

**THE ESTROGEN RECEPTOR 1 G594A
POLYMORPHISM IS ASSOCIATED WITH
MIGRAINE SUSCEPTIBILITY IN TWO
INDEPENDENT CASE/CONTROL GROUPS**

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Abstract

Migraine is a painful and debilitating disorder with a significant genetic component. Steroid hormones, in particular estrogen, have long been considered to play a role in migraine, as variations in hormone levels are associated with migraine onset in many sufferers of the disorder. Steroid hormones mediate their activity via hormone receptors, which have a wide tissue distribution. Estrogen receptors have been localized to the brain in regions considered to be involved in migraine pathogenesis, hence it is possible that genetic variation in the estrogen receptor gene may play a role in migraine susceptibility. This study thus examined the estrogen receptor 1 (ESR α) gene for a potential role in migraine pathogenesis and susceptibility. A population-based cohort of 224 migraine sufferers and 224 matched controls were genotyped for the G594A polymorphism located in exon 8 of the ESR1 gene. Statistical analysis indicated a significant difference between migraineurs and non-migraineurs in both the allele frequencies ($P = 0.003$) and genotype distributions ($P = 0.008$) in this sample.

An independent follow-up study was then undertaken using this marker in an additional population based cohort of 260 migraine sufferers and 260 matched controls. This resulted in a significant association between the two groups with regard to allele frequencies ($P = 8 \times 10^{-6}$) and genotype distributions ($P = 4 \times 10^{-5}$). Our findings support the hypothesis that genetic variation in hormone receptors, in particular the estrogen receptor 1 gene, may play a role in migraine.

Keywords: migraine, association, estrogen receptor gene

Introduction

Migraine is a genetically complex disease with a multifactorial mode of inheritance (1,2). It has a high prevalence with approximately 18% of women and 6% of men suffering from the disorder (3). Migraine is characterized by severe head pain with associated nausea, emesis, photophobia, phonophobia, and neurological disturbances. The International Headache Society (IHS) has classified various types of migraine according to their clinical features (4). The two main subtypes of migraine are migraine without aura (MO), occurring in ~70-75% of migraineurs, and migraine with aura (MA) which occurs in ~25% of migraineurs (4). Some people experience both types of attack in their lives (5). While the precise pathogenesis of migraine is unknown, it is widely accepted that short-term alterations in neuronal activity occur in relation to the attack, along with temporary changes in the cerebral vasculature. Trigeminal nerve activation is also considered pivotal to progression of a migraine attack (6).

There is significant evidence to indicate that the fluctuating hormones of the ovarian cycle are specific migraine triggers (7,8), although the precise role of hormones in the pathogenesis of migraine is yet to be established. To date, studies have focused on the hormonal milieu, in particular estrogen. The currently recognised mechanism of hormonally triggered migraine is estrogen withdrawal, however this theory is based on empirical evidence that attacks can be prevented by artificially stabilizing hormone levels (9).

Steroid hormones exert their effects via a cognate receptor. The classic mode of action of steroid hormone receptors is as ligand activated transcription factors, regulating gene expression via interaction with hormone response elements in the promoter region of sensitive genes. Recent research has also demonstrated non-genomic effects of steroid hormone receptors, resulting in rapid effects activated via intracellular second messenger systems (10). Furthermore there is evidence to indicate that steroid hormone receptors can be activated in the absence of hormones (11). Given the putative role of hormones in migraine, and the relationship between steroid hormones and their receptors, we postulated that genes encoding hormone receptors represent likely candidates for migraine studies.

ESR1 is located on chromosome 6q25.1. It has 8 exons (12), and is over 295 kilobases in size (13). ESR1 is expressed in various human brain regions including the hypothalamus, limbic system, hippocampus, cortices of the temporal lobe and the brainstem (14). It is expressed in serotonin neurons of some species (15). In addition to alternative splicing mechanisms, different promoters are used to regulate ESR1 in distinct neuronal populations (14). Along with its role in target gene transcription, ligand activated ESR1 has rapid effects on neuronal excitability via second messenger systems, resulting in a range of cellular effects including changes in Ca^{2+} currents and activation of endothelial nitric oxide synthase (11,16,17). As changes in neuronal excitability have been implicated in migraine pathogenesis, we hypothesized that genetic variation in the ESR1 may impact on expression or function, in turn influencing migraine susceptibility. To our knowledge, no published study exists on the potential role of this gene in

