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GENETIC POLYMORPHISM IN BRAF GENE ASSOCIATED WITH THE INCIDENCE OF BRAIN TUMORS IN A SAMPLE OF IRAQI PATIENTS

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ABSTRACT : A total number of 40 tissue sample from male and female suffering brain tumors were selected and subjected to study the Genetic polymorphism in the BRAF gene that cause lethal deterioration in its function. Tissue samples were collected from Neuroscience Hospital in 2017 and submitted to molecular analysis using specific primers designed for this purpose. Thirty mutations were identified in BRAF region 28 of them are single nucleotide polymorphism (SNPs) with missense translation and two deletions inframe type. All these mutation caused a pathogenic effect on the molecular consequence, located on chromosome 7, benign at nucleotide 140, 753,333 and ended at nucleotide 140, 781, 600. An important finding that was registered through NCBI with accession number LC421658 is a lethal stop codon at position 2032 on BRAF gene appeared through gene analysis of Iraqi patients. This mutation was not reported in previous studies. The sequence of BRAF gene isolated from Iraqi patient with brain tumor was registered in National Center for Biotechnology Information (NCBI) with accession number (NRM33).

Key words : BRAF gene, brain tumor, genetic polymorphism, oncogene.

INTRODUCTION

Gliomas are the most frequent primary brain tumors in children and adults (Gurney and Kadan-Lottick, 2001). Derived from neuroepithelial cells, gliomas are a heterogeneous group composed of diûerent histological and biological subtypes, including astrocytic, oligodendroglial and mixed oligoastrocytic tumors. These neoplasms are classiûed in four grades of malignancy according to the World Health Organization (WHO) classiûcation of brain tumors (Kleihues and Cavenee, 2000). Gliomas are the most common CNS tumors in children and adolescents, and they show an extremely broad range of clinical behavior. The majority of pediatric gliomas present as benign, slow growing lesions classified as grade I or II by the WHO classification of CNS tumors. Pediatric low-grade gliomas (LGGs) or glioneuronal tumors (WHO grade I or II) are a highly heterogeneous collection of entities accounting for 25% to 30% of all childhood CNS tumors. They are roughly as common as malignant gliomas and embryonal tumors combined (Louis *et al*, 2016). BRAF encodes a RAF kinase, which signal downstream of RAS and activate the MAPK pathway and has emerged as a major oncogenic driver and a potential therapy target in a wide variety of solid tumors and hematological malignancies (Vultur *et al*, 2011). BRAF signaling is critical for cell division and differentiation and activating BRAF mutations result in uncontrolled growth and tumorigenesis. 2–4 Over 90% of activating BRAF mutations in cancer cells occur within the kinase domain at amino acid V600, most commonly resulting in V600E, which is an approved target for the inhibitors dabrafenib and vemurafenib in the treatment of metastatic malignant melanoma (Cantwell *et al*, 2011). Some nonmelanoma malignancies with activating BRAF alterations such as V600E have responded to BRAF targeted therapy (Dadu *et al*, 2015). BRAF gene fusions represent a different mechanism of BRAF activation and have been described in several solid tumor types (Pettirossi *et al*, 2015).

MATERIALS AND METHODS

Sample collection

The study included 40 fresh tissues collected directly at operation room from male and female suffering brain tumor during the period started from March 2017 to December 2017 attending Neuroscience Hospital in Baghdad and five healthy persons (provided by forensic institute). Their age ranging from 20 to 70 years.

Table 1 : Sequences of primers used in the procedures of the present study with PCR product size and references.

No.	Primer name	Sequences 3′—— 5′	Product size bp	References
1.	BR-1-F	TGCATTTGGGATTGTTCTGTATGA	370	Designed in this study*
	BR-1-R	AAACGCACCATATCCCCCTG		
2.	BR-2-F	TGCATTTGGGATTGTTCTGTATG	301	Designed in this study*
	BR-2-R	TGTTTGGAAACCAGCCCGAT		
3.	BR-3-F	AGACGGGACTCGAGTGATGA	617	Designed in this study*
	BR-3-R	TCATACAGAACAATCCCAAATGC		
4.	BR-4-F	GCATTTGGGATTGTTCTGTATGA	370	Designed in this study*
	BR-4-R	GAAACGCACCATATCCCCCT		

DNA extraction

Total cellular DNA extracted from tissue samples using the Reliaprep tissue genomic DNA MiniPrep System from Geneaid/Korea, determination of concatenation and purity of the extracted DNA was done using nanodrop (Techne, UK).

