PREVALENCE, SPECTRUM, AND FOUNDER EFFECT OF BRCA1 AND BRCA2 MUTATIONS IN EPITHELIAL OVARIAN CANCER FROM THE MIDDLE EAST

Ismail A. Al-Badawi[1], Abdul K. Siraj[2], Rong Bu[2], Sandeep K. Parvathareddy[2], Khawla S. Al-Kuraya[2]

[1]Obstetrics - Gynecology Department, [2]Human Cancer Genomic Research, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

ABSTRACT

Epithelial ovarian cancer (EOC) remains the most fatal gynecological malignancies. Germline mutations in Breast Cancer susceptibility gene 1 and 2 (*BRCA1* and *BRCA2*) have previously been estimated to contribute to 13-18% of all EOC. The prevalence and influence of *BRCA1* and *BRCA2* mutations in EOC in Middle Eastern population is not fully explored. Ethnic differences of ovarian cancer genomics have prompted us to investigate the spectrum of *BRCA1* and *BRCA2* mutations among our Middle Eastern EOC patients. To characterize the prevalence of *BRCA* mutations in our patients, *BRCA* mutation screening was performed in over 400 unselected ovarian cancer patients using Capture and/or Sanger sequencing. Seventeen short tandem repeat (STR) markers were used for founder mutation analysis. A total of 19 different types of pathogenic mutations were identified in 50 cases (40 mutant cases in *BRCA1* and 10 in *BRCA2*). Nine mutations were recurrent accounting for 80% (40/50) of all mutant cases (9.8% (40/407) of the entire cohort). Haplotype analysis carried out on all unrelated cases with these mutations revealed only two (c.4136_4137 delCT and c.1140 dupG) sharing the same haplotypes thus representing founder Saudi mutations. *BRCA1* mutant cases were significantly associated with positive family history (p <0.0001), grade 3 tumors (p = 0.0003) and loss of BRCA1 protein expression (p <0.0001). Eighty five percent (34/40) of all BRCA1 mutant cases were of serous histology. *BRCA2* mutant cases were associated with loss of *BRCA2* protein expression (p=0.0102) and a trend towards younger age (p=0.0505). Identification of the mutation spectrum, prevalence and founder effect in Middle Eastern population facilitates genetic counseling, risk assessment and development of cost-effective screening strategy.

INTRODUCTION

- Ovarian cancer (OC) in Saudi Arabia ranks seventh among all cancers affecting females.
- OC in Saudi population has unique characteristics such as lower median age at presentation (55 years in Saudi vs 65 years in Western population), advanced stage and higher incidence of mucinous carcinomas (19% vs 3% in Western population).
- It is believed that nearly 5-12% of invasive EOC are caused by hereditary susceptibility
- Germline mutations of Breast Cancer susceptibility gene 1 and 2 (*BRCA1* and *BRCA2*) have been estimated to contribute to 13-18% of all OC.
- *BRCA1* and *BRCA2* mutations confers a lifetime risk of developing OC of approximately 50% (*BRCA1*) and 20% (*BRCA2*).
- To date, studies of germline mutations in *BRCA1* and *BRCA2* genes, their founder effect and contribution to EOC in general are very limited.
- This prompted us to analyze the prevalence, spectrum and founder effect of *BRCA1/2* gene mutations in EOC among Middle Eastern population and develop a panel for screening approach in order to improve genetic testing strategy, which could improve cancer prevention and risk assessment for patients from this ethnicity.

MATERIALS AND METHODS

- A total of 407 archived samples from unselected patients with EOC diagnosed between 1987 and 2017 at King Faisal Specialist Hospital and Research Center (KFSH&RC) were included in the study.
- Clinical and histological data were available for all these patients and are summarized in Table 1.
- DNAs were isolated from formalin-fixed, paraffin-embedded (FFPE) epithelial ovarian cancer non-tumor tissues using Gentra DNA isolation kit.
- 290 samples were screened for the germline mutations using the panel comprising mutations identified in 117 samples by capture sequencing. Detected mutations were further confirmed for their germline origin by independent PCR and Sanger sequencing.
- Genotype analyses were performed if the recurrent mutation was observed in unrelated families.
- BRCA1 & BRCA2 protein expression was assessed by immunohistochemistry on tissue microarray.

