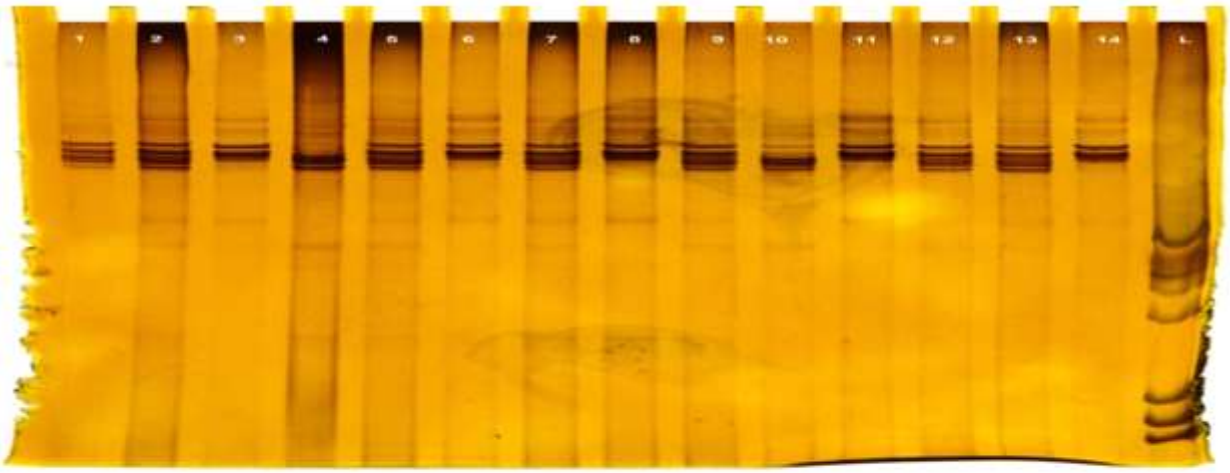

Figure (2) the genotyping of *Scn1A* gene by SSCP technique; 100 v for 6–12 h. lane L DNA ladder, lanes 1,2,5,7,9,10,12,13 A pattern ,lanes 3,6,8,11,14 B pattern and lane 4 C

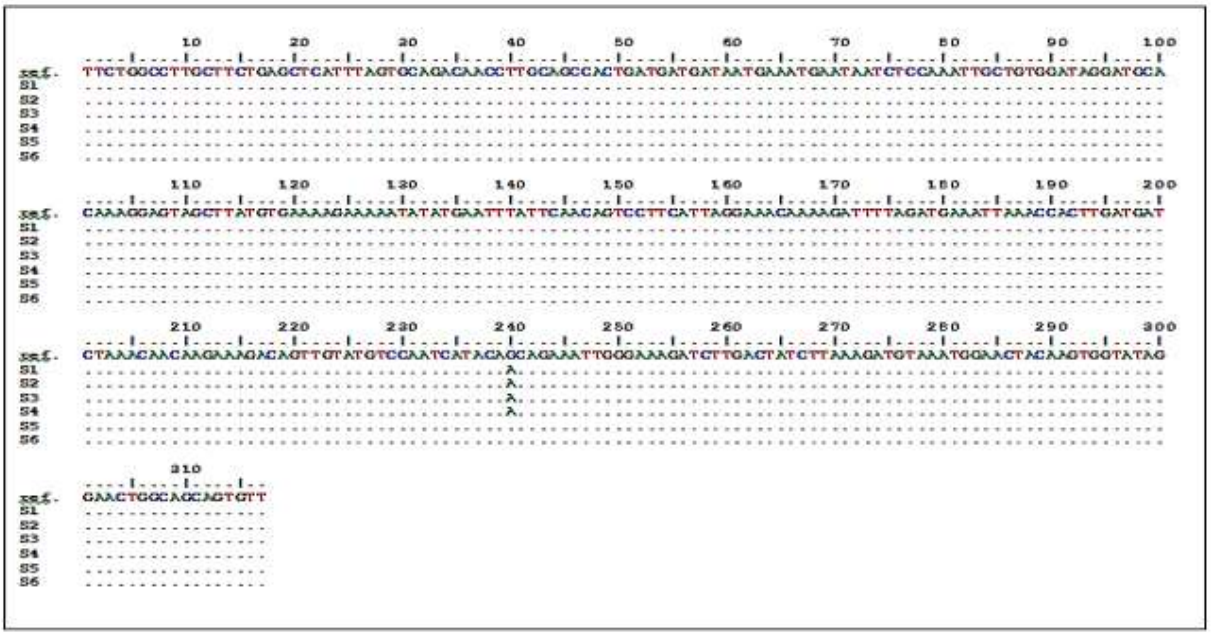

Figure (3) DNA sequences alignment of 6 genotyped samples with their corresponding reference sequences of the 317 bp amplicons of the downstream portion of the *SCN1A* gene. The symbol "ref." refers to the NCBI referring sequence, "S1-S6" refer to the genotyped samples 1 to 6, respectively.

