MS.ID-IJPBS-3439
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (2): 01-06
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

BRCA1/2 Mutation Genetic Screening and Hereditary Cancer Treatment

E.Priya*[1]
[1]Research Development Centre, Bharathiar University, Coimbatore – 641046, INDIA

Received: 12 Jan 2022 / Accepted: 6 March 2022/ Published online: 01 April 2022
***Corresponding Author Email:** priya_28122003@yahoo.co.in

Abstract
The new Genomics topic area and objectives for 2020 reflect the increasing scientific evidence supporting the health benefits of using genetic tests and family health history to guide clinical and public health interventions. Women with certain high-risk family health history patterns for breast and ovarian cancer could benefit from receiving genetic counselling to learn about genetic testing for BRCA1/2. About 5% to 10% of Pancreatic cancers are caused by germ-line mutations in BRCA1, BRCA2 or PALB2 genes, and this subset of tumors may demonstrate significant sensitivity to DNA damaging agents and PARP1 inhibitors. Multiple lines of evidence indicate that BRCA1-related cancers may derive less benefit from taxane-based treatment than other categories of cancer patients. Ongoing revolution in technologies of DNA analysis, particularly the invention of next-generation sequencing, allows expecting that dozens of new familial cancer genes will be identified in the near future. This article brings you the concept of mutation responsible for breast cancer, hereditary risk factors, genetic features of BRCA genes, methods for diagnosis, treatment procedures and different prevention methodologies.
Keywords
Genomics, Pegylated liposomal doxorubicin, DNA analysis, Breast and Ovarian cancer.

*****

INTRODUCTION:
BRCA1 and BRCA2 are human genes that belong to a class of genes known as tumor suppressors. Mutation of these genes has been linked to hereditary breast and ovarian cancer. The cancer risk caused by BRCA1 and BRCA2 mutations are inherited in a dominant fashion[1].A mutated BRCA gene can be inherited from either parent. Because they are inherited from the parents, they are classified as hereditary or germline mutations rather than acquired or somatic mutations(figure-1).Cancer caused by a mutated gene is a hereditary cancer rather than a sporadic cancer. Because humans have a diploid genome, each cell has two copies of the gene (one from each biological parent).Typically only one copy contains a disabling, inherited mutation, so the affected person is heterozygous for the mutation[2].
If the functional copy is harmed, however, then the cell is forced to use alternate DNA repair mechanisms, which are more error prone. The loss of the functional copy is called loss of heterozygosity (LOH).Any resulting errors in DNA repair may result in cell death or a cancerous transformation of the cell[3].The likelihood that a breast and or ovarian cancer is associated with a harmful mutation in BRCA1 or BRCA2 is highest in families with a history of multiple cases of breast cancer and ovarian cancer. Both men and women who inherit harmful BRCA1 or BRCA2 mutations, whether they develop cancer themselves or not, may pass the mutations on to their sons and daughters[4].In other words, a woman who has inherited a harmful mutation in BRCA1 or BRCA2 is about five times more likely to develop breast cancer than a woman who does not have such a mutation.So in this article we are going to discuss about the hereditary risk factors, prevention strategies, treatment procedures, methods of diagnosis in detail[5].

Hereditary risk factors:

Women have about a 1 in 7 chance of getting breast cancer in their lifetime. Most cancer is sporadic, about 5-10% of cases are genetically linked. Women inheriting mutation of *BRCA* gene have increased chance of disease also can lead to ovarian cancer[6]. The *BRCA1* associated lifetime risks include chances of getting breast cancer about 50 to 85%,second primary breast cancer about 40 to 60%,ovarian cancer about 15 to 45%,male breast cancer less than 1%,there may also be an increased chances of prostrate and colon cancer(figure-2).The *BRCA2* mutations may cause breast cancer 50 to 85%,ovarian cancer 10 to 20%,male breast cancer 5 to 10%,there may also be increased chances of prostrate, laryngeal and pancreatic cancers[7].

According to estimates of lifetime risk, about 12.0 percent of women (120 out of 1,000) in the general population will develop breast cancer sometime during their lives compared with about 60 percent of women (600 out of 1,000) who have inherited a harmful mutation in *BRCA1 or BRCA2.* In other words, a woman who has inherited a harmful mutation in *BRCA1 or BRCA2* is about five times more likely to develop breast cancer than a woman who does not have such a mutation [8].

Lifetime risk estimates for ovarian cancer among women in the general population indicate that 1.4 percent (14 out of 1,000) will be diagnosed with ovarian cancer compared with 15 to 40 percent of women (150–400 out of 1,000) who have a harmful *BRCA1 or BRCA2* mutation [9].