RESULTS

- In our cohort of 407 cases, a total of 19 different PVs were identified in 50 women (12.3%), 40 cases (9.8%) harboring *BRCA1* and 10 (2.5%) in *BRCA2* (Table 2, Figure 1).
- Out of these 19 PVs, nine were recurrent, six in *BRCA1* and three in *BRCA2* (Table 2).
- The nine recurrent PVs accounted for 80% (40/50) of all cases with PVs and the overall frequency of cases harboring recurrent PVs was 9.8% (40/407).
- Our results revealed that all the carriers of c.1140 dupG and c.4136_4137delCT mutations shared the same haplotypes indicating these two are founder mutations. This is the first report of founder mutations identified in Saudi EOC patients.
- In our study, these two founder mutations accounted for 42% (21/50) of all cases harboring with PVs.
- *BRCA1* PVs were significantly associated with positive family history (p<0.0001), grade 3 tumors (p=0.0003) and loss of BRCA1 protein expression (p<0.0001) (Table 3).
- Cases harboring *BRCA2* PVs were associated with loss of BRCA2 protein expression (p=0.0102) and showed a trend towards younger age (p=0.0505) (Table 4).
- By immunohistochemistry, loss of expression of BRCA1 protein was noted in 23.9% (96/407) of unselected EOC cases and was significantly associated with stage IV tumors (p=0.0043), *BRCA1* PVs (p<0.0001) and a trend was seen with Grade 3 tumors (p =0.0694) (Table 5).
- Loss of BRCA2 expression was seen in 15.7% (63/402) of the cases and was significantly associated with Stage III tumors (p=0.0019) and *BRCA2* PVs (p=0.0102) (Table 6).

TABLE 1: Clinicopathological variables for the Epithelial ovarian cancer patient cohort (n=407).

	n (%)
Age	
Median	50.0
Range (IQR)^	40.5 – 62.0
Histopathology	
Serous	288 (70.8)
Mucinous	61 (15.0)
Endometrioid	38 (9.3)
Clear cell	9 (2.2)
Undifferentiated	11 (2.7)
Grade	
Well differentiated	89 (21.9)
Moderately differentiated	137 (33.6)
Poorly differentiated	161 (39.6)
Unknown	20 (4.9)
Stage	
I	76 (18.7)
II	23 (5.7)
III	241 (59.2)
IV	58 (14.2)
Unknown	9 (2.2)
Progression free survival duration in months	
Median	14.0
Range	2.0 – 199.0

Abbreviations - ^ Inter quartile range

TABLE 2: Spectrum of BRCA Deleterious Mutations Identified.

Gene	Exon	Mutation	No. of Cases 1st Set[£]	2nd Set[¶]	Novel
BRCA1	6	c.414del7;p.L138fs	1	1	
	10	c.1066C>T;p.Q356X*		1	
	10	c.1140dupG;p.K381fs	4	5	
	10	c.2157dupA;p.E720fs	1		
	10	c.2405_2406delTG;p.V802fs	1		
	10	c.4069_4069delTCAA;.p.N1356fs	3		
	11	c.4136_4137delCT;. p.S1379X	5	7	
	17	c.5096C>T;p.R1699W	1	1	
	17	c.5152+1 G>C*		1	
	17	c.5152+2 T>G*		1	yes
	22	c.5431C>T;p.Q1811X	1		
	23	c.5530delC;p.L1844fs	1	5	
BRCA2	11	c.4177dupG;p.A1393fs	1		
	11	c.5271T>A;p.Y1757X	1	1	
	11	c.5760_5770delTTTTCTCAGCA;p.F1921fs	1	1	yes
	11	c.5826_5827delTT;p.S1943X*		1	
	11	c.6446dupA;p.V2151fs	1		yes
	13	c.7007G>A; p.Arg2336His	1	1	
	16	c.7647C>A;p.C2549X	1		

†: Full Gene Screening Assay; ¶: Specific Mutation Screening Assay
* Pathogenic variants detected only in the 2nd set of 290 cases.