Scn1a is also connected to several other syndromes, many of which are associated with major comorbidity. Scn1a is a syndrome associated with Scn1a. Two illnesses associated with Scn1a include hemiplegic migraine and autism spectrum disorder. In addition to epilepsy, DS sufferers are severely affected by different effects. Scn1a channelopathies are the most common channelopathies linked to epilepsy. It may produce a range of phénotypes, including febriles plus (GEFS+) hereditary epilepsy, from autocontained and pharmaceutical-responsive epilepsy to developmental and epileptic encephalopathy. There is a wide range of severity amongst developmental and epileptic encephalopathies (DEEs), ranging from myoclonic-atonic epilepsy (MAE) to DS, epilepsy of infancy with migrating focal seizures (EIMFS), and early onset Scn1a DEE. There are increasing indications that each has its own co-morbidity and prognosis, many of which are caused by operating faults [19].

For 10 controls in Tunisia to be tested and haplotype frequencies estimated, Fendri-Kriaa et al. selected three SNPs from their group: rs3812718 (intron 4), rs6432860 (exo 13), and rs2298771 (exon 13). The European Parliament (exon 16). The percent of ACA haplotype patients in the phase group was considerably greater than the anticipated 17-11,4% (6-28%) in the control group. The SNP haplotype in these two DS patients was found to be homozygous, indicating it to be a haplotype linked with illness. This haplotype has recently been discovered in a homozygous form in just one DS patient. In the other four febrile cases, the same haplotype was identified, albeit in a heterozygous form, which indicates a connection with the DS phenotty [20].

The rs2298771 polymorphism in the Scn1a exon area is a frequent polymorphism that leads to the conversion of threonin to alanine that may change structural and functional sodium-channel characteristics as well as the treatment for the SCB-AED of epilepsy [21].

A. *CaSR* rs1801725

The results that were reached are close to what were reached by the scientist and his group, as they explained the following in their research, The Lee study found no variants in CACNB4, CASR, GABRA1, GABRD, CLCN2, or EFHC1 that were compatible with predicted Mendelian Juvenile myoclonic epilepsy (JME) inheritance. A lot of causes may cause this startling result in a family study. The probability of monogenic disorders with changes in penetration and expression of cause-genes is difficult to differentiate between harmful variations. Although the coverage of certain areas stays high and thorough, comprehensive coverage is still challenging because the variations in copy numbers (CNV) may be detected in multi-gene panel sequence information. Pathogenic variable studies of recessive disorders like those seen in DF families are especially important for parental testing [22]