migraine. Our pilot study has investigated a common synonymous polymorphism located in exon 8 of the ESR1 gene (NCBI mRNA ref seq MN_000125) (18). This polymorphism, first described by Roodi *et al.*, (1985), occurs in codon 594 of exon 8 and consists of a guanine to adenine change at nucleotide 2014 (SNP rs 2228480) (18, 19). This polymorphism has previously shown an association with breast cancer, a disease in which complex hormonal influences are considered to play a role (20). Given the putative role of hormones in migraine, we chose this polymorphism as a candidate for our current investigations. The investigation was undertaken using a population based case-control approach. Due to past problems with non-replication of positive associations, we performed an additional study on an independent population based cohort using the same marker.

Materials and Methods

Study Population

Research was approved by the Griffith University Ethics Committee for experimentation on human subjects. All 1150 participants of the study gave informed consent prior to participation. All participants were interviewed, and completed a detailed questionnaire providing information including personal and family medical history, migraine symptoms, age of onset, frequency, severity and treatment as previously described (21, 22). This questionnaire revealed that 78% of individuals in the migraine group had a known family history of migraine. Migraineurs were diagnosed by a clinical neurologist as having either migraine

with aura (MA) or migraine without aura (MO) based strictly on the widely accepted criteria specified by the International Headache Society (4). The first study population was comprised of 275 migraineurs and 275 unrelated control individuals. The controls were matched for sex, age [\pm 5 years], and ethnicity [Caucasian] to avoid the potential bias of population stratification, and were recruited in parallel at a similar time and geographical location (East Coast of Australia) as the case group. The follow-up second study population consisted of 300 migraineurs similarly diagnosed and matched with 300 controls. All participants provided a blood sample from which DNA was extracted by a modification of the salting out method used by Miller *et al.* (1988) (23).

Genotyping

Genotyping for the ESR1 G594A marker was undertaken by polymerase chain reaction (PCR) and restriction enzyme digestion. Oligonucleotide primers used were those previously described by Curran *et al* (2001), sense 5'GAG GAG ACG GAC CAA AGC CAC3' and antisense 5'GCC ATT GGT GTT GGA TGC ATG C3' resulting in a 227 base pair fragment following PCR (20). The 20 μ l PCR reaction mix contained 50 ng genomic DNA, 0.25 μ M of each primer, 1 x PCR buffer, 3.75 mM MgCl₂, 0.2mM dNTPs and DNA polymerase. Thermocycler conditions were 94 °C for 2 minutes 30 seconds, 5 cycles of 94 °C for 45 seconds, 69 °C for 1minute, and 72 °C for 2 minutes, followed by 30 cycles of 94 °C for 30 seconds, 67 °C for 30 seconds and 72 °C for 45 seconds, with a final step of 72 °C for 5 minutes. The G allele at codon 594 in the ESR1 gene introduces a restriction site for the *BtgI* enzyme, resulting in fragments of 129 and 98 base pairs. Following amplification, 10 μ l of product was digested with *BtgI*

overnight at 37 °C. After digestion, the product was loaded into a 5% Agarose gel stained with ethidium bromide and electrophoresed at 90V for 60 minutes. An undigested sample indicated presence of the 594A allele. An electrophoretogram of the digested PCR product illustrating all genotypes appears in Figure 1.

Statistical Analysis

Genotype data and allele frequencies were compared between the two populations using standard chi-square analysis. Only when results were available from both matched pairs ie. migraine affected and age, sex matched controls, were they included in the genotypic analyses. Odds ratios and 95% confidence intervals were calculated. Due to multiple testing, the Bonferroni correction for 5 tests was applied, which set the level of significance at 0.01 (ie. 0.05/5). All genotype frequencies were tested for Hardy Weinberg Equilibrium.