Mutations in several other genes, including *TP53, PTEN, STK11/LKB1, CDH1, CHEK2, ATM, MLH1, and MSH2,* have been associated with hereditary breast and/or ovarian tumors[10].However, the majority of hereditary breast cancers can be accounted for by inherited mutations in *BRCA1 and BRCA2*.Overall, it has been estimated that inherited *BRCA1 and BRCA2* mutations account for 5 to 10 percent of breast cancers and 10 to 15 percent of ovarian cancers among white women in the United States[11].

Genes associated with breast cancer:

***BRCA-1* (Breast Cancer Gene 1)**

This gene helps to repair the damaged DNA or destroy the cells if DNA cannot repair. It Interacts with other proteins including tumor suppressors and regulates the cell division. It normally produces the protein that protects from cancer[12].Damage of the *BRCA 1* due to mutation or hereditary factors leads to increase the risk of breast cancer (figure-3)

***BRCA 2* (Breast Cancer Gene -2)**

BRCA-2 appears to be a cancer-causing gene when altered. *BRCA-2* appears to account for as many cases of breast cancer as does *BRCA-1.BRCA-2* apparently triggers breast cancer in males as well as in females.

Diagnostic methods for breast cancer:

In men breast cancer can be detected by Monthly self-examination of the breast, annual clinician breast exam, annual mammography or adhering to population screening guidelines for prostate cancer [13].

GENETIC TESTING:

DNA isolation and PCR amplification for the different exons:

Blood samples (3 ml each) were collected from the Genomic DNA was extracted from peripheral blood lymphocytes. Universal primers were used to amplify four regions of the *BRCA1* gene (exons 2, 8, 13 and 22) and one region of *BRCA2* gene (exon 9)[14].Then the Mutation detected by using the following techniques like Single strand conformation polymorphism assay (SSCP) and heteroduplex analysis.

Single-strand conformation polymorphism (**SSCP**):

Single-strand chain polymorphism, is defined as conformational difference of single-stranded nucleotide sequences of identical length as induced by differences in the sequences under certain experimental conditions[15].This property allows sequences to be distinguished by means of gel electrophoresis, which separates fragments according to their different conformations(Figure-4).

Heteroduplex Analysis:

A heteroduplex analysis is a double-stranded (duplex) molecule of nucleic acid originated through the genetic recombination of single complementary strands derived from different sources, such as from different homologous chromosomes or even from different organisms [16].One such example is the heteroduplex DNA strand formed in hybridization processes, usually for biochemistry-based phylogenetical analyses(figure-5).

Treatment and prevention methods:

The prevention methods include increased surveillance, lifestyle changes, chemoprevention and prophylactic surgery [17].In surveillance *BRCA* analysis test has to be done or clinical breast examination every six months or mammograms or magnetic resonance imaging once in a year has to be done[18].Surveillance for ovarian cancer includes having semiannual pelvic exams, transvaginal ultrasound imaging and blood tests to measure your cancer antigen 125 levels. The lifestyle changes include having first child at a younger age,having more children than average and Breastfeeding for more than one year decreases the risk of breast cancer for an average-risk woman.The prevention

methods also includes chemoprevention methods which includes use of the drug called tamoxifen[19].

CHEMOPREVENTION:

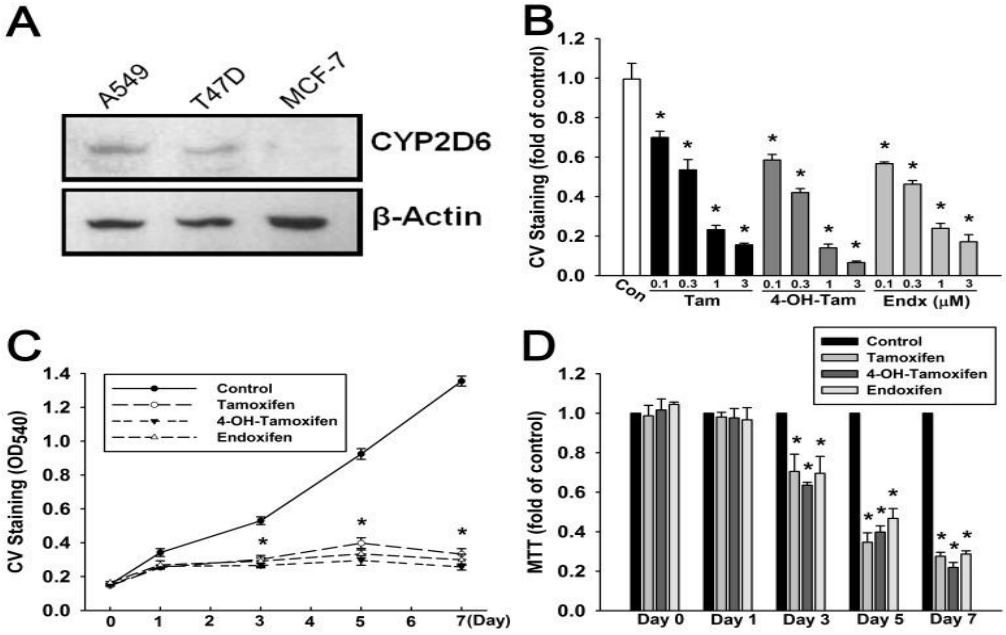
Tamoxifen:

