Archives of Physiology and Biochemistry
The Journal of Metabolic Diseases

ISSN: 1381-3455 (Print) 1744-4160 (Online) Journal homepage: https://www.tandfonline.com/loi/iarp20

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To cite this article: Amira M. Nageeb, Magdy M. Mohamed, Lobna R. Ezz El Arab, Mohamed K. Khalifa & Menha Swellam (2020): Next generation sequencing of *BRCA* genes in glioblastoma multiform Egyptian patients: a pilot study, Archives of Physiology and Biochemistry, DOI: 10.1080/13813455.2020.1729814

To link to this article: https://doi.org/10.1080/13813455.2020.1729814

Published online: 26 Feb 2020.

ARCHIVES OF PHYSIOLOGY AND BIOCHEMISTRY
https://doi.org/10.1080/13813455.2020.1729814

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ORIGINAL ARTICLE

Next generation sequencing of *BRCA* genes in glioblastoma multiform Egyptian patients: a pilot study

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ABSTRACT

Background: Germ line mutations of *BRCA1* and *BRCA2* were correlated with a variety of cancer Authors aimed to use next-generation sequencing (NGS) to detect *BRCA1* and *BRCA2* germ line mutations in glioblastoma multiform (GBM) Egyptian patients.

Materials and methods: Genomic DNA was extracted from six GBM cases, amplified using Ion AmpliSeq *BRCA1* and *BRCA2* panel. DNA libraries were pooled, barcoded and finally sequenced using Ion Torrent Personal Genome Machine sequencer.

Results: *BRCA1* the previously reported rs1799966, rs1799950, rs16941 were found in five cases and they are in a linkage disequilibrium forming two distinct haplotypes, which might support their role in cancer predisposition. Out of the 18 reported variants in *BRCA2*, three *denovo* mutations were detected which leads to frame shift.

Conclusion: Further studies on large number of GBM patients and control cases to determine *BRCA1* and *BRCA2* germline mutations and haplotypes; diagnostic and prognostic role are encouraged.

ARTICLE HISTORY
Received 20 December 2019
Revised 6 February 2020
Accepted 10 February 2020
Published online 25 February 2020

KEYWORDS
Glioblastoma multiform; *BRCA* genes; NGS; mutations; tumour suppressor; sequencing

Introduction

The most aggressive type of primary brain tumour is glioblastoma multiform (GBM; astrocytoma grade IV; Ivanka *et al*. 2014) which has been characterised with a high degree of tumour heterogeneity and easy invasion to other surrounding tissues (Denysenko *et al*. 2010). Current treatment strategies for GBM include radical surgical resection, standard radiation therapy, and/or chemotherapy using temozolomide (TMZ) which has been able to improve survival period for GBM patients with median survival from 14 to 16 months (Kast *et al*. 2013) but still the degree is unsatisfactory as only few survive more than 5 years (Stupp *et al*. 2009).

Breast cancer type 1 and 2 susceptibility (*BRCA1* and *BRCA2*) genes are essential molecules responsible for homologous recombination – depended double-strand break repairing pathway – as they participate in cellular resistance to alkylating agents (Kondo *et al*. 2010, Quiros *et al*. 2011, Short *et al*. 2011, Zhang *et al*. 2012). It has been reported that mutations in *BRCA1* and/or *BRCA2* genes resulted in unfavourable survival (Lee *et al*. 2014). Thus introducing polymerase inhibitor agents as poly(ADP-ribose) polymerase-1 (PRAPi) targeting mutations may lead to failure of DNA damage repair leading to apoptosis in *BRCA1* and *BRCA2* defective cells. To accomplish this assignment, identification of all mutations in these genes should be tested. Using conventional genetic testing methods is a Labour intensive and non-specific in some cases, in addition by considering the gene size and the mutation variants of *BRCA1* (1706 mutations among 5592 bp gene size) and of *BRCA2* (1446 mutations among 10,257 bp gene size). Their high-allelic heterogeneity and the deficiency of mutation hotspots revealed the challenging in genetic testing for the two genes.

Introducing high-throughput and cost-effective screening technologies as next-generation sequencing (NGS) that have been emerged as a prevailing device for the detection of causative mutations and novel disease-related genes defects and are speedily affecting genetic diagnostics providing fast, inexpensive, and detailed genetic information. In the current study, authors aimed to carry out preliminary study using a semiconductor bench top NGS platform (Personal Genome Machine [PGM], Life Technologies, Eugene, OR, USA) with commercially available ready-to-use IonAmpliseq*BRCA1* and *BRCA2* panel (Life Technologies) for detection of to detect *BRCA1* and *BRCA2*germline mutations in Blood samples from GBM Egyptian patients to identify if there are a predisposition germline mutations risk relate to GBM.

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Materials and methods

Sample collection and GBM criteria

In this prospective study, six adult patients newly diagnosed with GBM cases were enrolled and their diagnosis was based on magnetic resonance imaging (MRI) and pathological analysis, their performance were $\leq$2 according to Ester Clinical Oncology Group, none of the GBM patients had other brain tumours or other malignancies. The validation criteria for selected GBM patients were those who have not received any treatments (adjuvants therapy including radio- or chemotherapy) those who were available for follow-up and post-operational survival time was more than 1 month. Blood samples were collected after patients signed their informed consent according to the appropriate protocols approved by Medical Ethical Committee from National Research Centre 17111) in accordance with the Helsinki Doctrine.