Results

Statistical analysis revealed a significant difference between genotyped migraineurs and the matched control group with regard to allele frequencies ($P = 0.003$) and genotype frequencies ($P = 0.008$). Results of comparisons between male case and control groups (allele frequency $P = 0.034$; genotype frequency $P = 0.046$), and female case and control groups (allele frequency $P = 0.032$; genotype frequency $P = 0.064$) indicated that no significant gender effect was evident. Furthermore, the association was seen in both subgroups, MA (allele frequency $P = 0.013$; genotype frequency $P = 0.025$) and MO (allele frequency $P = 0.019$;

genotype frequency $P = 0.007$). Consequently, the significant association seen in the case-control analysis occurred similarly in both males and females, and in the MA and MO subgroups. Results are displayed in Table 1. The follow-up independent study also revealed a significant difference between genotyped migraineurs and the matched control group with regard to allele frequencies ($P = 8 \times 10^{-6}$) and genotype frequencies ($P = 4 \times 10^{-5}$). This significant association occurred in females (allele frequency $P = 3 \times 10^{-6}$; genotype frequency $P = 2 \times 10^{-5}$), and in the MA subgroup (allele frequency $P = 1 \times 10^{-6}$; genotype frequency $P = 7 \times 10^{-6}$). Although the association did not occur in males (allele frequency $P = 0.717$; genotype frequency $P = 0.127$) and the MO subgroup (allele frequency $P = 0.529$; genotype frequency $P = 0.818$), this may be due to small numbers in these subgroups (males $n = 36$, MO $n = 39$). Alternatively, estrogen and its receptor may play a lesser role in male migraineurs. Results are displayed in Table 2. Allele frequencies in both study populations did not deviate from Hardy-Weinberg equilibrium ($P = 0.14$; $P = 0.88$), and each independent sample cohort showed similar frequencies in the case and control groups. Internal controls using random repeat samples and negative controls were used to confirm genotypes and to exclude the potential for genotyping errors, which in our hands have been estimated to be $<5\%$. Only when results for both of a matched pair were obtained, were they included in the analysis. Results of odds ratio calculations based upon the Mantel Haenszel method of combining the datasets (22) comparing the G/G genotypes with the G/A and A/A genotype frequencies together, indicated that individuals who carried the 594A allele were 2 times more likely to suffer from migraine [OR = 1.96, 95% CI = 1.43-2.68] than those who did not carry this allele. Similarly, odds ratios were calculated on the subgroups comparing G/G

genotypes with the G/A and A/A genotype frequencies together. Results were as follows: MA subgroup OR = 1.97, [95% CI = 1.41-2.77]; MO subgroup OR = 1.80 [95% CI = 1.10 to 2.94]; males OR = 1.95 [95% CI = 0.95 to 3.98]; females OR = 1.96 [95% CI = 1.39 to 2.78].

In summary, we performed association analyses in two independent study populations. Results of these studies, which have remained significant after we have applied a Bonferroni correction for multiple testing ($\alpha = 0.01$), provide evidence for association of the Estrogen Receptor 1 G594A polymorphism with migraine susceptibility.

Discussion

Migraine is considered a genetically complex disorder showing strong familial aggregation. A higher concordance in monozygotic twins than dizygotic twins indicates a strong genetic component along with complex inheritance (25).

Approximately 50% of susceptibility is attributed to multiple genes while the balance is attributed to environmental factors (26). Although the genetic basis for migraine is largely unknown, investigations of various genes involved in key biological pathways have been undertaken. Results so far have been mixed, although this may be attributed in part to the heterogeneous nature of the disorder. Current understanding of migraine is that a number of genes and/or environmental factors may each contribute to an individual's migraine susceptibility (26).

Steroid hormones, in particular estrogen, have long been considered to play a role in migraine pathogenesis. The present study considered that this role could be related to or exacerbated by variation in the estrogen receptor gene. We have

conducted a case-control association study to investigate the estrogen receptor α gene as a candidate in migraine susceptibility. This method is considered useful for investigating complex diseases (27) and for detecting susceptibility genes of modest effect (28). The study tested two large carefully matched case-control populations for an ESR1 exon 8 SNP. Results of this study have indicated a positive association of an ESR1 exon 8 polymorphism with migraine in two independent cohorts. This positive association was seen equally in all subgroups in the initial study group and in the female and MA subgroups in the follow-up group. Lack of association in males may have been related to the limited number of males in the second population, however it is also possible that hormonal factors may play a different role in the two genders in migraine, and estrogen and its receptor may play a lesser role in male migraineurs.