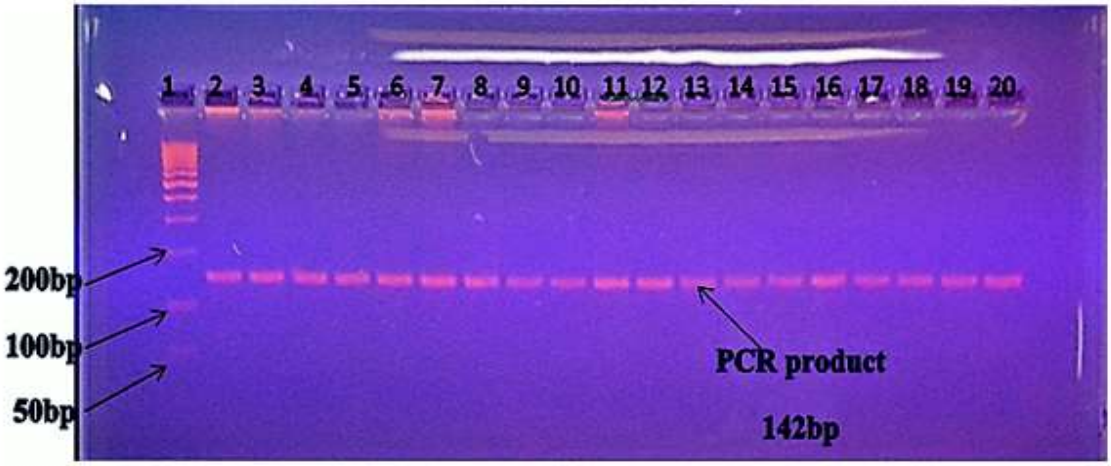

Figure (4) The electrophoresis pattern of PCR product for *Casr* rs1801725, this amplification product was one band for both patient and control, 0.8% agarose, 75 V, 20mA for 60min.Lane 1 DNA ladder, Lanes 2-14 patients, lanes 15-20 control.

The polymorphism in this SNP was studied by using PCR-RFLP ;the target restriction sit for *ScaI* restriction enzyme figure(5) show the three genotypes GG genotype (142bp); TT enotype (23 and 119 bp)and GT genotype (142 ,119 and 23 pb).

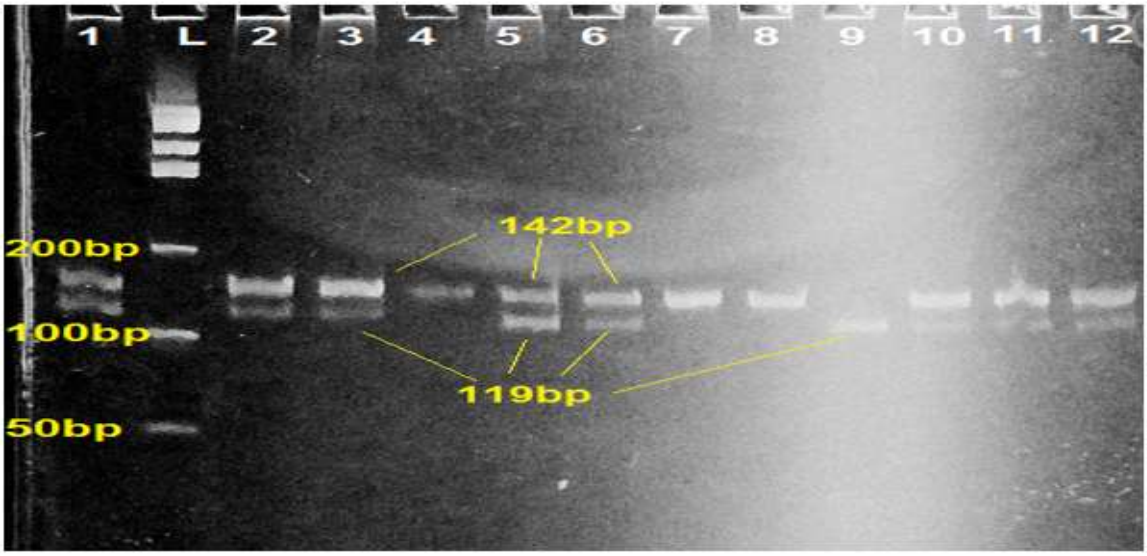

Figure (5) polyacrylamide gel electrophoresis of restriction fragments for rs1801725 genotyping by PCR-RFLP technique.lane L DNA ladder; lanes 4,7,8 GG genotype; lane9 TT genotype, other lanes GT genotype

Allele frequency for patient and control groups are listed in Table (5), The results showed that there was no significant differences in allele frequency between the patients and control groups.

Table (5): Allele frequency and allelic association of rs1801725.

Allele frequency							
Allele	Control		Patients		Odd Ratio	*CI 95%*	*p-value*
	No.	%	No.	%			
G	83	75	114	85	1.854	0.974-3.529	0.057
T	27	25	20	15	0.539	0.283-1.027	

The results from Hardy-Weinberg equilibrium exact test clarified that there was no significant difference among the three genotypes for both patients and control (Table 6); The frequencies of GG,TG and TT of *Casr* rs1801725 polymorphism were 71.6% ,26.9% and 1.5% in the patient with epilepsy group, and 58.2%,34.5% and 7.3% in the healthy group.