Treatment strategy

Regarding the six primary GBM adult cases reported in the current study, MRI was applied first for all patients for diagnosis. GBM patients were treated with surgical resection or near total resection were ($n = 4$) while the remaining went for excision biopsy ($n = 2$). Afterward, all were treated with standard therapy protocol which involved radiotherapy (total dose of 60 Gy, given in 30 fractions over 6 weeks) with concomitant TMZ chemotherapy (100 mg/day for 45 days), followed by six cycles of TMZ treatment at a dose of 150 mg/sq m body surface area for day 1–5. These patients were followed up at regular intervals and evaluated clinically and radiologically by MRI.

DNA extraction

Extraction of DNA was carried out from EDTA blood samples following the manufacturer's instructions using QIAamp DNA mini blood kit (Cat No # 51104, Qiagen, Hilden, Germany) based on spin column for DNA extraction method. The purity and the concentration of the extracted DNA were detected before further investigation using nano-drop spectrophotometer (Quawell, Q-500, Scribner, NE, USA) then stored at $-80\,^{\circ}$C till further assessments.

Library preparation and purification

The target regions for the investigated genes BRCA1 and BRCA2 were amplified using Ion AmpliSeq *BRCA1* and *BRCA2* Panel (Life Technologies) which consists of three primer pools (167 amplicons) covering the entire coding region with 10–20 bp of intronic flanking sequences around the coding sequence for both genes. Amplification process was carried out in 20 µl scale using Ion AmpliSeq Library kit (Cat No# 4480441 Life Technologies). Reaction was carried out using 4 µl of 5× Ion AmpliSeq HiFi master mix, 10 µl of 2× Ion AmpliSeq primer pool (each of the three primers in a separate well for each sample ID 1–6), 10 ng of genomic DNA/reaction (2 µl of 5 ng/µl from the sample stock), and finally 4 µl of nuclease-free water. The amplification reaction was performed using the following thermal conditions: 99 °C for 2 min, for one cycle for activating the enzyme followed by (99 °C for 2 s, and 60 °C for 4 min.) for 16 cycles using thermal cycler (SureCycler 8800, Agilent, Santa Clara, CA, USA). After amplification, the primers were digested using FuPa Reagent and thermal conditions: 50 °C for 10 min, 55 °C for 10 min, and 60 °C for 10 min, then ligated with both adaptors (Cat No# A29751 Life Technologies) and barcodes with Ion Xpress Barcode Adaptors 1–16 kit (Cat No# 4471250 Life Technologies). Then, amplification was carried out using thermal conditions: 22 °C for 30 min, 68 °C for 5 min, and 72 °C for 5 min, as mentioned in Ion AmpliSeq Library kit user guide. Purification of prepared libraries was done and quantified using Ion Library TaqMan Quantitation kit (Cat No# 4468802 Life Technologies) by quantitative PCR using real-time PCR system (Max3005P QPCR system, Stratagene, Agilent Biotechnology, USA). The QPCR was carried out according to the following thermal conditions: 50 °C for 2 min, 95 °C for 2 min, and 40 cycles as 95 °C for 15 s, 60 °C for 1 min. The amplified libraries were further preceded to template preparation.

Template preparation

Purified and quantified libraries were pooled on a molar equivalent rations to yield at least 500× depth of coverage for each sample. The pooled libraries were clonally amplified using Ion PGM Hi-Q view OT2 kit (Cat No# A29900 Life Technologies) on the Ion OneTouch 2 instrument (Life Technologies) according to manufacturer instructions. Then, template ion sphere particles (ISP) were enriched using Ion PGM enrichment beads (Cat No# 4478525 Life Technologies) using Ion OneTouch ES system (Life Technologies) according to the manufacturer's instructions, the positive ISP Quality was assayed on Qubit 2.0 Fluorometer (Life Technologies) then proceeded for performing the sequencing process.

Sequencing using ion torrent PGM platform

After calibrations and adjustments of the pH according to manufacturer instructions using Ion PGM Hi-Q View Sequencing kit (Cat No# A30044 Life Technologies), all barcoded enriched samples were sequenced on Ion Torrent PGM Platform (Ion Torrent PGM, Life Technologies) using Ion 318 Chip Kit V2 BC (Cat No# 4488150 Life Technologies) the depth of coverage was calculated to be at least 500×.

Data analysis

Alterations of *BRCA1*and *BRCA2* genes were annotated using cloud-based Ion Reporter Suit software (version 5.4.0 Life Technologies) after aligned with hg19 reference human genome (Genome reference Consortium GRCh37) using Ion Torrent Server and variant caller plugin, using manufacture recommended parameters for *BRCA1* and *BRCA2* panels. Analysis of variants was applied without bias and the

Table 1. Demographic and clinical criteria for GBM (grade IV) patients.

Pat. ID	Gender	Age	Mass, size	ECGO	Type of surgery	Cyc. CT	Response	OS/mon.
1	Female	59	Rt. Frontal mass	1	Total excision	6	PR	16
2	Female	65	Rt. Tempro-partial	2	Biopsy	2	PD	6
3	Female	33	Lt. Frontal mass.	1	Total excision	6	CR	20
4	Male	39	Rt. Frontal mass temporal	1	Sub-total excision	None	SD	12
5	Male	42	Rt. Partial mass occipital	1	Sub-total excision	6	PR	14
6	Female	78	Lt. Front temporal mas	2	Biopsy	None	SD	7

Cyc.: cycles; CT: chemotherapy; CR: complete response; PR: partial response; PD: progressive disease; OS: overall survival; SD: Stable disease.

reference sequences for *BRCA1* and *BRCA2* were NM_007300.3 and NM_000059.3, respectively.

For linkage disequilibrium (LD) and haplotype analysis all the reported known variants in both *BRCA1* and BRCA2 were submitted to the LDlink tool against all population databases because there is no specific Egyptian population database available till now (Machiela and Chanock 2015).