TABLE 3: Correlation of *BRCA1* Mutation with clinico-pathological parameters in EOC.

	Total		BRCA1 Mutant		BRCA1 WT		p value
	N	%	N	%	N	%	
Total Number of Cases	407		40	9.8	367	90.2	
Age							
≤ 50 years	205	50.5	24	11.7	181	88.3	0.2038
> 50 years	201	49.5	16	8.0	185	92.0	
Family History							
Positive	22	10.1	13	59.1	9	40.9	<0.0001
Negative	195	89.9	21	10.8	174	89.2	
Histopathology							
Serous	288	70.8	34	11.8	254	88.2	0.0025
Mucinous	61	15.0	0	0.0	61	100.0	
Endometrioid	38	9.3	4	10.5	34	89.5	
Clear cell	9	2.2	0	0.0	9	100.0	
Undifferentiated	11	2.7	2	18.2	9	81.8	
FIGO Grade							
Well differentiated	89	23.0	2	2.3	87	97.7	0.0003
Moderately Differentiated	137	35.4	9	6.6	128	93.4	
Poorly Differentiated	161	41.6	26	16.2	135	83.8	
Tumour Stage							
Stage I	76	19.1	4	5.3	72	94.7	0.1540
Stage II	23	5.8	2	8.7	21	91.3	
Stage III	241	60.5	22	9.1	219	90.9	
Stage IV	58	14.6	10	17.2	48	82.8	
BRCA1 IHC							
Present	305	76.1	16	5.3	289	94.7	<0.0001
Absent	96	23.9	24	25.0	72	75.0	
PFS – Median (months)			20		20		0.2775

TABLE 4: Correlation of *BRCA2* Mutation with clinico-pathological parameters in EOC.

	Total		BRCA2 Mutant		BRCA2 WT		p value
	N	%	N	%	N	%	
Total Number of Cases	407		10	2.5	397	97.5	
Age							
≤ 50 years	205	50.5	8	3.9	197	96.1	0.0505
> 50 years	201	49.5	2	1.0	199	99.0	
Family history							
Positive	22	10.1	1	4.6	21	95.4	0.6185
Negative	195	89.9	5	2.6	190	97.4	
Histopathology							
Serous	288	70.8	9	3.1	279	96.9	0.3391
Mucinous	61	15.0	0	0.0	61	100.0	
Endometrioid	38	9.3	1	2.6	37	97.4	
Clear cell	9	2.2	0	0.0	9	100.0	
Undifferentiated	11	2.7	0	0.0	11	100.0	
FIGO Grade							
Well differentiated	89	23.0	1	1.1	88	98.9	0.1805
Moderately Differentiated	137	35.4	2	1.5	135	98.5	
Poorly Differentiated	161	41.6	7	4.4	154	95.6	
Tumour Stage							
Stage I	76	19.1	0	0.0	76	100.0	0.0903
Stage II	23	5.8	0	0.0	23	100.0	
Stage III	241	60.5	9	3.7	232	96.3	
Stage IV	58	14.6	1	1.7	57	98.3	
BRCA2 IHC							
Present	339	84.3	5	1.5	334	98.5	0.0102
Absent	63	15.7	5	7.9	58	92.1	
PFS – Median (months)			14		20		0.2961

TABLE 5: Correlation of BRCA1 protein expression with clinico-pathological parameters in EOC.

