GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association of *BRCA2* variants with cardiovascular disease in Saudi Arabia

M. Alanazi[1], N.P. Reddy[1], J.P. Shaik[1], S.A. Ajaj[2], A.A.A. Jafari[1], H. Saeed[1], Z. Khan[1] and A.P. Khan[1]

[1]Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
[2]Family Medicine Department, College of Medicine, King Saud University, Riyadh, Saudi Arabia

Corresponding author: A.P. Khan
E-mail: apathan@ksu.edu.sa

Genet. Mol. Res. 13 (2): 3876-3884 (2014)
Received September 16, 2013
Accepted December 9, 2013
Published May 16, 2014
DOI http://dx.doi.org/10.4238/2014.May.16.13

ABSTRACT. Abnormalities in the breast cancer tumor suppressor genes (*BRCA1* and *BRCA2*) are associated with breast and ovarian cancer. Recently, two single nucleotide polymorphisms (SNPs; rs11571836 and rs1799943) were identified, both located in untranslated regions of chromosome 13, associated with cardiovascular disease (CVD) in a multi-ethnic population. We examined the association between these *BRCA2* polymorphisms and traits of CVD patients from Saudi Arabia. We genotyped rs11571836 and rs1799943 in 159 unrelated CVD patients and 176 healthy controls. The genotype and allele distributions in the overall population revealed a statistically significant association between rs1799943 and CVD (P = 0.01-0.022), whereas no risk association was identified for rs11571836. Additionally, haplotype analysis using both SNPs demonstrated no association between the SNPs and CVD. The genotype distribution of the 2 SNPs in the normal Saudi population deviated significantly (P < 0.000001) from that of the 6 different HapMap populations (CEU, CHB-Han, JPT, YRI, GIH, and MKK), except for the JPT population for rs1799943. This is the

first study to examine the association between these SNPs and CVD in a Saudi population. Our results suggest that the increased health risk associated with the rs11571836 genotype is specific to male patients suffering from CVD. Stratification of patients and controls based on gender revealed no association between rs1799943 and the risk of CVD in either gender. These SNPs should be evaluated in larger cohorts in different populations to determine their suitability as screening markers for predicting CVD risk earlier in life to implement necessary preventive measures.

Key words: Association study; BRCA2; Cardiovascular disease; HapMap; Saudi Arabian population; Single nucleotide polymorphism

INTRODUCTION

Abnormalities in the tumor suppressor genes breast cancer gene 1 (*BRCA1*) and breast cancer gene 2 (*BRCA2*) are associated with breast and ovarian cancers (King et al., 2003). *BRCA* genes play important roles in repairing DNA that has been damaged during replication or by genotoxic stress, allowing the cells to grow normally. Germline mutations in these genes significantly increase the susceptibility for familial breast cancers (Liede et al., 2004), which account for up to 10% of all breast cancers. Alterations in these genes in both men and women have revealed a modulatory role in other cancers and diseases (Mai et al., 2009). While an aberrant *BRCA1* gene in men confers a slightly higher risk of developing prostate cancer, men with abnormal *BRCA2* are 7 times more likely to acquire the disease (Breast Cancer Linkage Consortium, 1999; Thompson and Easton, 2002; PDQÒ Cancer Information Summary, 2009). The risk of other cancers, such as those of the skin or digestive tract has been reported as slightly higher in men with abnormal *BRCA1* or *BRCA2* genes (Breast Cancer Linkage Consortium, 1999; Thompson and Easton, 2002; PDQÒ Cancer Information Summary, 2009). For women, in addition to increasing the risk of breast cancer, abnormalities in *BRCA1* or *BRCA2* genes increase the risk of developing ovarian, colon, pancreatic, and thyroid cancers, as well as melanoma (Lynch et al., 2008). Recently, Shukla et al. (2011) demonstrated that *BRCA1* and *BRCA2* mutation carriers are also at increased risk of cardiac failure. Cardiomyocyte-specific knockout of *BRCA1* in mice results in poor ventricular function and increased mortality following ischemic or genotoxic stress, suggesting that BRCA dysfunction plays a role in cardiac damage during myocardial infarction and genotoxin-induced cardiotoxicity (Shukla et al., 2011). Similarly, mice treated with doxorubicin showed targeted disruption of BRCA2 in cardiomyocytes, resulting in greater cardiac dysfunction and mortality (Shukla et al., 2011). Moreover, using data from Study of Health Assessment and Risk Evaluation (SHARE) studies that included multi-ethnic populations (Aboriginals, South Asians, Europeans, and Chinese), Zbuk et al. (2012) identified an association between single nucleotide polymorphisms (SNPs) (rs11571836 and rs1799943) in *BRCA2* and cardiovascular disease (CVD). The SHARE studies also demonstrated that Aboriginals and South Asians had the highest prevalence of CVD among the 4 ethnic groups tested (Zbuk et al., 2012). A large number of SNPs that are common in *BRCA1/2* have been reported (Szabo et al., 2000).