Results

Clinical data of patients

Among the primary GBM enrolled six cases, four cases were females (66.7%) versus two males (33.3%) and their ages were categorised as $\leq$ 60 years (66.7%) versus >60 (33.3%). Performance status using ECGO system reported four cases (66.7%) were <2, while the remaining (33.3%) were equal 2. Two patients reported as frontal mass while three patients were diagnosed with temporal or partial mass and one with occipital mass. Regarding surgery treatment, four GBM patients underwent for excision either total or subtotal, while the remaining two patients received excision biopsy. Then, five patients received radiotherapy and chemotherapy for six cycles while only one patient (patient ID 6) radiotherapy only. Their responses were varied as three showed responses to treatment (2 GBM patients with partial response [PR], one patient with complete response [CR]) while the other three patients; two reported stable disease [SD] and one showed progressive disease [PD], their clinical features were summarised in Table 1.

Genetic variations among BRCA1 and BRCA2 genes

Thirty-one genetic variations were reported among the two genes as summarised in Table 2. For *BRCA1* (NM_007300.3, NG_005905.2); 13 single nucleotide variations (SNVs) were reported (NM_007300.3), 3 (23%) were intronic and 10 (77%) were exonic as shown in Table 2. All *BRCA1* detected SNV were previously reported in the dbSNP database. Among the 13 detected SNV, there were 8 SNP segregate in haplotype like patterns so LDmatrix of the LDlink tool was used to determine if there are any LD between those SNPs using all population data. A strong LD was found between 7 out of 13 *BRCA1* SNPs with both r^2 and D' greater than 0.96 when using all population database Figure 1 showed the results for the 13 SNPs and Figure 2 for the 8 SNPs only, the rs799917 reached D' value of 0.97 with all the other 7 SNPs but with reduced r^2 around 0.42. The r^2 and D' matrixes for the 13 *BRCA1* SNPs presented in Tables 3 and 4 respectively, and for the strongly linked 8 SNPs the r^2 and D' matrixes are showed in Tables 5 and 6, respectively.

The LDhap tool was used to determine the haplotypes and their frequencies for those 8 SNPs, out of 17 haplotypes previously reported for those SNPs across all population Table 7, there were five common haplotypes Figure 3 among them two haplotypes where presents in the studied patients. The C-G-C-C-A-G-A-G haplotype which were present in three out of six (50%) as a homozygote, and as a heterozygote haplotype in another two patients (33.3%). The only patient who was expressing the wild type T-A-T-T-G-A-G-A haplotype as a homozygote for *BRCA1* where found to carry a *denovo* frameshift deletion in *BRCA2*.

Regarding *BRCA2* gene (NM_000059.3, NG_012772.3), a total 18 genetic variations were reported Table 2; they were 16 SNVs (7 [43.75%] intronic and 9 exonic [56.24%]) 15 SNVs out of them were previously reported in dpSNP except for the c(0).7069-29A > T which was *denovo*. For the Indels two *denovo* insertion/deletion (indels) were detected, one intronic (g.22999del ATATCT) while the other was exonic (g.22709delT) which leads to frameshift converting serine 570 to leucine and premature stop codon at 571.

For *BRCA 2*, the LD analysis for the detected known variations showed that there is no LD between those variations using all population database Figure 4. The output matrixes for r^2 and D' showed in Tables 8 and 9, respectively.

Discussion

BRCA1 and *BRCA2*are tumour suppressor genes found to be involved in different cancer types more over those two genes become promising targets for anticancer drugs. So the germ line genetic signature of BRCA1&2 can carry answers for cancer predisposition risk and the drug strategy choice. NGS technologies that scalable with higher throughput capable carryout genomic profiling for all types of variants in a single run (Hamblin *et al*. 2017). Thus, the current study aimed to preliminary establish the sequencing ofgermline *BRCA1&2* genes using Ion AmpliSeq *BRCA1* and *BRCA2* Panel from DNA extracted from blood samples of GBM patients using Ion PGM sequencer (Life Technologies).

Previously it has been reported that DNA repair pathway is promising pathway for GBM susceptibility as there were several SNPs were associated with GBM risk (Chang *et al*. 2008). In an agreement, Zhu *et al*. reported the presence of certain BRCA1 rs799917 SNP is significance with the GBM risk. And he also concluded that there is a LD between

Table 2. Variations detected in *BRCA1* & *BRCA2* genes.