Table (6): Genotype frequency of rs1801725 and deviation from Hardy- Weinberg equilibrium.

genotype	Control		Patients		Odd Ratio	*CI 95%*	*p-value*
	No.	%	No.	%			
GG	32	58.2	48	71.6	1	-	0.16
TG	19	34.5	18	26.9	0.63	0.29-1.38	
TT	4	7.3	1	1.5	0.17	0.02-1.56	

Further analysis for the data was tested to study the association of each genotypes with epilepsy under different models of inheritance. The result showed that there were no significant association between any genotype and epilepsy under any of studies inheritance model table (7).

Table (7): Association of rs1801725 genotype with epilepsy under different models of inheritance.

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	G/G	32 (58.2%)	48 (71.6%)	1.00	0.16*
	T/G	19 (34.5%)	18 (26.9%)	0.63 (0.29-1.38)	
	T/T	4 (7.3%)	1 (1.5%)	0.17 (0.02-1.56)	
Dominant	G/G	32 (58.2%)	48 (71.6%)	1.00	0.12
	T/G-T/T	23 (41.8%)	19 (28.4%)	0.55 (0.26-1.17)	
Recessive	G/G-T/G	51 (92.7%)	66 (98.5%)	1.00	0.17*
	T/T	4 (7.3%)	1 (1.5%)	0.19 (0.02-1.78)	
Overdominant	G/G-T/T	36 (65.5%)	49 (73.1%)	1.00	0.36
	T/G	19 (34.5%)	18 (26.9%)	0.70 (0.32-1.51)	

* Fisher´s Exact Test

Therefore, the polymorphism at this site of the gene may not have a direct effect on the inheritance of epilepsy, but this gene may affect the calcium and vitamin D receptors, and thus its effect is indirect on the development of epilepsy in children at Babylon province.

The research consisted of a genome-wide investigation of a 153-gene panel, with its most significant findings revealing that 51 genes that have been previously seen to be highly expressed in the brain, 14 genes with known expression that is thought to be under expression, and 88 with known to be average expression in the brain. CASR is one of the 14 low-expression genes that has been linked to epilepsy [23].

B. *CaSR* rs1801726

The CASR gene is one of the six primary genes responsible for the inheritance of the syndrome juvenile myoclonic epilepsy (JME), which is one of the neurogenic syndromes of generalized epilepsies. Adolescence is what most people go through at some stage. A lifetime hereditary generalized seizure disease happens in the majority of cases at adolescence. Grand myoclonic and jerk seizures recur in 20% of non-prophylactic seizure patients if antiepileptic drugs (AED) are discontinued[24].

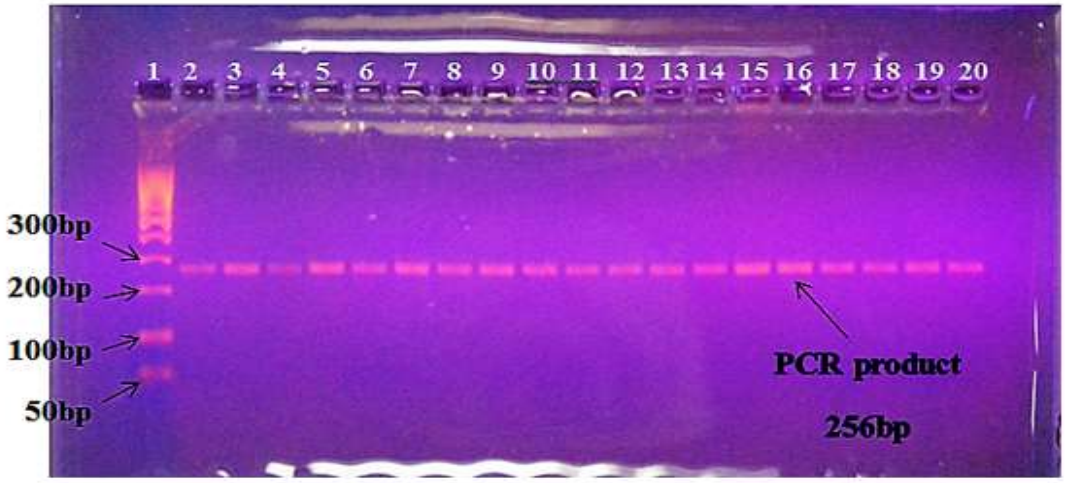

Figure (6) The electrophoresis pattern of PCR product for *Casr* gene (rs1801726), this amplification product was one band for both patient and control, 0.8% agarose, 75 V, 20 mA for 60min. Lanel DNA ladder, lane 2-14 patients, lane 15-20 control.