In the present study, we examined the association between 2 SNPs, rs11571836 and

rs1799943, which are located in the untranslated region of *BRCA2* and cardiovascular disease by comparing the genotypic distribution of these SNPs in 159 CVD cases to 176 healthy subjects from Saudi Arabia.

MATERIAL AND METHODS

Study population

We analyzed data from 159 subjects attending the Cardiology Clinics at King Khalid University Hospital, Riyadh, using gender-stratified random sampling. An interviewer-administered questionnaire was used to obtain data on self-reported CVD. Trained nurses performed blood pressure and anthropometric measurements. The 159 subjects with clinically confirmed CVD formed the patient group. For controls, we randomly selected 176 subjects from the general population who did not have a personal or family history of CVD or any other heart-related disorders. All patients and controls had been residents of Riyadh for more than 3 generations. Cases and controls were age-matched and sub-grouped based on gender. Blood samples were obtained from both cases and controls at King Khalid University Hospital before the start of treatment. Written informed consent was obtained from all participants, and approval was received from the King Khalid University Hospital ethics review committee.

Clinical parameters of subjects

A total of 335 subjects were involved in our study, including 159 cardiovascular disease patients and 176 controls. Clinical parameters of the cardiovascular disease patients are summarized in Table 1. There were no significant differences in age and gender distribution between cardiovascular disease patients and controls ($P > 0.05$).

DNA extraction

Approximately 3 mL blood samples were collected in sterile tubes containing ethylenediaminetetraacetic acid (EDTA) from each subject enrolled in the study. Genomic DNA was isolated from blood samples using the QIAmp DNA blood Mini Kit (Qiagen; Hilden, Germany) following manufacturer instructions. After extraction and purification, the DNA was quantified on a NanoDrop 8000 to determine the concentration, and its purity was examined using standard A_{260}/A_{280} and A_{260}/A_{230} ratios (NanoDrop Technologies; Wilmington, DE, USA) (Sambrook et al., 1989).

Genotyping

Two SNPs (rs11571836 and rs1799943) in the *BRCA2* gene were genotyped using a TaqMan allelic discrimination assay (Livak, 1999). For each sample, 20 ng DNA was mixed with 5.6 μL 2X Universal Master Mix and 200 nM primers (Applied Biosystems; Foster City, CA, USA). All genotypes were determined by endpoint reading on an ABI 7500 apparatus (Applied Biosystems). The primers and probe mix were purchased directly through the assays-on-demand service of Applied Biosystems. Five percent of the samples were randomly

selected and subjected to repeat analysis as a quality control measure to verify the genotyping procedures.