Although this marker showed significant differences in allele frequencies in the two test versus control populations, association studies by virtue of their design do not determine whether it is the marker itself or a variant in linkage disequilibrium that is responsible for the association. Further genetic and functional analyses will be needed to determine the causal mechanism for our detected estrogen receptor association. It is possible that other polymorphisms and mutations in this gene may be associated with migraine, in particular those in linkage disequilibrium with this particular marker. Previous population genetics studies performed in our laboratory have shown that markers in exon 1 and exon 4 were not in linkage disequilibrium with the exon 8 variant (20). This information suggests that the migraine susceptibility haplotype is specific to the 3' region of the ESR gene. To our knowledge this is the first study to report an association of a hormone related

gene variant with migraine and these data suggest that the estrogen receptor gene may be an integral player in mechanisms that are relevant to migraine pathogenesis.

Steroid hormones, via their receptors, have many well-known effects on the central nervous system, particularly in relation to sexual differentiation and reproductive function. New information is emerging indicating a much wider range of effects than previously understood. Furthermore, these effects have the potential to impact on factors involved in migraine pathogenesis. Estradiol can exert behavioural and electrophysiological effects by binding to neuronal membranes, in particular the central serotonergic and opioid neurons. This mechanism may lead to a disturbance of pain perception (29). According to animal models, sex steroid hormones may also cause changes in regions involved in the neurovascular headache pathway (30). Estrogen changes in the rat have been associated with altered mRNA expression in sensory neurons (31), while estrogen injections appear to alter the size of the receptive area of the trigeminal mechanoreceptors in rats (32).

A further possible explanation for the role of estrogen receptor in migraine is its influence on calcium channels (10). Due to the discovery of mutations in the CACNA1A calcium channel gene in familial hemiplegic migraine, a rare subtype of migraine, the role of calcium channels and calcium homeostasis in the pathogenesis of common migraine is also possible. Calcium and other ion channels are significant factors in the mechanism of neurotransmitter release and cortical spreading depression (33), thus impaired function of calcium channels

and calcium homoeostasis could trigger an attack. Furthermore, an altered density of calcium channels could result in excitation of the periaqueductal grey, raphe nuclei or locus coeruleus neurons that are considered to be in the region responsible for initiation of migraine attacks (33).

L-type Ca^{2+} channels are one of the main pathways of intercellular calcium entry in the brain. Johnson *et al.* (1997) demonstrated in an animal model that the density of cardiac L-type Ca^{2+} channels is regulated by the estrogen receptor (34), while Mermelstein *et al.* (1996) demonstrated that estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor (35). A further consideration is that ESR can mediate changes in vascular tone. Chen *et al.* (1998) demonstrated that ESR1 mediates the non-genomic activation of endothelial nitric oxide synthase, causing the rapid dilation of blood vessels. Furthermore, the observed response was evident at concentrations well below those found in normal cycling women (17). Obviously these hypotheses would require extensive analysis, as estrogen/ESR action is understood to be tissue specific. Nevertheless they demonstrate potential mechanisms whereby genetic variation in the estrogen receptor gene could have an impact on mechanisms that may be crucial to migraine pathogenesis.

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Experiments comply with the current laws in Australia.

References

1. Terwindt G, Haan J, Ophoff R, Frants R, Ferrari M (1997) The quest for migraine genes. *Curr Opin Neurol* 10:221-225.
2. Gardner K (1999) The genetic basis of migraine: how much do we know? *Can J Neurol Sci* 26:Supp3-S37-S43.
3. Stewart WF, Schechter A, Rasmussen BK (1994) Migraine prevalence: a review of population-based studies. *Neurology* 44(suppl 4):S17-S23.
4. Headache Classification Committee of the International Headache Society (1988) Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalgia* 8 (Suppl 7):20.
5. Ferrari MD (1998) Migraine. *Lancet* 351(9108):1043-1051.
6. Moskowitz MA (1991) The visceral organ brain: implications for the pathophysiology of vascular head pain. *Neurology* 41(2(Pt 1)):182-186.
7. MacGregor EA (1996) "Menstrual" migraine: towards a definition. *Cephalgia* 16:11-21.