PCR protocols

Extracted DNA from tissue samples and healthy was subjected to PCR amplification using specific primers designed specifically to amplify BRAF gene using the following program: initial denaturation at 94^{0}C for 5 min., 35 cycles of denaturation at 94^{0}C for 1 min, annealing at 55^{0}C for 1 min, extension at 72^{0}C for 1 min. and final extension at 72^{0}C for 10 min.

DNA sequencing

The purified PCR products of the amplified BRAF gene were sent to Macrogen, Korea for sequencing. The obtained sequences of these samples were analyzed at the National Center for Biotechnology Information (NCBI) website using the BLAST search and analysis tool and examined for the presence of SNP.

RESULTS

BRAF gene amplification

Samples of fresh tissue were subjected to amplification through PCR using four specific primers designed specifically for this purpose. Amplicons resulted were 370bp, 301bp, 617bp and 370bp in size, respectively shown in Fig. 1.

Molecular analysis of BRAF gene

Detection of BRAF gene mutations by sequencing

After amplification of genomic fragments related to BRAF gene located on chromosome 7, 30 SNPs were identified. About 28 of these genetic changes were of missense type detected on introns caused by single nucleotide change, whereas 2 deletions were found in these introns also. The molecular consequence of these SNPs was a lethal effect and disrupted completely BRAF function. Table 2 shows the details of SNPs identified with their location and effect.

Open reading frame analysis

Normal BRAF gene plays majore role in cell cycle control. This comes from affecting serine/ therionin kinase function which regulates cell division. Expresion of BRAF

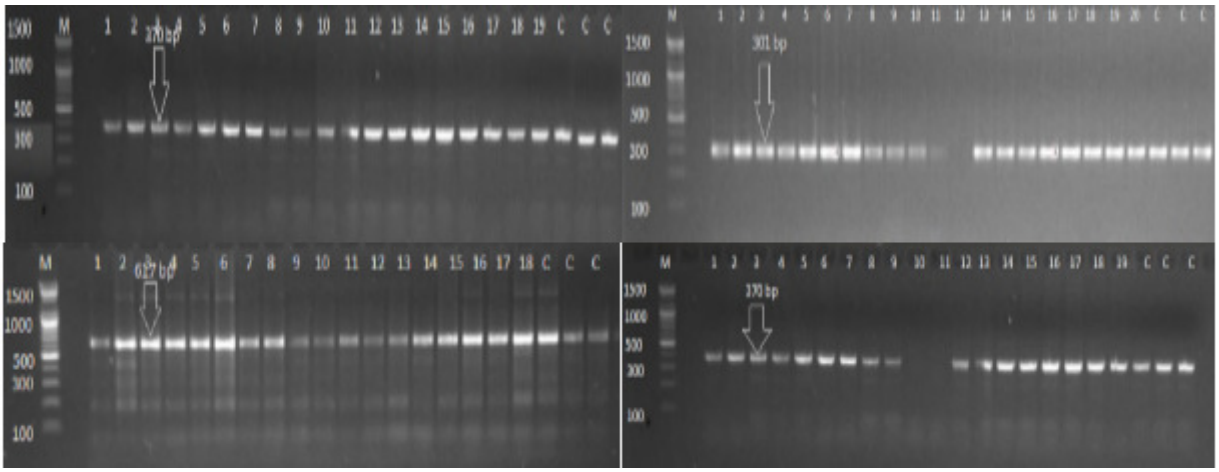

Fig. 1 : Specific PCR amplification of BRAF gene using primers designed in this study. M: DNA Lader 100bp, Lance1-19: patients, Lance C: Control (healthy).

Table 2 : Details of SNPs identified on BRAF gene.