	Total		BRCA1 loss		BRCA1 normal		p value
	N	%	N	%	N	%	
Total Number of Cases	401		96	23.9	305	76.1	
Age							
≤ 50 years	202	50.5	52	25.7	150	74.3	0.4096
> 50 years	198	49.5	44	22.2	154	77.8	
Family History							
Positive	22	10.3	8	36.4	14	63.6	0.2962
Negative	191	89.7	49	25.7	142	74.3	
Histopathology							
Serous	285	71.1	74	26.0	211	74.0	0.1001
Mucinous	59	14.7	7	11.9	52	89.1	
Endometrioid	38	9.5	11	29.0	27	71.0	
Clear cell	9	2.2	1	11.1	8	88.9	
Undifferentiated	10	2.5	3	30.0	7	70.0	
FIGO Grade							
Well differentiated	85	22.2	13	15.3	72	84.7	0.0694
Moderately Differentiated	137	35.9	33	24.1	104	75.9	
Poorly Differentiated	160	41.9	45	28.1	115	71.9	
Tumour Stage							
Stage I	76	19.4	11	14.5	65	85.5	0.0043
Stage II	22	5.6	1	4.6	21	95.4	
Stage III	236	60.2	64	27.1	172	72.9	
Stage IV	58	14.8	18	31.0	40	69.0	
***BRCA1* mutation**							
Present	40	10.0	24	60.0	16	40.0	<0.0001
Absent	361	90.0	72	19.9	289	80.1	
PFS – Median (months)			21		19		0.5371

TABLE 6: Correlation of BRCA2 protein expression with clinico-pathological parameters in EOC.

	Total		BRCA2 loss		BRCA2 normal		p value
	N	%	N	%	N	%	
Total Number of Cases	402		63	15.7	339	84.3	
Age							
≤ 50 years	201	50.1	38	18.9	163	81.1	0.0771
> 50 years	200	49.9	25	12.5	175	87.5	
Family History							
Positive	21	9.9	2	9.5	19	90.5	0.6833
Negative	192	90.1	24	12.5	168	87.5	
Histopathology							
Serous	287	71.4	40	13.9	247	86.1	0.4990
Mucinous	59	14.7	12	20.3	47	79.7	
Endometrioid	36	9.0	6	16.7	30	83.3	
Clear cell	9	2.2	3	33.3	6	66.7	
Undifferentiated	11	2.7	2	18.2	9	81.8	
FIGO Grade							
Well differentiated	88	23.0	17	19.3	71	80.7	0.6034
Moderately Differentiated	134	35.1	22	16.4	112	83.6	
Poorly Differentiated	160	41.9	23	14.4	137	85.6	
Tumour Stage							
Stage I	74	18.8	12	16.2	62	83.8	0.0019
Stage II	23	5.9	2	8.7	21	91.3	
Stage III	239	60.8	44	18.4	195	81.6	
Stage IV	57	14.5	1	1.8	56	98.2	
***BRCA2* mutation**							
Present	10	2.5	5	50.0	5	50.0	0.0102
Absent	392	97.5	58	14.8	334	85.2	
PFS – Median (months)			25		20		0.2126

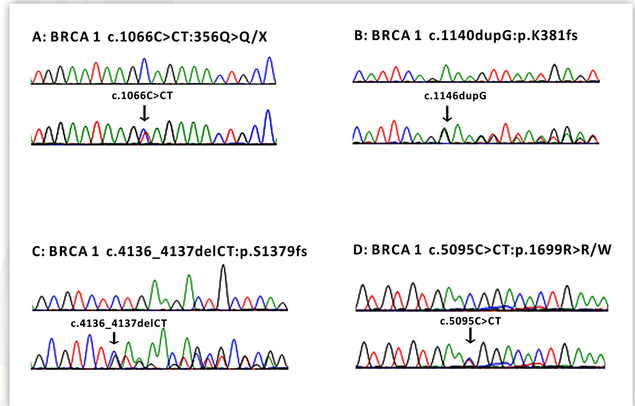

FIGURE 1: Sequence chromatogram of four representative mutations in *BRCA1* gene detected in our cohort.

CONCLUSIONS

- Even though in this study we have found two founder mutations in *BRCA1* gene in Middle Eastern population, we cannot rule out the possibility that other founder mutations in *BRCA1* and/or *BRCA2* genes may also exist if targeted capture sequencing is conducted in all samples or a larger patient cohort is involved.
- However, we think this study should pave the road for future studies in Middle Eastern ethnicity in view of the economic advantages of analyzing founder mutations instead of full gene screening testing, particularly for Middle Eastern countries with limited economic resources.

Designed by: RC Scientific Information Office