8. MacGregor EA (1997) Menstruation, sex hormones, and migraine. *Neurol Clin* 15:125-141.
9. Massiou H, MacGregor, EA (2000) Evolution and treatment of migraine with oral contraceptives. *Cephalgia* 20:170-174.
10. Kelly MJ, Levin ER (2001) Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol Metab* 12:152-156.
11. Cenni B, Picard D (1999) Ligand-independent activation of steroid receptors: new roles for old players. *Trends Endocrinol Metab* 10(2):41-46.
12. Iwase H, Greenman JM, Barnes DM, Hodgson S, Bobrow L, Mathew CG (1996) Sequence variants of the estrogen receptor gene found in breast cancer patients with ER negative and progesterone receptor positive tumours. *Cancer Lett* 108:179-184.
13. Weizmann Institute of Science GeneCards, available <http://bioinfo.weizmann.ac.il/cards/index.html>
14. Osterlund MK, Grandien K, Keller E, Hurd YL (2000) The human brain has distinct regional expression patterns of estrogen receptor α mRNA isoforms derived from alternative promoters. *J Neurochem* 75(4):1390-1398.

15. Bethea CL, Lu NZ, Gundlach C, Streicher JM (2002) Diverse actions of ovarian steroids in the serotonin neural system. *Front Neuroendocrinol* 23:41-100.
16. Luconi M, Forti G, Baldi E (2002) Genomic and non-genomic effects of estrogens: molecular mechanisms of action and clinical implications for male reproduction. *J Steroid Biochem Mol Biol* 80:369-381.
17. Chen Z, Yuhanna I, Galcheva-Gargova Z, Karas R, Mendelsohn M, Shaul P (1999) Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest* 103(3):401-406.
18. National Centre for Biotechnology Information, available <http://www.ncbi.nlm.nih.gov/>
19. Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, Parl FF (1995) Estrogen receptor gene analysis in estrogen receptor positive and receptor negative primary breast cancer. *J Natl Cancer Inst* 87(6):446-451.
20. Curran JE, Lea RA, Rutherford S, Weinstein SR, Griffiths LR (2001) Association of estrogen receptor and glucocorticoid receptor gene polymorphisms with sporadic breast cancer. *Int J Cancer (Pred Oncol)* 95:271-275.

21. Lea RA, Dohy A, Jordan K, Quinlan S, Brimage PJ, Griffiths LR (2000) Evidence for allelic association of the dopamine b-hydroxylase gene (DBH) with susceptibility to typical migraine. *Neurogenetics* 3:35-40.
22. Johnson MP, Lea RA, Curtain RP, Macmillan JC, Griffiths LR (2003) An investigation of the 5-HT_{2C} receptor gene as a migraine candidate gene. *Am J Med Genet* 117B:86-89.
23. Miller SA, Dykes DD, Plensky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16(3):1215.
24. Mantel N, and Haenszel W (1959) Statistical Aspects of the Analysis of Data from Retrospective Studies of Disease. *J Natl Cancer Inst* 22:719 - 748.
25. Haan J, Terwindt GM, Ferrari MD (1997) Genetics of migraine. *Neurol Clin* 15:43-59.
26. Peroutka SJ (2002) Sympathetic look at genetic basis of migraine. *Headache* 42(5):378-381.

27. Xu J, Wiesch DG, Meyers DA (1998) Genetics of complex human diseases: genome screening, association studies and fine mapping. *Clin Exp Allergy* 28(5):1-5.
28. Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. *Science* 273:1516-1517.
29. Silberstein SD, Merriam GR (2000) Physiology of the menstrual cycle. *Cephalgia* 20:148-154.
30. Marcus DA (1995) Interrelationships of neurochemicals, estrogen, and recurring headache. *Pain* 62:129-139.
31. Sohrabji F, Miranda RC, Toran-Allerand CD (1994) Estrogen differentially regulates estrogen and nerve growth factor receptor mRNAs in adult sensory neurons. *J Neurosci* 14:459-471.
32. Bereiter DA, Stanford LR, Barker DJ (1980) Hormone-induced enlargement of receptive fields in trigeminal mechanoreceptive neurons: II possible mechanisms. *Brain Res* 184:411-423.
33. Edvinsson L (1999) On migraine pathophysiology. In: Edvinsson L (ed). *Migraine and headache pathophysiology*. Martin Dunitz Ltd London, pp 3-5.