Variant ID	Location	Variant type	Gene	Molecular consequences	Most severe clinical significance	Alleles
rs397507484	140,753,333	single nucleotide variant	BRAF	intron variant, non-coding transcript variant, missense variant	Pathogenic	T, A, G
rs121913364	140,753,334	single nucleotide variant	BRAF	missense variant, non coding transcript variant, intron variant	Pathogenic	T, C, G
rs113488022	140,753,336	single nucleotide variant	BRAF	missense variant, non coding transcript variant, intron variant	Pathogenic	A, T, C, G
rs121913378	140,753,337	single nucleotide variant	BRAF	missense variant, non coding transcript variant, intron variant	Pathogenic	C, A, T
rs121913375	140,753,339	single nucleotide variant	BRAF	missense variant, non coding transcript variant, intron variant	Pathogenic	G, A, C
rs121913366	140,753,345	single nucleotide variant	BRAF	missense variant, non coding transcript variant, intron variant	Pathogenic	A, T, C
rs121913369	140,753,346	single nucleotide variant	BRAF	synonymous variant, missense variant, non coding transcript variant, intron variant	Pathogenic	G, A, C
rs397507483	140,753,348	single nucleotide variant	BRAF	non coding transcript variant, intron variant, missense variant	Pathogenic	C, A
rs121913361	140,753,349	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	C, A, G
rs121913341	140,753,350	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T, C
rs794729219	140,753,352	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, G
rs121913337	140,753,353	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T
rs121913338	140,753,354	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	T, A, C
rs397516896	140,753,355	single nucleotide variant	BRAF	intron variant, missense variant, non-coding transcript variant	Pathogenic	C, T
rs397516895	140,753,392	single nucleotide variant	BRAF	non coding transcript variant, missense variant, intron variant	Pathogenic	A, T
rs869025607	140,781,598 - 140,781,600	deletion	BRAF	in frame variant, non-coding transcript variant	Pathogenic	TGT, TGT
rs397516890	140,781,601 - 140,781,603	deletion	BRAF	non coding transcript variant, in frame variant	Pathogenic	TCC, TCC
rs113488022	140,753,336	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T, C, G
rs121913378	140,753,337	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	C, A, T
rs121913375	140,753,339	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	G, A, C
rs121913366	140,753,345	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T, C
rs121913369	140,753,346	single nucleotide variant	BRAF	synonymous variant, missense variant, non-coding transcript variant, intron variant	Pathogenic	G, A, C

Table 2 continued....

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rs397507483	140,753,348	single nucleotide variant	BRAF	non coding transcript variant, intron variant, missense variant	Pathogenic	C, A
rs121913361	140,753,349	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	C, A, G
rs121913341	140,753,350	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T, C
rs794729219	140,753,352	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, G
rs121913337	140,753,353	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	A, T
rs121913338	140,753,354	single nucleotide variant	BRAF	missense variant, non-coding transcript variant, intron variant	Pathogenic	T, A, C
rs397516896	140,753,355	single nucleotide variant	BRAF	intron variant, missense variant, non-coding transcript variant	Pathogenic	C, T