Genotype pattern studied by PCR-SSCP show the presence of the three genotypes pattern A:heterozygous CG, B: homozygous CC and C : homozygous GG; figure(7).

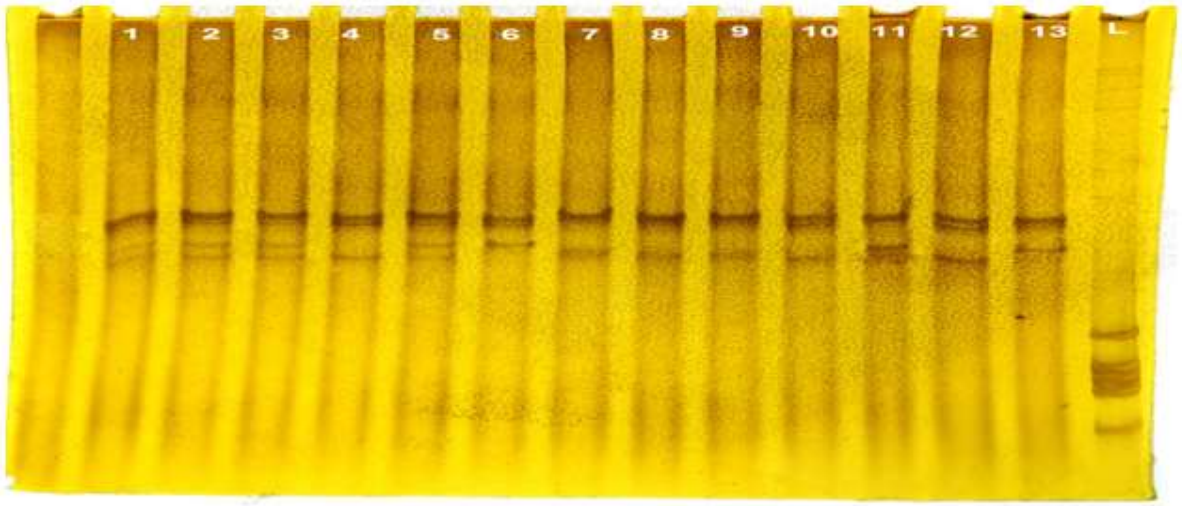

Figure (7) the genotyping of *Casr* gene (rs1801726) by PCR-SSCP technique; 100 v for 12 h. lane L DNA ladder, lanes 1,2,3,5 and 11 A pattern, lanes 6 and 13 B pattern, lane 4,7,8,9,12 and 10 C pattern

Allele frequency for patient and control groups are listed in Table (8), The results showed that there was no significant differences in allele frequency between the patients and control groups.

Table (8): Allele frequency and allelic association of *CaSR* rs1801726.

Allele frequency							
Allele	Control		Patients		OR	*CI 95%*	*p-value*
	No.	%	No.	%			
C	62	56	75	56	0.984	0.592-1.636	0.950
G	48	44	59	44	1.016	0.611-1.689	

The results from Hardy-Weinberg equilibrium exact test clarified that there was no significant difference among the three genotypes for both patients and control (Table 9);The present study indicated the appearance of the genotype CG, GG in epileptic patients were (49.2%, 19.4% respectively) while in the control group were (47.3% ,20% respectively).

Table (9): Genotype frequency of rs1801726 and deviation from Hardy- Weinberg equilibrium.

genotype	Control		Patients		OR	*CI 95%*	*p-value*
	No.	%	No.	%			
CC	18	32.7	21	31.34	1	-	0.98
CG	26	47.3	33	49.25	1.09	0.48-2.45	
GG	11	20	13	19.4	1.01	0.37-2.81	

Further analysis for the data was tested to study the association of each genotypes with epilepsy under different models of inheritance. The result showed that there were no significant association between any genotype and epilepsy under any of studies inheritance model table (10).

Understanding the *Casr's* (how it works) relative to its potential global Ca^{2+} control in the brain is difficult. However, it is likely that the *Casr* has Ca^{2+} concentration in the brain. The *Casr* has a PTH (parathyroid Hormone Ca^{2+} regulation) response. The SNP (on exon seven, which is called rs1801725, and two on the adjacent) were found to be strongly correlated with the existence of Alzheimer's disease [25].

Table (10): Association of rs1801726 genotype with epilepsy under different models of inheritance.