Gene	Position	Consequence	Variation type	cDNA	LRG	AA change	Frequency	dbSNP 144	Homozygotes	Heterozygots	Total cases	Clinivar		BRCA exchange
												Significance	Disease names	
BRCA1	Intron	–	SNV	c.5075-53C > T	LRG_292:g.153980C > T		0.17	rs8176258	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon	Nonsynonymous	SNV	c.4598G > T	LRG_292:g.143513G > T	p.Ser1533Ile	0.17	rs1800744	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
		Nonsynonymous	SNV	c.1067A > G	LRG_292:g.123520A > G	p.Gln356Arg	0.17	rs1799950	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intron 8	–	SNV	c.442-34C > T	LRG_292:g.118070C > T		0.5	rs799923	0/6	3/6	3/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon 11B	Nonsynonymous	SNV	c.2077G > A	LRG_292:g.124530G > A	p.Asp693Asn	0.67	rs4986850	1/6	3/6	4/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon 16	Nonsynonymous	SNV	c.4900A > G	LRG_292:g.146907A > G	p.Ser1634Gly	0.83	rs1799966	3/6	2/6	5/6	Likely-benign	Hereditary cancer-predisposing syndrome	Not yet Reviewed
	Exon 13	Synonymous	SNV	c.4308T > C	LRG_292:g.135531T > C	p.Ser1436=	0.83	rs1060915	3/6	2/6	5/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon 11C	Nonsynonymous	SNV	c.3113A > G	LRG_292:g.125566A > G	p.Glu1038Gly	0.83	rs16941	3/6	2/6	5/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon 11B	Synonymous	SNV	c.2311T > C	LRG_292:g.124764T > C	p.Leu771=	0.83	rs16940	3/6	2/6	5/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intron	–	SNV	c.-19-115T > C	LRG_292:g.93754T > C		0.83	rs3765640	3/6	2/6	5/6	Benign		Benign/Little Clinical Significance
	Exon 11D	Nonsynonymous	SNV	c.3548A > G	LRG_292:g.126001A > G	p.Lys1183Arg	1	rs16942	3/6	2/6	5/6	Uncertain-Significance	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon11c	Nonsynonymous	SNV	c.2612C > T	LRG_292:g.125065C > T	p.Pro871Leu	1	rs799917	3/6	2/6	5/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exon 11B	Synonymous	SNV	c.2082C > T	LRG_292:g.124535C > T	p.Ser694=	1	rs1799949	3/6	2/6	5/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
BRCA2	Intronic	–	SNV	c.-26G > A	LRG_293:g.5956G > A		0.17	rs1799943	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Synonymous	SNV	c.231T > G	LRG_293:g.8761T > G	p.Thr77=	0.17	rs114446594	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intronic	–	SNV	c.681 + 56C > T	LRG_293:g.19069C > T		0.17	rs2126042	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intronic	–	INDEL	c.2136 + 91-96 delATATCT	LRG_293:g.22999del ATATCT		0.17	Denovo	0/6	1/6	1/6			
	Intronic	–	SNV	c.7069-29A > T	LRG_293:g.34050A > T		0.17	Denovo	0/6	1/6	1/6			
	Exonic	Synonymous	SNV	c.7242A > G	LRG_293:g.44616A > G	Ser2414=	0.17	rs1799955	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intronic	–	SNV	c.8487 + 47C > T	LRG_293:g.60125C > T		0.17	rs11571744	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Nonsynonymous	SNV	c.9875C > T	LRG_293:g.87909C > T	p.Pro3292Leu	0.17	rs56121817	0/6	1/6	1/6	Conflicting-Interpretations-Of-Pathogenicity	Hereditary cancer-predisposing syndrome	Not yet Reviewed
	Intronic	–	SNV	c.1910-74T > C	LRG_293:g.25712T > C		0.33	rs2320236	0/6	2/6	2/6	Benign		Benign/Little Clinical Significance
	Exonic	Synonymous	SNV	c.3396A > G	LRG_293:g.27272A > G	p.Lys1132=	0.33	rs1801406	0/6	2/6	2/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intronic	–	SNV	c.7806-14T > C	LRG_293:g.52030T > C		0.33	rs9534262	0/6	2/6	2/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Intronic	–	SNV	c.8755-66T > C	LRG_293:g.68772T > C		0.33	rs4942486	0/6	2/6	2/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Nonsynonymous	INDEL	c.1711delT	LRG_293:g.22709delT	p.Ser570fsX1	0.5	Denovo	2/6	0/6	2/6			
	Exonic	Nonsynonymous	SNV	c.1114A > C	LRG_293:g.22113A > C	p.Asn372His	1	rs144848	0/6	1/6	1/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Synonymous	SNV	c.3807T > C	LRG_293:g.27683T > C	p.Val1269=	1	rs543304	1/6	5/6	6/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Synonymous	SNV	c.4563A > G	LRG_293:g.28439A > G	p.Leu1521=	1	rs206075	5/6	1/6	6/6	Likely-benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic	Synonymous	SNV	c.6513G > C	LRG_293:g.30389G > C	p.Val2171=	1	rs206076	5/6	1/6	6/6	Benign	Hereditary cancer-predisposing syndrome	Benign/Little Clinical Significance
	Exonic		SNV	c.7397T > C	LRG_293:g.44771C > T	p.Val2466Ala	1	rs169547	5/6	1/6	6/6	Benign	Hereditary cancer-predisposing syndrome	Not YET Reviewed