Statistical analysis

Genotype and allelic frequencies were computed and evaluated for deviations from Hardy-Weinberg equilibrium. Case-control and other genetic comparisons were performed using the chi-square test (χ^2), and allelic odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the Fisher 2-tailed exact test. Statistical analysis was conducted using SPSS 16.0 for Windows (SPSS, Inc.; Chicago, IL, USA). We considered a P value of <0.05 to be significant. The (χ^2 test was used to compare the observed genotype distribution of the 2 *BRCA2* SNPs (rs11571836 and rs1799943) with their expected values. Allele and genotype frequencies of polymorphisms in the Saudi population (SAUDI) were compared with the HapMap population (www.hapmap.org). For example, we included Utah residents of Northern and Western European ancestry (CEU) from the CEPH collection, Han Chinese in Beijing, China (HCB), Japanese in Tokyo, Japan, (JPT), Yoruba in Ibadan, Nigeria (YRI), Gujarati Indians in Houston, Texas (GIH), Maasai in Kinyawa, Kenya (MKK), and Saudi population residing in the Riyadh region of central Saudi Arabia (SAUDI). Pairwise chi-square tests were performed between the SAUDI population and other populations using the allele frequencies in a 2 x 2 contingency table to determine whether a significant difference between the Saudi and other populations exists.

RESULTS

A total of 159 CVD cases and 176 healthy controls were included in this study. Clinical characteristics of CVD cases are shown in Table 1. In the present study, concentrations of triglycerides, cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol were in the desirable range in both male and female CVD patients (Table 1), whereas the levels of cardiac enzyme profiles differed between the 2 genders. Levels of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were higher than the desirable range in males but were within the normal range in females (Table 1). Creatine kinase (CK) levels were within the normal limits in both male and female CVD patients. The genotype frequencies observed showed no significant departure from Hardy-Weinberg expectations for both polymorphic loci examined in this study.

Table 1. Clinical data of the genotyped study subjects.

	Male	Female	Desirable range
Age	64 ± 12.63	64 ± 10.03	-
Lipid profiling (mM)			
Triglycerides	1.4	1.24	<1.70
Total cholesterol	4.2	3.83	<5.18
HDL-cholesterol	1.01	1.21	>1.04
LDL-cholesterol	2.58	2.25	<2.59
Cardiac enzymes (U/L)			
Aspartate aminotransferase	69.85	20.1351	6-40
Lactate dehydrogenase	222.79	163.773	90-200
Creatine kinase	76.65	75.28	10-150

HDL = High-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol.

The homozygous ancestral allele was used as a reference to determine the odds of acquiring CVD relative to patients with other genotypes. The genotype distribution of the SNPs analyzed along with the corresponding OR and significance level are shown in Table 2. We found a significant difference in the distribution of *BRCA2* genotype (rs1799943) between CVD cases and the matched healthy controls (Table 2). The distribution of the 3 genotypes, AA, AG, and GG, for rs1799943 in CVD cases was 24 (0.15), 78 (0.49), and 57 (0.36), respectively, compared to 36 (0.20), 100 (0.57), and 40 (0.23), respectively, in healthy controls. The homozygous (GG) variant (OR = 2.13, $\chi^2 = 5.23$, P = 0.02) in CVD patients was associated with a significant risk when compared to healthy individuals (Table 2). The frequency of the G allele for rs1799943 was higher in CVD cases (0.6) compared to that in healthy controls (0.51) (OR = 1.456, $\chi^2 = 5.78$, P = 0.016) (Table 2). The genotypes for rs11571836 showed no significant difference between CVD cases and matched healthy controls.

Table 2. Genotype frequencies of BRCA2 gene polymorphism in cardiovascular disease cases and controls.

Genotype	Cases	Controls	OR	95%CI	χ^2	P
rs1799943						
AA (wild)	24 (0.15)	36 (0.20)	Ref			
AG	78 (0.49)	100 (0.57)	1.170	0.645-2.122	0.27	0.60507
GG (variant)	57 (0.36)	40 (0.23)	2.138	1.109-4.119	5.23	0.02226
AG+GG	135 (0.85)	140 (0.8)	1.446	0.820-2.553	1.63	0.20137
A	126 (0.4)	172 (0.49)	Ref			
G	192 (0.6)	180 (0.51)	1.456	1.071-1.979	5.78	0.01623
rs11571836						
AA (wild)	5 (0.03)	7 (0.04)	Ref			
AG	54 (0.33)	45 (0.26)	1.680	0.499-5.656	0.71	0.39848
GG (variant)	103 (0.64)	119 (0.70)	1.212	0.373-3.934	0.10	0.74888
AG+GG	157 (0.97)	164 (0.96)	1.340	0.417-4.311	0.24	0.62211
A	64 (0.2)	59 (0.17)	Ref			
G	260 (0.8)	283 (0.83)	0.847	0.572-1.253	0.69	0.40566