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	C/C	18 (32.7%)	21 (31.3%)	1.00	0.98
	C/G	26 (47.3%)	33 (49.2%)	1.09 (0.48-2.45)	
	G/G	11 (20%)	13 (19.4%)	1.01 (0.37-2.81)	
Dominant	C/C	18 (32.7%)	21 (31.3%)	1.00	0.87
	C/G-G/G	37 (67.3%)	46 (68.7%)	1.07 (0.50-2.29)	
Recessive	C/C-C/G	44 (80%)	54 (80.6%)	1.00	0.93
	G/G	11 (20%)	13 (19.4%)	0.96 (0.39-2.36)	
Overdominant	C/C-G/G	29 (52.7%)	34 (50.8%)	1.00	0.83
	C/G	26 (47.3%)	33 (49.2%)	1.08 (0.53-2.21)	

The present investigation focused on chromosome 3 CASR gene sequences. The protein encoded by this gene, called G-protein-coupled receptor, has several roles in regulating calcium homeostasis (https://www.uniprot.org/uniprot/P41180). NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) confirmed the identification of this genomic segment based on the sequencing reactions. About 99.5% sequence similarity was found between the sequenced samples and the reference target sequences, which partially cover the coding regions for the G-protein-coupled receptor, when analyzing the 256 bp amplicons using the NCBI BLASTn engine. Alignment findings for the 256 bp samples showed that some samples differed from the reference DNA sequences by a single base pair (figure 11). This investigation revealed an intriguing nucleic acid polymorphism (SNP) in the examined samples: the substitution of adenine for guanine at position 116 (G116C).

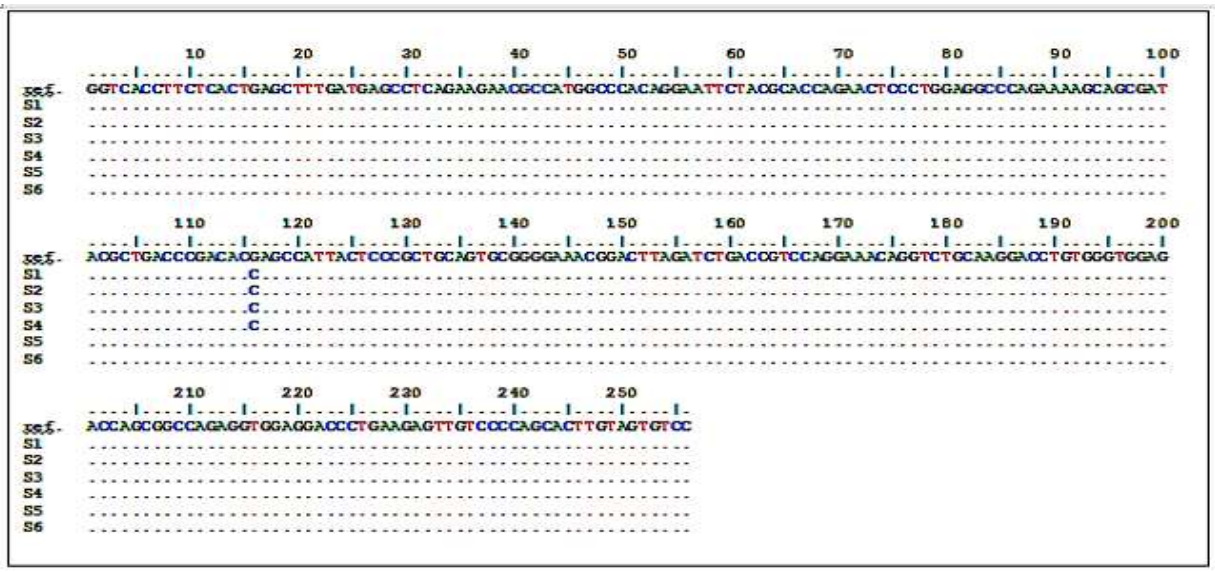

Figure (11) DNA sequences alignment of 6 genotyped samples with their corresponding reference sequences of the 256 bp amplicons of the coding sequences of the *CASR* gene. The symbol "ref." refers to the NCBI referring sequence, "S1-S6" refer to the genotyped samples 1 to 6, respectively.

The PCR amplicon location of the sequence containing the detected variant was shown alongside the corresponding chromatogram and extensive comments. Nonetheless, S1 and S2 showed a heterozygous G/C genotype for this SNP, whereas S3 and S4 were found to be homozygous C/C, and S5 and S6 were found to be homozygous G/G (Figure 11).