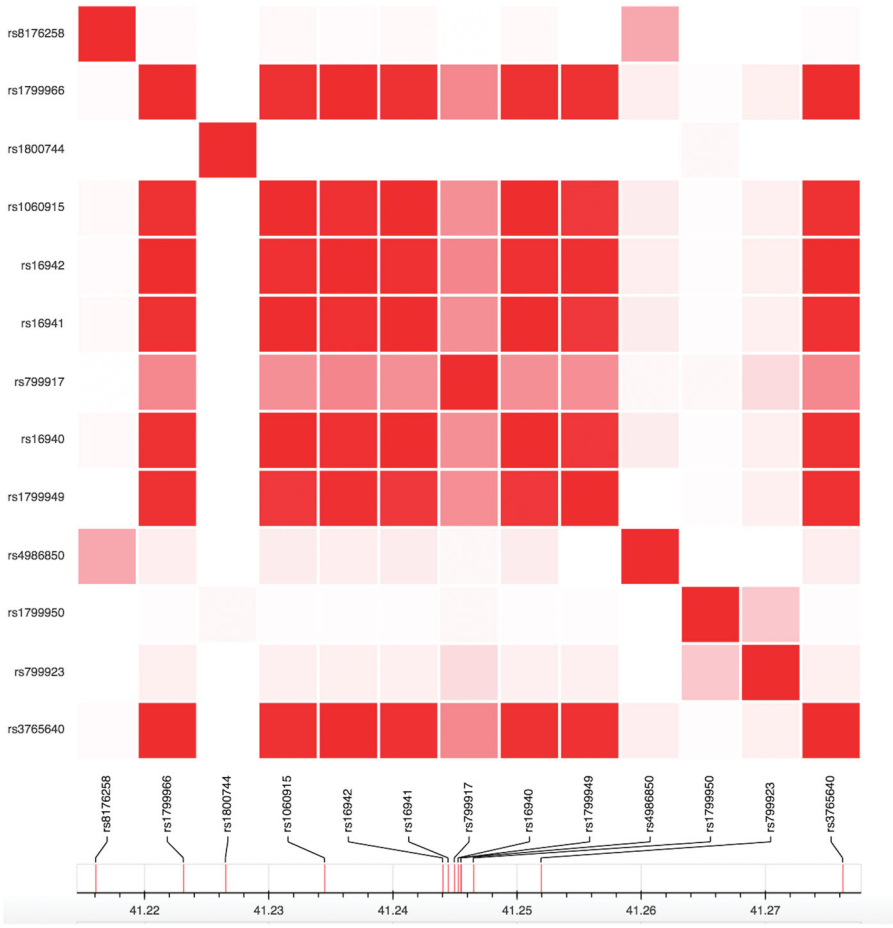

Figure 1. LD plot showing the LD between the 13 detected variants in *BRCA1*.

rs799917 and the rs1799966, the same thing which was reported in the current study (Chang *et al*. 2008, Zhu *et al*. 2017). The current study reported that the rs799917 and the rs1799966 are located on the same LD block and they are along with the other six SNPs are representing a distinct haplotype, and this haplotype was detected among five GBM studied patients (5/6: 83%) in this study which may indicate their association GBM risk.

These findings point out the association of the presence of these haplotypes and GBM predisposition. Future studies are in progress for reporting the presence of these SNPs among Egyptian populations.

Previously, it was reported in genome-wide associations reports that seven genes can proliferation glioma risk (*TERT*, *CCDC26*, *EGFR*, *CDKN2A*, *CDKN2B*, *RTEL1*, and *PHLDB1*), but *BRCA* genes were not among them (Shete *et al*. 2009, Sanson *et al*. 2011, Boukerroucha *et al*. 2015) While in another studies a link has been reported between mutations of familial *BRCA1* and progressed multicentric GBM (Elmariah Sarina *et al*. 2006). Moreover, in a previous study carried out by Radiation Therapy Oncology Group (Brown *et al*. 2016). a link has been reported between low expression levels of *BRCA1*proteinand prolonged survival of GBM patients as this was referred to the concept that low DNA repair triggers cancer cells to be more vulnerable to DNA-damaging cancer treatment. Also, Shoua *et al*. (2018) reported the presence of *BRCA* mutation in patients with GBM this association directs the addition of targeted therapies as PRAPi to patients resistant to TMZ (Koji *et al*. 2012, Lee *et al*. 2014). These findings have strengthened the detection for the link between *BRCA* mutations and GBM.

In the current pilot study, 31 mutations were reported in BRCA1&2 among GBM patients; 13 for *BRCA1* and 18 for *BRCA2*(3 were *denovo*).

Regarding *BRCA1* 13 SNVs, eight SNPs where found to be in LD which might reflect the light on a haplotype risk considering that five out of the six patients where carrying either homozygote mutant haplotype or heterozygote haplotype and the only patient who was caring the homozygotes wild type haplotype was found to carry a deleterious frameshift mutation in BRCA2 gene. One of the major SNPs in this haplotype block is the rs1799966 which was previously reported to carry a risk of colorectal cancer and associated also with poor cancer prognosis (Zhu *et al*. 2017).

For *BRCA2* gene, all detected genetic variations in this gene were ranging from benign to likely benign except for rs56121817 it was Conflicting-Interpretations-Of-Pathogenicity but it did not show in any of the six patients except for one patient.

LD and haplotype analysis for the detected SNPs in the *BRCA2* resulted in no significance between the SNPs and/or a specific haplotype.