34. Johnson BD, Zheng W, Korach KS, Scheuer T, Catterall WA, Rubanyi GM (1997) Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. *J Gen Physiol* 110:135-140.

35. Mermelstein PG, Becker JB, Surmeier DJ. (1996) Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J Neurosci* 16(2):595-604.

Table 1. Distribution of ESR Exon 8 Codon 594 ACG/ACA Polymorphism frequencies in migraineurs and controls of original sample

Group	Genotypes			N (alleles)	Alleles	
	GG	GA	AA		G	A
Migraine	81 (36%)	120 (54%)	23 (10%)	448	282 (63%)	166 (37%)
Male	18 (32%)	33 (58%)	6 (10%)	114	69 (61%)	45 (39%)
MA	11 (31%)	19 (53%)	6 (16%)	72	41 (57%)	31 (43%)
MO	7 (33%)	14 (67%)	0	42	28 (68%)	14 (32%)
Female	63 (38%)	87 (52%)	17 (10%)	334	213 (64%)	121 (36%)
MA	44 (43%)	47 (46%)	12 (11%)	206	135 (66%)	71 (34%)
MO	19 (30%)	40 (62%)	5 (8%)	128	78 (61%)	50 (39%)
Control	112 (50%)	99 (44%)	13 (6%)	448	323 (72%)	125 (28%)
Male	28 (49%)	28 (49%)	1 (2%)	114	84 (74%)	30 (26%)
Female	84 (50%)	71 (43%)	12 (7%)	334	239 (72%)	95 (28%)
Total Case V Control	$\chi^2 = 9.77$ $p = 0.008$			$\chi^2 = 8.56$ $p = 0.003$ $df = 1$		

Table 2. Distribution of ESR Exon 8 Codon 594 ACG/ACA Polymorphism frequencies in independent sample

Group	Genotypes			N (alleles)	Alleles	
	GG	GA	AA		G	A
Migraine	103 (40%)	125 (48%)	32 (12%)	520	331 (64%)	189 (36%)
Male	15 (42%)	19 (53%)	2 (5%)	72	49 (68%)	23 (32%)
MA	11 (37%)	17 (56%)	2 (7%)	60	39 (65%)	21 (35%)
MO	4 (67%)	2 (33%)	0	12	10 (83%)	2 (17%)
Female	88 (39%)	106 (47%)	30 (14%)	448	282 (63%)	166 (37%)
MA	71 (37%)	93 (49%)	27 (14%)	382	235 (62%)	147 (38%)
MO	17 (52%)	13 (39%)	3 (9%)	66	47 (71%)	19 (29%)
Control	152 (58%)	93 (36%)	15 (6%)	520	397 (76%)	123 (24%)
Male	20 (55%)	11 (31%)	5 (14%)	72	51 (71%)	21 (29%)
Female	132 (59%)	82 (37%)	10 (4%)	448	346 (77%)	102 (23%)
Total Case V Control	$\chi^2 = 20.26$ $p = 4 \times 10^{-5}$			$\chi^2 = 19.95$ $p = 8 \times 10^{-6}$ $df = 1$		

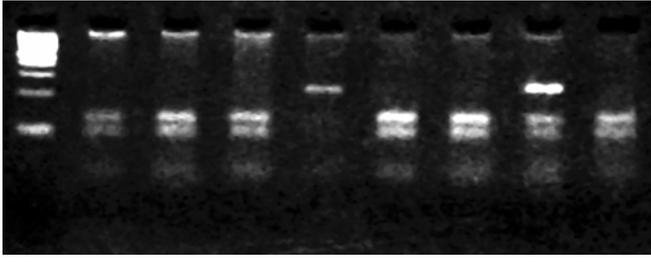


Fig 1. Agarose gel electrophoretogram of ESR1 gene exon 8 PCR product after digestion with *BtgI* in 8 migraineur samples. Lane 1 shows the 100 base pair ladder. Lanes 2, 3, 4, 6, 7, and 9 show 129 and 98 base pair fragments of a homozygote for the G allele. Lane 5 shows a 229 base pair fragment of a homozygote for the A allele. Lane 8 shows 227, 129, and 98 base pair fragments of a heterozygote.