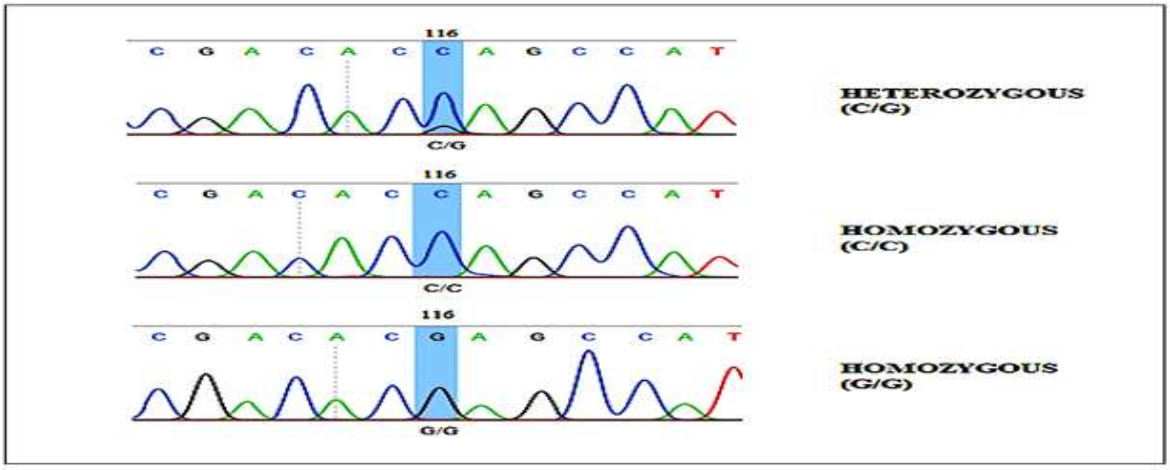

Figure (12): The pattern of the detected G116C SNP within the DNA chromatogram of the targeted 256 bp amplicons of the *CASR* gene. The identified SNP was highlighted according to its position in the PCR amplicons. S1 / S2, S3 / S4, and S5 / S6 samples exhibited the G/C, C/C, and G/G states respectively in the highlighted polymorphic locus.

C. Haplotype analysis of *CaSR* gene (rs1801725 and rs1801726) :

Haplotypes are groups of alleles at distinct loci on the same chromosome that are inherited together. The fact that alleles are allocated to a chromosome distinguishes haplotypes from individual genotypes at SNPs. Each person has two haplotypes for each length of the genome, which reflect the maternal and paternal chromosomes, respectively [26]

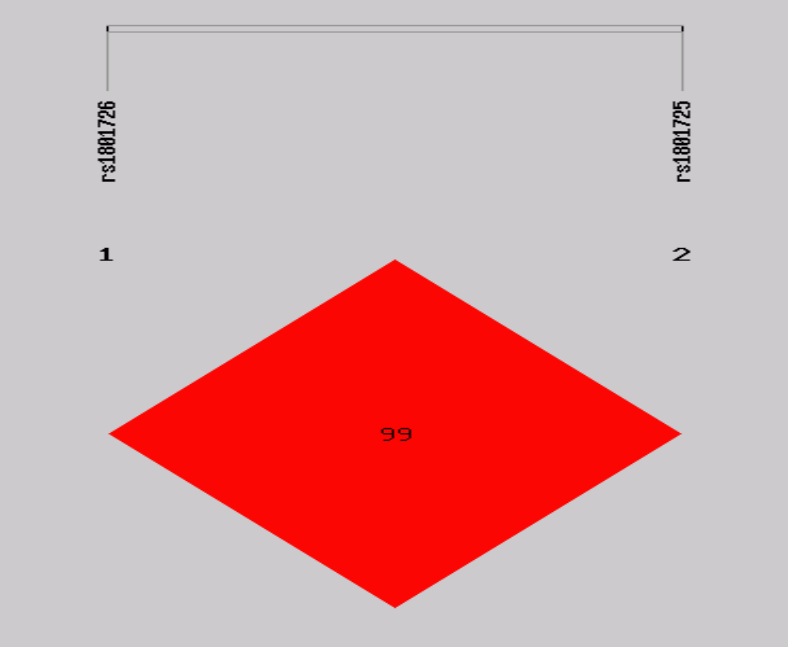

Figure (13) Haplotype analysis of *CaSR* (rs1801725 and rs1801726)

Linkage disequilibrium between (rs1801725 and rs1801726) shows the strong linkage between the two SNPs D' (99)figure (13), this is logical due to the short genomic distance between the two SNPs (75bp), Figure (14) the genomic location of the two rs1801725 and rs1801726 according to NCBI.