We examined the association between CVD risk and individual SNPs based on patient gender. The genotype distributions in the male (N = 89) and female (N = 70) CVD patient groups were compared with those of the respective male and female control subjects. Interestingly, the increased CVD risk associated with the GG genotype in the overall population for rs1799943 was not observed after stratification by gender (Table 3).

In contrast, rs11571836 was found to increase the risk of CVD in males. While none of the genotypes of rs11571836 were associated with disease risk in females, both the heterozygous AG (OR = 11.9; CI = 0.609-233.68; P = 0.0277) and homozygous GG (OR = 10.9; CI = 0.575-207.62; P = 0.0307) genotypes were significantly associated with a higher risk of acquiring CVD in males (Table 3).

Haplotype analysis was conducted to determine whether a combination of both the rs1799943 and rs11571836 SNPs in the *BRCA2* gene was associated with CVD risk. We found no significant differences in haplotype distributions in the comparison of CVD patients with controls (Table 4; P > 0.05).

Genotype and allele frequencies of *BRCA2* variants in Saudis and other populations

We compared the genotypic and allelic frequencies of the *BRCA2* SNPs rs1799943

and rs11571836 in a normal healthy Saudi population with those of subjects in the HapMap project study groups. Allelic frequencies for both SNPs were significantly different in the Saudi population compared with the 6 populations represented in the HapMap project (Tables 5 and 6). Only the Japanese population showed similarities in the genotype and allele distributions comparable with Saudi Arabians for rs1799943. For rs1799943, the A (wild-type) and G (variant) allele frequencies were 0.49 and 0.51, respectively in the Saudi population. The variant G allele frequency varied from 0.535 among the Japanese population to 0.947 in the Nigerian (MKK) population. Thus, except for the Japanese population, the allelic frequencies of rs1799943 in all of the HapMap populations, including CEU, HCB, YRI, GIH, and MKK, were found to be significantly different from that of the Saudi population (Table 5).

Table 3. Genotype frequencies of BRCA gene polymorphism in cardiovascular disease cases between male and female samples.

Genotype	Cases	Control	OR	95%CI	χ^2	P
rs1799943	Male	Male				
AA (wild)	12 (0.14)	15 (0.2)	Ref			
AG	43(0.48)	42 (0.56)	1.280	0.536-3.055	0.31	0.57800
GG (variant)	34(0.38)	18 (0.24)	2.361	0.913-6.106	3.20	0.07346
AG+GG	77(0.86)	60 (0.8)	1.604	0.699-3.682	1.26	0.26225
A	67 (0.38)	72 (0.48)	Ref			
G	111 (0.62)	78 (0.52)	1.529	0.984-2.377	3.58	0.05856
rs1799943	Female	Female				
AA (wild)	12(0.17)	21 (0.21)	Ref			
AG	35(0.50)	58 (0.57)	1.056	0.463-2.407	0.02	0.89682
GG (variant)	23(0.33)	22 (0.22)	1.830	0.730-4.587	1.67	0.19574
AG+GG	58(0.83)	80 (0.78)	1.269	0.578-2.784	0.35	0.55212
A	59 (0.42)	100 (0.49)	Ref			
G	81 (0.58)	102 (0.51)	1.346	0.872-2.078	1.80	0.17952
rs11571836	Male	Male				
AA (wild)	0	4 (0.05)	Ref			
AG	28(0.31)	21 (0.28)	11.93	0.609-233.686	4.85	0.02771
GG (variant)	62 (0.69)	51 (0.67)	10.922	0.575-207.62	4.67	0.03072
AG+GG	90 (1)	72 (0.95)	11.234	0.595-212	4.85	0.02759
A	28 (0.16)	29 (0.19)	Ref			
G	152 (0.84)	123 (0.81)	1.280	0.723-2.266	0.72	0.39634
rs11571836	Female	Female				
AA (wild)	5 (0.07)	3 (0.03)	Ref			
AG	26(0.36)	24 (0.25)	0.650	0.140-3.017	0.31	0.58040
GG (variant)	41 (0.57)	68 (0.72)	0.362	0.082-1.594	1.93	0.16427
AG+GG	67 (0.93)	92 (0.97)	0.437	0.101-1.892	1.29	0.25650
A	36 (0.25)	30 (0.16)	Ref			
G	108 (0.75)	160 (0.84)	0.562	0.327-0.968	4.38	0.03630