Tamoxifen is used as a chemoprevention agent in the treatment of high-risk cancer. Data from three studies suggest that tamoxifen may be able to help in lowering the risk of breast cancer in *BRCA1 and BRCA2* mutation carriers. Two of these studies examined the effectiveness of tamoxifen in helping to reduce the development of cancer in the opposite breast of women undergoing treatment for an initial breast cancer [20].

Tamoxifen is a selective estrogen receptor modulator (SERM) and as a partial agonist or antagonist it binds to ER. Tamoxifen has been used for endocrine therapy for the treatment of early and advanced breast cancer in pre and post menopause women. Some of the side effects of Tamoxifen such as hot flushes and sweats, fatigue, painful joints and mood changes may sometimes lead to the discontinuation of the treatment [21,22]. These symptoms are relieved by serotonin reuptake inhibitors (SSRIs). Tamoxifen is metabolized in the liver by CYP2D6 isoenzyme. Tamoxifen metabolism is decreased by the inhibition of CYP2D6 isoenzyme [23,24] which in turn adversely affects breast cancer treatment. Evidence shows that coadministration of fluoxetine decreases the plasma concentration of tamoxifen. Tamoxifen exerts its cytotoxic effect through cytostatic rather than cytocydal. Tamoxifen induces growth inhibition through accumulation of cells in the G1 phase of the cell cycle. Cytostasis, induced by cell cycle arrest, is a condition that is poorly tolerated by any cell and must either be escaped or resolved by cellular death.

Tamoxifen exerts cytotoxic effect while Risperidone does not affect cell viability in T47D breast cancer cells

To prove that T47D human breast cancer is suitable for the study, several human breast cancer cells have been examined for whether they express CYP2D6 enzyme [Figure 1A]. A549 human lung cancer cell line has been used as a positive control and it shows prominent CYP2D6 protein expression. Significant amount of CYP2D6 protein was present in human breast cancer cells while MCF-7 were not expressed with any detectable protein. Hence, T47D has been used as the main material in the experiment. Cytotoxic effect has been exhibited in a low dosage range (0.1-3 µM) [Figure 1B]by 4-OH- Tamoxifen and Endoxifen showing that T47D cells might metabolize Tamoxifen to active metabolites. Tamoxifen showed its cytotoxicity after 3 days as a cytostatic agent[Figure 1C and 1D].



T47D human breast cancer cells exhibit tamoxifen-induced cytotoxic effect dose- and time-dependently.

(A) T47D human breast cancer cells but not MCF-7 cells expressed significant amount of CYP2D6 protein. A549 human lung cancer was loaded as positive control which shows prominent protein expression. (**B**) Cells were treated with tamoxifen, 4-OH-tamoxifen, and endoxifen (0.1–3 µM) for 7 days. Cell viability of T47D cells was examined by crystal violet (CV) staining (**C, D**) Tamoxifen (1 µM), 4-OH-tamoxifen (1 µM), and endoxifen (1 µM) markedly inhibited cell viability from Day3 to Day7 in T47D cells, measured by both crystal violet (CV) staining and MTT assay. Graphs show mean ± S.E.M. of at least three independent experiments. *****, $p<0.05$ to control group; t-test. Tam, tamoxifen; 4-OHTam, 4-hydroxy-tamoxifen.

Sodium selenite:

Sodium Selenite has been the spotlight of recent research into the role of protein phosphatases as dephosphorylating agents that limit pro-angiogenic signaling molecules.

The role of angiogenesis as a catalyst in the growth and spread of malignant tumors has been a topic of extensive study. Decades of research have proven that protein kinases play a key role in angiogenetic activity [25]. These kinases exhibit reversible phosphorylation that controls angiogenesis much like a switch. As a result, a variety of antibodies and small molecule kinase inhibitors have been developed to target pro-angiogenic signaling.

New research is shifting focus and investigating protein phosphatases that subsequently dephosphorylate the protein kinases. One intracellular phosphatase of particular interest is PP2A.PP2A has been shown to negatively regulate proteins key to angiogenic signaling. Particularly, PP2A is highly disruptive to the P13K/Akt and MAPK pathways. Research has proven that sodium selenite is an ideal stimulator of PP2A that shows significant effectiveness is inhibiting angiogenetic signaling.