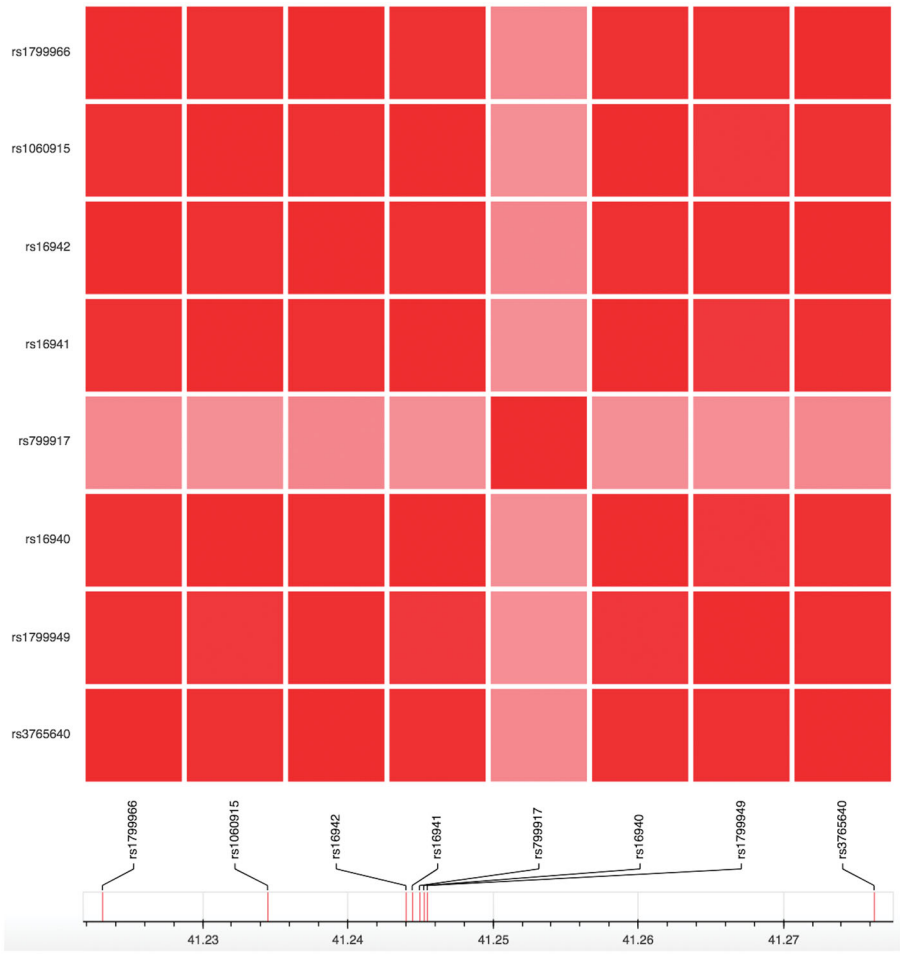

Figure 2. LD plot showing the LD between the eight detected variants in *BRCA1*.

Table 3. The calculated r^2 matrix for the 13 detected variants in *BRCA1*.

RS-number	rs8176258	rs1799966	rs1800744	rs1060915	rs16942	rs16941	rs799917	rs16940	rs1799949	rs4986850	rs1799950	rs799923	rs3765640
rs8176258	1	0.02	0	0.022	0.02	0.022	0.009	0.022	0.001	0.32	0	0.001	0.02
rs1799966	0.02	1	0	0.912	0.984	0.913	0.451	0.908	0.913	0.063	0.012	0.06	0.982
rs1800744	0	0	1	0	0	0	0.001	0	0	0	0.027	0.005	0
rs1060915	0.022	0.912	0	1	0.916	0.996	0.419	0.99	0.843	0.069	0.011	0.055	0.914
rs16942	0.02	0.984	0	0.916	1	0.921	0.457	0.922	0.927	0.064	0.012	0.06	0.99
rs16941	0.022	0.913	0	0.996	0.921	1	0.419	0.995	0.847	0.069	0.011	0.055	0.918
rs799917	0.009	0.451	0.001	0.419	0.457	0.419	1	0.421	0.423	0.029	0.027	0.131	0.45
rs16940	0.022	0.908	0	0.99	0.922	0.995	0.421	1	0.85	0.069	0.011	0.055	0.913
rs1799949	0.001	0.913	0	0.843	0.927	0.847	0.423	0.85	1	0.004	0.011	0.055	0.92
rs4986850	0.32	0.063	0	0.069	0.064	0.069	0.029	0.069	0.004	1	0.001	0.004	0.062
rs1799950	0	0.012	0.027	0.011	0.012	0.011	0.027	0.011	0.011	0.001	1	0.203	0.012
rs799923	0.001	0.06	0.005	0.055	0.06	0.055	0.131	0.055	0.055	0.004	0.203	1	0.06
rs3765640	0.02	0.982	0	0.914	0.99	0.918	0.45	0.913	0.92	0.062	0.012	0.06	1

Table 4. The calculated D' matrix for the 13 detected variants in *BRCA1*.

RS-number	rs8176258	rs1799966	rs1800744	rs1060915	rs16942	rs16941	rs799917	rs16940	rs1799949	rs4986850	rs1799950	rs799923	rs3765640
rs8176258	1	1	1	1	1	1	1	1	0.205	1	1	1	1
rs1799966	1	1	1	0.997	0.999	0.999	0.986	0.997	0.997	1	1	1	0.996
rs1800744	1	1	1	1	1	1	1	1	1	1	1	1	1
rs1060915	1	0.997	1	1	0.993	0.999	0.993	0.997	0.919	1	1	1	0.994
rs16942	1	0.999	1	0.993	1	0.996	1	0.998	0.998	1	1	1	0.997
rs16941	1	0.999	1	0.999	0.996	1	0.995	0.998	0.922	1	1	1	0.997
rs799917	1	0.986	1	0.993	1	0.995	1	0.997	0.997	1	1	1	0.99
rs16940	1	0.997	1	0.997	0.998	0.998	0.997	1	0.925	1	1	1	0.995
rs1799949	0.205	0.997	1	0.919	0.998	0.922	0.997	0.925	1	0.255	1	0.994	0.996
rs4986850	1	1	1	1	1	1	1	1	0.255	1	1	1	0.991
rs1799950	1	1	1	1	1	1	1	1	1	1	1	1	1
rs799923	1	1	1	1	1	1	1	1	0.994	1	1	1	1
rs3765640	1	0.996	1	0.994	0.997	0.997	0.99	0.995	0.996	0.991	1	1	1

Table 5. The calculated r^2 matrix for the eight variants representing the discussed haplotype in *BRCA1*.

RS-number	rs1799966	rs1060915	rs16942	rs16941	rs799917	rs16940	rs1799949	rs3765640
rs1799966	1	0.912	0.984	0.913	0.451	0.908	0.913	0.982
rs1060915	0.912	1	0.916	0.996	0.419	0.99	0.843	0.914
rs16942	0.984	0.916	1	0.921	0.457	0.922	0.927	0.99
rs16941	0.913	0.996	0.921	1	0.419	0.995	0.847	0.918
rs799917	0.451	0.419	0.457	0.419	1	0.421	0.423	0.45
rs16940	0.908	0.99	0.922	0.995	0.421	1	0.85	0.913
rs1799949	0.913	0.843	0.927	0.847	0.423	0.85	1	0.92
rs3765640	0.982	0.914	0.99	0.918	0.45	0.913	0.92	1

Table 6. The calculated D' matrix for the eight variants representing the discussed haplotype in *BRCA1*.