Table 4. Major haplotype frequencies of BRCA2 gene polymorphism in cardiovascular disease cases and controls.

Haplotype	Variant	CVD Cases	Controls	OR	CI	χ^2 value	P
H1	AA	29	43	Ref			
H2	AG	132	145	1.350	0.797-2.286	1.25	0.26336
H3	GG	160	159	1.483	0.882-2.493	2.22	0.13601

The A (wild-type) and G (variant) allele frequencies for rs11571836 were 0.17 and 0.83, respectively, in the Saudi population. The variant G allele frequency varied from 0.066 among Nigerians (YRI) to 0.471 in Japanese (JPT) subjects. Allelic frequencies for rs11571836 in all HapMap populations were significantly different from that in the Saudi population according to the results of the pairwise χ^2 test (Table 5).

Table 5. Allele and genotype frequencies of rs11571836 in Saudi and other populations.

Population	AA	AG	GG	A	G	P
CEU (N = 226)	0.708	0.274	0.018	0.845	0.155	<0.000001
HCB (N = 86)	0.442	0.488	0.070	0.686	0.314	<0.000001
JPT (N = 172)	0.244	0.570	0.186	0.529	0.471	<0.000001
YRI (N = 226)	0.876	0.115	0.009	0.934	0.066	<0.000001
GIH (N = 176)	0.648	0.330	0.023	0.812	0.188	<0.000001
MKK (N = 286)	0.832	0.161	0.007	0.913	0.180	<0.000001
SAUDI	0.04	0.26	0.70	0.17	0.83	refs

CEU = Utah residents with Northern and Western European ancestry from the CEPH collection; HCB = Han Chinese in Beijing China; JPT = Japanese in Tokyo Japan; YRI = Yoruba in Ibadan Nigeria; GIH = Gujarati Indians in Houston Texas; MKK = Maasai in Kinyawa Kenya; SAUDI = Saudi population residing in Riyadh region of central Saudi Arabia; a Chi-square test statistic value more than 3.841 at 5% significance level showed that populations are significantly different from Saudi population.

Table 6. Allele and genotype frequencies of rs1799943 in Saudi and other populations.

Population	AA	AG	GG	A	G	P
CEU (N = 226)	0.027	0.345	0.628	0.199	0.801	<0.000001
HCB (N = 86)	0.070	0.419	0.512	0.279	0.721	0.001244
JPT (N = 172)	0.174	0.581	0.244	0.465	0.535	0.658945
YRI (N = 226)	0.009	0.088	0.903	0.053	0.947	<0.000001
GIH (N = 176)	0.034	0.432	0.534	0.250	0.750	<0.000001
MKK (N = 286)	0.154	0.846	0.752	0.077	0.923	<0.000001
SAUDI	0.20	0.57	0.23	0.49	0.51	refs

CEU = Utah residents with Northern and Western European ancestry from the CEPH collection; HCB = Han Chinese in Beijing China; JPT = Japanese in Tokyo Japan; YRI = Yoruba in Ibadan Nigeria; GIH = Gujarati Indians in Houston Texas; MKK = Maasai in Kinyawa Kenya; SAUDI = Saudi population residing in Riyadh region of central Saudi Arabia; a Chi-square test statistic value more than 3.841 at 5% significance level showed that populations are significantly different from Saudi population.