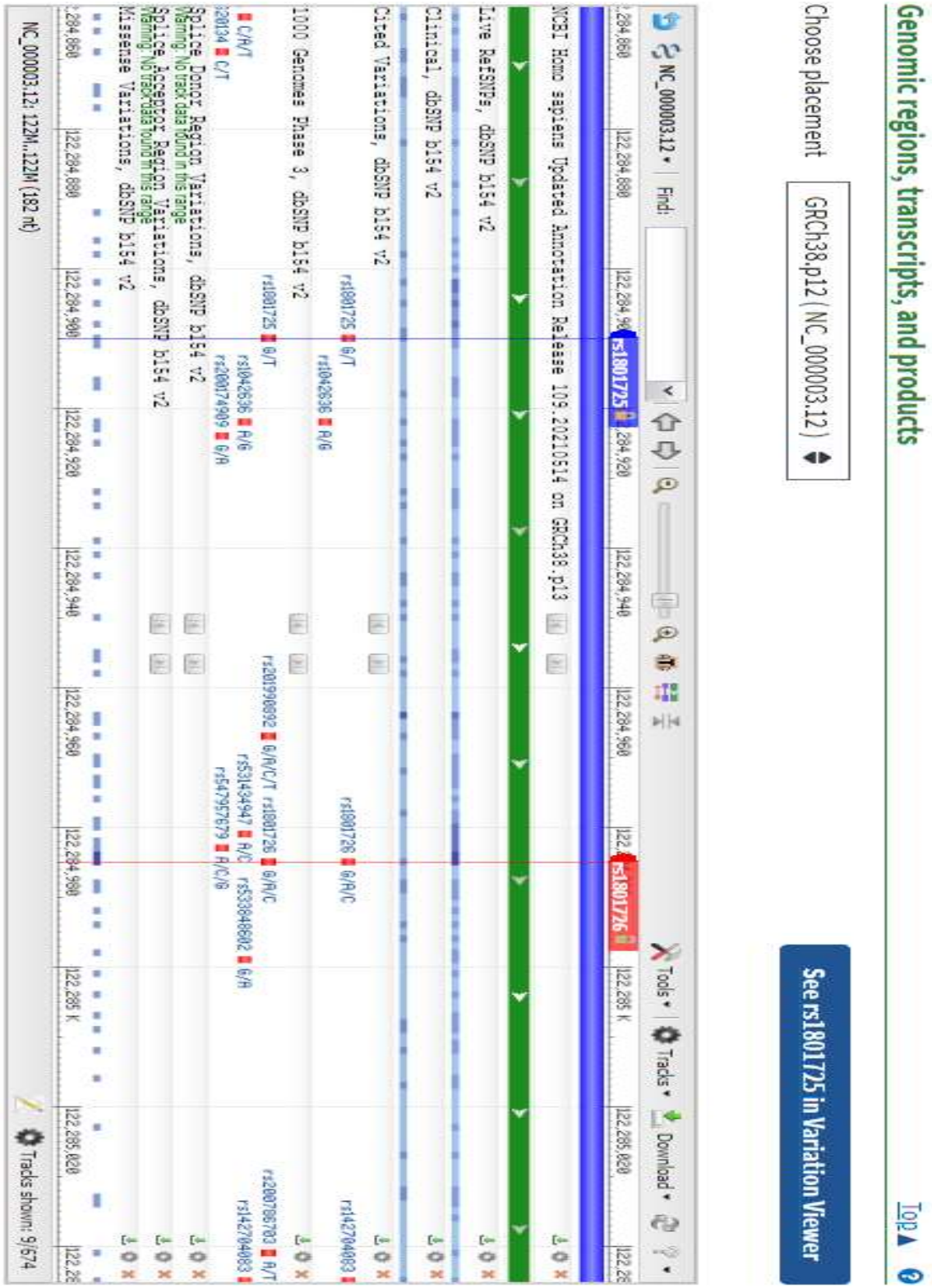

Figure (14) the genomic location of the two rs1801725 and rs1801726

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