RS-number	rs1799966	rs1060915	rs16942	rs16941	rs799917	rs16940	rs1799949	rs3765640
rs1799966	1	0.997	0.999	0.999	0.986	0.997	0.997	0.996
rs1060915	0.997	1	0.993	0.999	0.993	0.997	0.919	0.994
rs16942	0.999	0.993	1	0.996	1	0.998	0.998	0.997
rs16941	0.999	0.999	0.996	1	0.995	0.998	0.922	0.997
rs799917	0.986	0.993	1	0.995	1	0.997	0.997	0.99
rs16940	0.997	0.997	0.998	0.998	0.997	1	0.925	0.995
rs1799949	0.997	0.919	0.998	0.922	0.997	0.925	1	0.996
rs3765640	0.996	0.994	0.997	0.997	0.99	0.995	0.996	1

Table 7. The reported 17 haplotypes for the detected *BRCA1* 8 variants.

Haplotype	Count	Frequency
T_A_T_T_G_A_G_A	2266	0.4525
C_G_C_C_A_G_A_G	1591	0.3177
T_A_T_T_A_A_G_A	949	0.1895
C_A_C_T_A_A_A_G	89	0.0178
C_G_C_C_A_G_G_G	82	0.0164
C_A_T_T_A_A_G_A	6	0.0012
C_A_T_T_G_A_G_A	6	0.0012
C_G_T_C_G_A_G_G	4	0.0008
T_A_T_T_G_A_G_G	4	0.0008
T_G_T_T_A_A_G_A	3	0.0006
C_G_C_C_A_G_A_A	2	0.0004
C_G_C_C_A_G_G_A	1	0.0002
C_G_T_T_G_A_G_A	1	0.0002
T_A_C_C_A_G_A_G	1	0.0002
T_A_T_T_G_A_A_A	1	0.0002
T_A_T_T_G_G_A_A	1	0.0002
T_A_T_T_G_G_G_A	1	0.0002

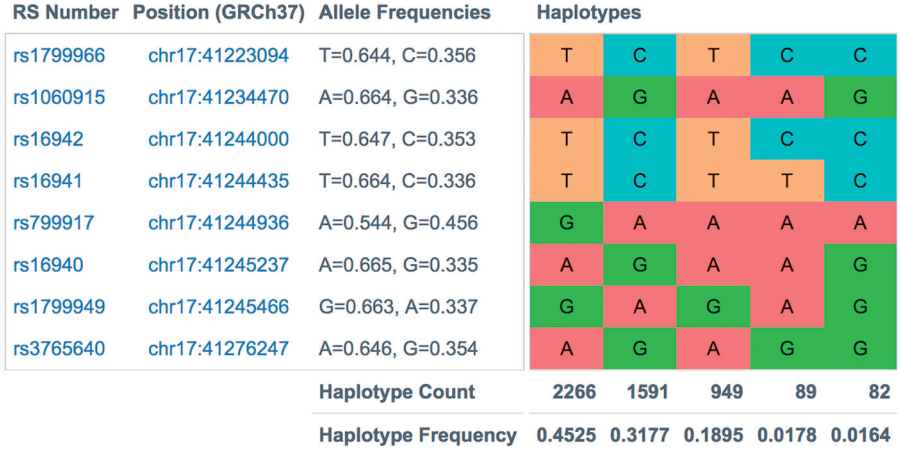

Figure 3. LD plot showing the LD between the 15 detected variants in *BRCA2*.

Among the reported *denovo* mutations in *BRCA2*, one was indel mutation and resulted in frameshift converting serine 570 to leucine and premature stop codon at 571, which may account for the patient did not respond to TZM or for his late stage.

In conclusion, to our knowledge, it is the first study on *BRCA* genes using genomic DNA extract of GBM Egyptian patients by NGS as high throughput technology. Although the small sample size can be considered as a limitation of this study but so far authors have there are some limitation