DISCUSSION

Deficiencies in the function of the double-strand DNA damage repair gene *BRCA2* have been linked to several malignancies (Yoshida and Miki, 2004). Recent findings indicate a potential correlation between CVD and cancer, but this correlation requires further investigation (Thompson and Easton, 2002). For instance, endothelin-1 signaling, which plays a role in CVD, has also been implicated in several cancers (Anand et al., 2001). Additionally, DNA repair pathways, which play a clear role in the development of cancers, have recently gained attention for their roles in CVDs (Wang, 2007). Overexpression of topoisomerase-II-α in unstable plaques from human carotid atheromas (Slevin et al., 2006) as well as involvement of BRCA1/2 in the pathogenesis of coronary atherosclerosis and plague rupture implicates DNA repair pathways in CVDs.

In this study, we examined the association between 2 *BRCA2* SNPs, rs1799943 and rs11571836, and the risk of CVDs. This is the first report to demonstrate a possible association between *BRCA2* variants and CVDs in a Saudi Arabian population. We found a statistically significant association between rs1799943 and CVDs in the overall population. Individuals with the GG genotype at rs1799943 were at a 2-fold higher risk of developing CVDs compared to those with the AA genotype at that locus. Although our analysis was statistically significant,

this observation requires further confirmation because our sample size was small. Moreover, genotypic analysis after stratification of patients and controls based on gender revealed no association between rs1799943 and CVD risk in either gender. However, rs11571836, which was not associated with CVD risk in the overall population, showed statistical significance for the increased risk of CVD in males. Analysis of these SNPs in a larger cohort and different ethnic populations may be useful for determining the significance of these SNPs as screening markers to predict CVD risk. It is widely accepted that cardiovascular disease is associated with elevated blood levels of LDL, total cholesterol, and triglycerides (Criqui et al., 1993), and that a low level of HDL is a risk factor for mortality from CVD; however, no such changes were observed in the present study, indicating the non-specific effect of stress and hypertension rather than of serum lipids. According to the guidelines of the American Heart Association (2012), the following values are considered risk factors for CVD: total cholesterol <200 mg/dL; triglycerides <200 mg/dL; HDL > 40 mg/dL; and LDL < 130 mg/dL. Surprisingly, these parameters were within the normal range in our CVD patients. In addition to lipids, elevated levels of serum cardiac enzymes such as serum glutamic oxalo-acetic transaminase (SGOT), LDH, and creatine phospho-kinase, have been reported in the initial phase of stroke (Dubo et al., 1967). As shown in Table 1, AST and LDH were above the normal range in males, but were within the prescribed limits in female patients. Thus, AST and LDH may be indicators of CVD in males, but lack significance in females. Levels of creatine kinase in both male and female CVD patients were within the normal range and may not be a strong indicator of the disease in our patient population. Notably, rs11571836 as well as levels of AST and LDH were associated with CVD only in males. Whether a link exists between rs11571836 in *BRCA2* and these enzyme levels in male patients require further investigation.

We compared the genotype and allele distributions of rs1799943 and rs11571836 in a Saudi population with those of other populations represented in the HapMap project. The allelic distribution for both SNPs was highly significantly different in the Saudi population compared to those of other populations as shown in Tables 5 and 6. Only the Japanese population showed similar allelic frequencies with no significant difference for rs1799943 compared to the Saudi population. Thus, the genetic makeup of the Saudi Arabian population appears to differ from other ethnicities in the HapMap study groups. Examining these SNPs in other populations may not yield similar results.