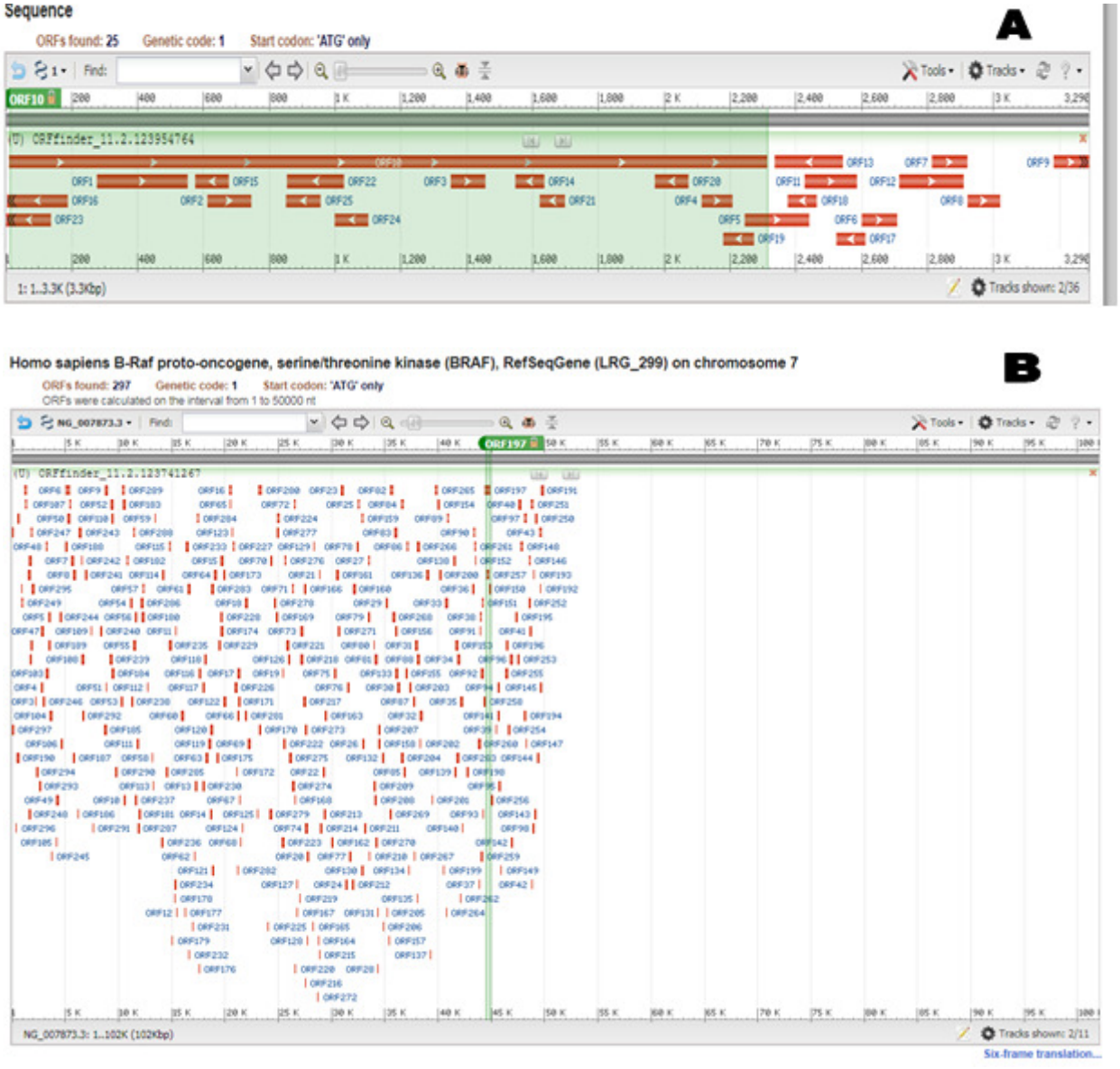

Fig. 2 : A detailed comparison of ORFs between normal BRAF gene (A) and mutated BRAF gene (B). The figure shows complete disruption of ORFs in mutated BRAF gene causing non-significant expression compared with the wild type of the gene.