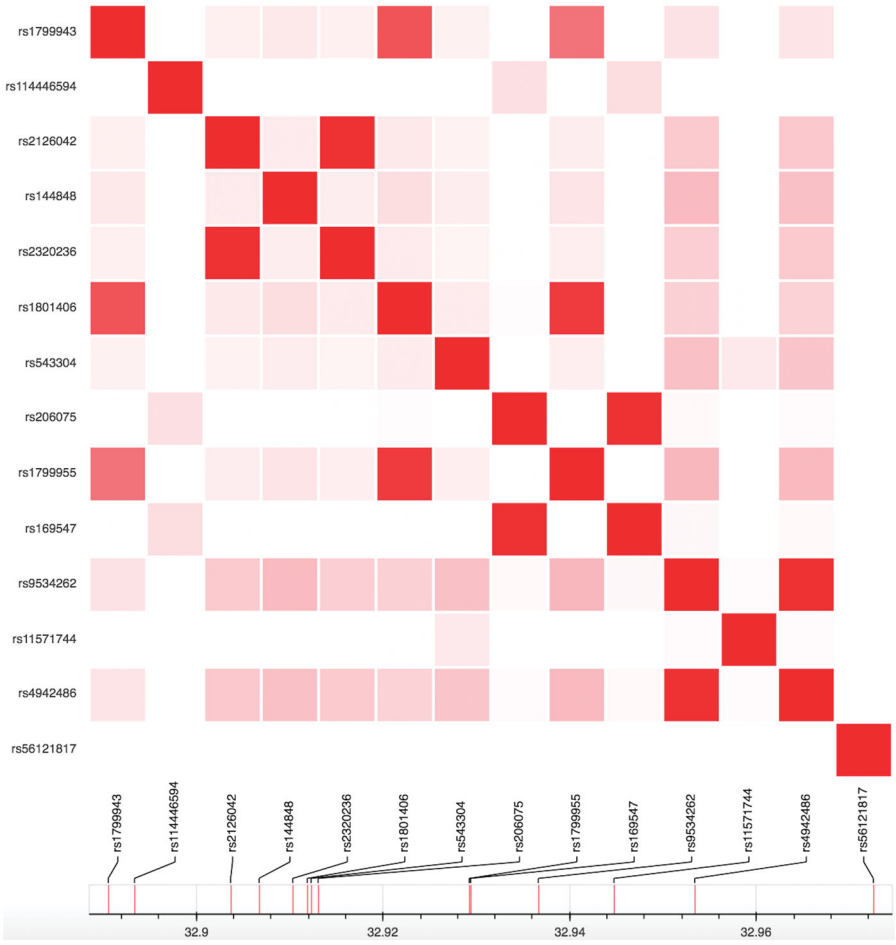

Figure 4. The frequent five reported *BRCA1* haplotypes represented by the eight variants.

Table 8. The calculated r^2 matrix for the 14 detected known variants in *BRCA2*.

RS-number	rs1799943	rs114446594	rs2126042	rs144848	rs2320236	rs1801406	rs543304	rs206075	rs1799955	rs169547	rs9534262	rs11571744	rs4942486	rs56121817
rs1799943	1	0.001	0.059	0.081	0.054	0.681	0.052	0.007	0.538	0.007	0.106	0.004	0.099	0
rs114446594	0.001	1	0.001	0.001	0.001	0.001	0.001	0.113	0.001	0.121	0.003	0	0.003	0
rs2126042	0.059	0.001	1	0.074	0.913	0.082	0.046	0.006	0.067	0.006	0.192	0.004	0.2	0
rs144848	0.081	0.001	0.074	1	0.068	0.12	0.066	0.009	0.099	0.008	0.25	0.005	0.226	0
rs2320236	0.054	0.001	0.913	0.068	1	0.074	0.043	0.006	0.062	0.005	0.177	0.003	0.191	0
rs1801406	0.681	0.001	0.082	0.12	0.074	1	0.074	0.01	0.825	0.008	0.169	0.006	0.164	0.001
rs543304	0.052	0.001	0.046	0.066	0.043	0.074	1	0.005	0.061	0.005	0.228	0.079	0.211	0
rs206075	0.007	0.113	0.006	0.009	0.006	0.01	0.005	1	0.008	0.913	0.022	0	0.019	0
rs1799955	0.538	0.001	0.067	0.099	0.062	0.825	0.061	0.008	1	0.008	0.266	0.005	0.256	0.001
rs169547	0.007	0.121	0.006	0.008	0.005	0.008	0.005	0.913	0.008	1	0.027	0	0.024	0
rs9534262	0.106	0.003	0.192	0.25	0.177	0.169	0.228	0.022	0.266	0.027	1	0.019	0.912	0
rs11571744	0.004	0	0.004	0.005	0.003	0.006	0.079	0	0.005	0	0.019	1	0.017	0
rs4942486	0.099	0.003	0.2	0.226	0.191	0.164	0.211	0.019	0.256	0.024	0.912	0.017	1	0
rs56121817	0	0	0	0	0	0.001	0	0	0.001	0	0	0	0	1

in the current study as to report current study reported 31 mutations among *BRCA* genes; three mutations were similar to the reference HGMD and three were *denovo* mutations one was related to the severity of the disease and hence may affect their future treatment and prognosis. Also, it is a breakthrough to report the first haplotyping report of *BRCA1* genes using genomic DNA samples from Egyptian GBM patients to realise variant mutations.

Further studies on a large number of patients and control are needed to investigate the role of different *BRCA1* haplotypes in the predisposition risk for GBM especially and other cancer types generally.

Patent

Using NGS as technology for identification of *BRCA* genes mutations among GBM patients through blood samples has been submitted for patent ID: 410/2019, no. 765806, date 13.3.2019. Academy of Scientific Research & Technology, Ministry of Scientific Research, Arab Republic of Egypt.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Table 9. The calculated *D'* matrix for the 14 detected known variants in *BRCA2*.

RS-number	rs1799943	rs114446594	rs2126042	rs144848	rs2320236	rs1801406	rs543304	rs206075	rs1799955	rs169547	rs9534262	rs11571744	rs4942486	rs56121817
rs1799943	1	1	0.985	0.962	0.978	0.967	0.989	1	0.785	1	0.674	1	0.625	1
rs114446594	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rs2126042	0.985	1	1	0.987	0.993	0.992	1	1	0.986	1	0.977	1	0.958	1
rs144848	0.962	1	0.987	1	0.986	0.997	0.99	1	0.993	1	0.815	1	0.806	1
rs2320236	0.978	1	0.993	0.986	1	0.979	1	1	0.985	1	0.976	1	0.972	1
rs1801406	0.967	1	0.992	0.997	0.979	1		1	0.995	0.969	0.725	1	0.687	1
rs543304	0.989	1	1	0.99	1	1	1	0.954	1	0.951	0.998	0.985	0.998	1
rs206075	1	1	1	1	1	1	0.954	1	1	0.992	0.855	1	0.835	1
rs1799955	0.785	1	0.986	0.993	0.985	0.995	1	1	1	1	0.998	1	0.94	1
rs169547	1	1	1	1	1	0.969	0.951	0.992	1	1	0.984	1	0.968	1
rs9534262	0.674	1	0.977	0.815	0.976	0.725	0.998	0.855	0.998	0.984	1	1	0.994	1
rs11571744	1	1	1	1	1	1	0.985	1	1	1	1	1	1	1
rs4942486	0.625	1	0.958	0.806	0.972	0.687	0.998	0.835	0.94	0.968	0.994	1	1	1
rs56121817	1	1	1	1	1	1	1	1	1	1	1	1	1	1

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