In conclusion, our findings highlight the role of *BRCA2* gene variants in CVD susceptibility in the Saudi Arabian population. We identified an association between rs1799943 in the overall study population as well as rs11571836 in males with CVD in the Saudi Arabian population. Our findings provide a new perspective for investigating the pathogenesis of CVD and may lead to the development of early diagnostic and screening markers for CVD. However, further studies with familial (*BRCA1* or *BRCA2* mutations) cases of breast cancer and an examination of the cardiac history of all *BRCA1* or *BRCA2* mutation-positive members, irrespective of the presence or absence of breast cancer, will help to clearly reveal any association between *BRCA1* and *BRCA2* and heart disease.

ACKNOWLEDGMENTS

Research supported by the King Saud University, Deanship of Scientific Research, College of Science Research Center.

REFRENCES

American Heart Association (2012). What do my Cholestorol Levels Mean? Available at [http://www.americanheart.org]. Accessed December 12, 2012.

Anand SS, Yusuf S, Jacobs R, Davis AD, et al. (2001). Risk factors, atherosclerosis, and cardiovascular disease among Aboriginal people in Canada: the Study of Health Assessment and Risk Evaluation in Aboriginal Peoples (SHARE-AP). *Lancet* 358: 1147-1153.

Breast Cancer Linkage Consortium (1999). Cancer risks in *BRCA2* mutation carriers. *J. Natl. Cancer Inst.* 91: 1316 -1310.

Criqui MH, Heiss G, Cohn R, Cowan LD, et al. (1993). Plasma triglyceride level and mortality from coronary heart disease. *N. Engl. J. Med.* 328: 1220-1225.

Dubo H, Park DC, Pennington RJ, Kalbag RM, et al. (1967). Serum-creatine-kinase in cases of stroke, head injury, and meningitis. *Lancet* 2: 743-748.

King MC, Marks JH and Mandell JB (2003). Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. *Science* 302: 643-646.

Liede A, Karlan BY and Narod SA (2004). Cancer risks for male carriers of germline mutations in *BRCA1* or *BRCA2*: a review of the literature. *J. Clin. Oncol.* 22: 735-742.

Livak KJ (1999). Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet. Anal.* 14: 143-149.

Lynch HT, Silva E, Snyder C and Lynch JF (2008). Hereditary breast cancer: part I. Diagnosing hereditary breast cancer syndromes. *Breast J.* 14: 3-13.

Mai PL, Chatterjee N, Hartge P, Tucker M, et al. (2009). Potential excess mortality in BRCA1/2 mutation carriers beyond breast, ovarian, prostate, and pancreatic cancers, and melanoma. *PLoS One* 4: e4812.

PDQÒ Cancer Information Summary (2009). National Cancer Institute; Bethesda, MD. Genetics of Breast and Ovarian Cancer (PDQÒ) - Health Professional. Available at [http://www.cancer.gov/cancertopics/pdq/genetics/breast-and-ovarian/HealthProfessional]. Accessed December 15, 2012.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Shukla PC, Singh KK, Quan A, Al-Omran M, et al. (2011). *BRCA1* is an essential regulator of heart function and survival following myocardial infarction. *Nat. Commun.* 2: 593.

Slevin M, Elasbali AB, Miguel TM, Krupinski J, et al. (2006). Identification of differential protein expression associated with development of unstable human carotid plaques. *Am. J. Pathol.* 168: 1004-1021.

Szabo C, Masiello A, Ryan JF and Brody LC (2000). The breast cancer information core: database design, structure, and scope. *Hum. Mutat.* 16: 123-131.

Thompson D and Easton DF (2002). Cancer Incidence in *BRCA1* mutation carriers. *J. Natl. Cancer Inst.* 94: 1358-1365.

Wang W (2007). Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat. Rev. Genet.* 8: 735-748.

Yoshida K and Miki Y (2004). Role of *BRCA1* and *BRCA2* as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci.* 95: 866-871.

Zbuk K, Xie C, Young R, Heydarpour M, et al. (2012). *BRCA2* variants and cardiovascular disease in a multi-ethnic study. *BMC Med. Genet.* 13: 56.