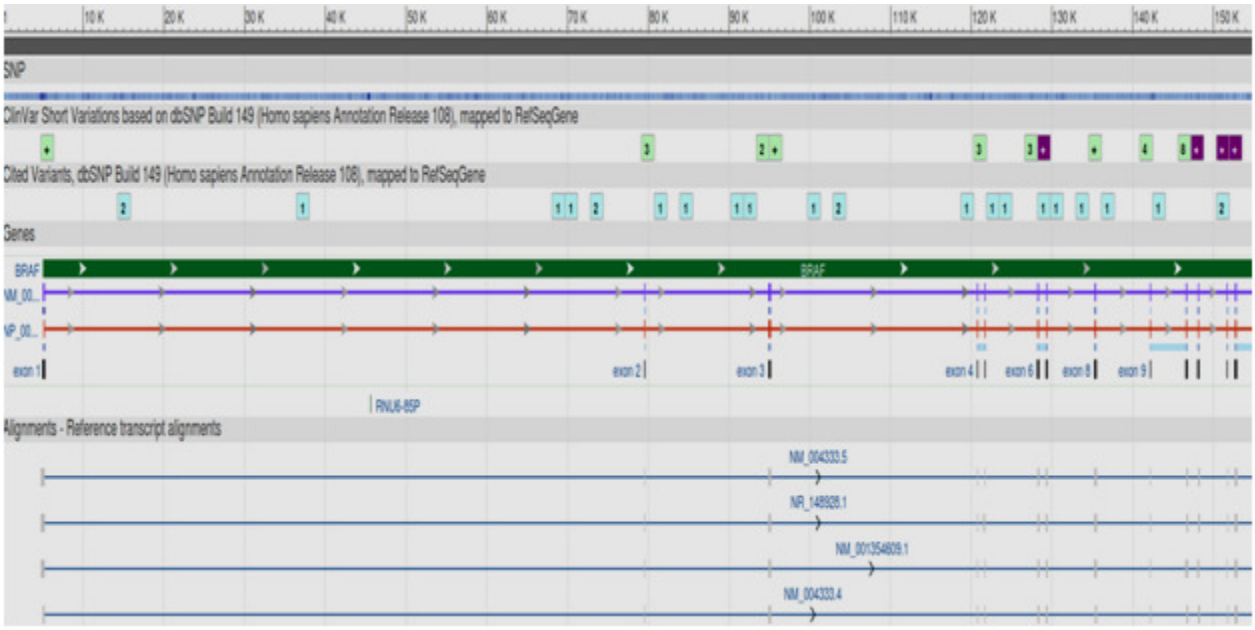

Fig. 3 : Distribution of mutations along BRAF gene. Locations identified with purple color reefer to lethal mutations, while locations identified with light blue color are likely benign mutations.

comes through 25 open reading frames (ORFs) that produce normal enzymes representing BRAF function. However, in this study, ORFs, were completely disrupted, and instead of normal 25 ORFs,we found the presence of 297 ORFs lack the normal function and expression of BRAF. Fig. 2-A shows normal ORFs of wild type BRAF, compared to the mutated type shown in Fig. 2-B.

Distribution of mutation on BRAF gene

This study meant to be thorough in identification of pathogenic mutation along BRAF gene. Analysis of data obtained showed that lethal mutations identified distributed on exons 7, 11, 12, 15, 17 and 18, while other exons may bear likely benign mutation on their nucleotide sequence. Fig. 3 shows distribution of lethal mutation on BRAF region.

DISCUSSION

BRAF geneencodes a serine/threonine-specific protein kinases (RAF, which signal downstream of RAS and activate the mitogen-activated protein kinase pathway) and has developed as a major oncogenic driver and a potential therapy target in a wide variety of solid braintumors and hematological malignancies, BRAF signing is critical for cell division, separation and differentiation and triggering BRAF mutations result in unrestrained growth and tumorigenesis, over 90% of activating BRAF mutations in cancer cells occur within the kinase domain at amino acid V600, most frequently resulting in V600E, which is an approved target for the inhibitors in the treatment of metastatic malignant melanoma (Trubini *et al*, 2018).

Genetic factors are essential for the disease in many samples of patients. The exons of BRAF and gene show mutations and some region of the gene shows a common mutation in some bases in some patient samples. The mutations detected in exons region of BRAF gene of brain tumor patients give evidence that these mutations play a part in this brain tumor.

In most type of Gangliogliomas, dysembryoblastic neuroepithelial tumors (DNT) and astrocytomas are due to BRAF V600E mutation,and mostly benign tumors that harbor BRAF V600E mutations in 20–60, 30 and 5% respectively of the examined cases (Fang *et al*, 2018).

Mutations in BRAF wereclinically relevantmutations with frequency 50% in epitheloid glioblastomas and 50–78% in pleomorphic xanthoastrocytomas (PXA) (including anaplastic variants) (Pakneshan *et al*, 2014).

Louis *et al* (2016) was analysed a single amino acid substitution at position 600, which detected in 14 of the 31-brain tumoras a result of a BRAF mutation in 3 gangliogliomas and screened a total of 31 tumors for activating mutations in exons 11 and 15.

Activating mutations in BRAF have been identified in approximately 66% of malignant gliomas, BRAF synthesis and constitutive beginning of BRAF in many brain tumor types like pilocytic astrocytoma's, as well as a restricted number of ûbrillary (grade II) astrocytoma's (Sievert *et al*, 2009).

According to statistic release by IraqiMinistry of Health in2015 the incidence of brain cancerin all patients within fourth grade is 1.584 case and (6.1%) from the

population while breast cancer in first grade, in the female the recorded cases 773 which represent (5.5%) and considered to be the secondtype of tumor after breast cancer. The Iraqi males' brain tumor ranked at the fifth position after colorectal cancer, leukemia, bladder cancer, and bronchuslung cancer with 769 cases that represent (6.9%) (Ministry of Health, 2018).

CONCLUSION

BRAF showed pathogenic mutation that severely affected its function directing cell division. These mutations affected the expression of enzymes involving cell cycle control and it was obvious through disrupted ORFs. Most of lethal mutations detected were at exon 15, whereas the other mutations were accompanied with likely benign allele mutationswhich involved in diminished BRAF function.

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