Pharmacological inhibitors of the enzyme poly ADP ribose polymerase (PARP):

DNA is damaged thousands of times during each cell cycle and that damage must be repaired.

BRCA1, BRCA2 and PALB2 are proteins that are important for the repair of double-strand DNA breaks by the error-free homologous recombinational repair, or HRR, pathway. When the gene for either protein is mutated, the change can lead to errors in DNA repair that can eventually cause breast cancer. When subjected to enough damage at one time, the altered gene can cause the death of the cells [26].

PARP1 is a protein that is important for repairing single-strand breaks ('nicks' in the DNA). If such nicks persist unrepaired until DNA is replicated (which must precede cell division), then the replication itself can cause double strand breaks to form.

Pegylated liposomal doxorubicin:

This is more effective against BRCA-mutated ovarian cancer patients. Doxorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication.

NATURAL CHEMOPREVENTION:

Cucurbitacins:

These are tetracyclic triterpenes isolated from plants that inhibit BRCA1 overexpressed breast cancer cells. Cucurbitacin is any of a class of biochemical compounds that some plants — notably members of the family Cucurbitaceae, that includes the common pumpkins and gourds — developed in order to defend themselves from herbivores. Cucurbitacins are chemically classified as steroids, formally derived from cucurbitane, a triterpene hydrocarbon — specifically, from the unsaturated variant cucurbita-5-ene, or 19-(10→9β)-abeo-10α-lanost-5-ene. They often occur as glycosides. Cucurbitacins are generally cytotoxic and poisonous to some animals, and some of them are among the bitterest tastes to humans[27].

Withaferin A (WA):

Withaferin A is a steroidal lactone which binds to and inhibits vimentin. It was first isolated from Winter cherry (Withania somnifera)and is the first member of the withanolides to be discovered. Withaferin A is a potent inhibitor of angiogenesis. Withaferin A inhibits both NF-κB and Sp1 Transcription factor activity. Withaferin A also down regulates VEGF gene expression. Withaferin A can affect calcium signaling [28].

Prophylactic surgery:

Prophylactic mastectomy and oophorectomy are two controversial procedures. In that Bilateral prophylactic mastectomy should reduce the Breast Cancer risk by at least 90%(figure.6).Salpigo-oophorectomy upon completion of childbearing should reduce the Breast Cancer risk by at most 50% and it Should reduce the Ovarian Cancer risk by at least 95%[29].

Finally for protecting ourselves from getting cancer certain food habits can be followed,they include taking brussel sprouts regularly in our diet,taking broccoli with garlic,including coffee in our diet and doing regular exercise are some of the simple preventive measures that can be adopted in our day to day life(figure.7).

Physical Activity: Numerous studies have examined the relationship between physical activity and breast cancer risk, and most of these studies have shown that physical activity, especially strenuous physical activity, is associated with reduced risk [30]. This decrease in risk appears to be more pronounced in premenopausal women and women with lower-than-normal body weight(figure.8).

Alcohol: There is substantial evidence that alcohol consumption is associated with increased breast cancer risk. However, it is uncertain whether reducing alcohol consumption would decrease breast cancer risk.

Dietary Fat: Although early studies suggested a possible association between a high-fat diet and increased breast cancer risk, more recent studies have been inconclusive. In the WHI, a low-fat diet did not help reduce breast cancer risk(figure.9,10).

CONCLUSION:

In this article we have seen in detail about the *BRCA* mutation, Risk factors of *BRCA* gene mutations, Genetic features of *BRCA* mutation, Methods of diagnosis, Treatment and Prevention procedures. Research studies are being conducted to find newer and better ways of detecting, treating, and preventing cancer in *BRCA1* and *BRCA2* mutation carriers. Additional studies are focused on improving genetic counseling methods and outcomes. Our knowledge in these areas is evolving rapidly. We have already tested these diagnostic methods and treatment procedures in the *BRCA* mutated ovarian cancer patients. In future we will prove the efficiency of this diagnostic procedures and treatment methods in Breast cancer patients.

Conflicts of interest:

The author declares that there are no conflicts of interest.

Financial support and sponsorship: NIL

Acknowledgement:

I sincerely acknowledge my parents and daughter for their help and support althrough the preparation of this article.

REFERENCES:

[1] Jeffrey N. Weitzel, Veronica I. Lagos, Carey A. Cullinane, Patricia J. Gambol, Julie O. Culver, Kathleen R. Blazer, Melanie R. Palomares, Katrina J. Lowstuter, Deborah J. MacDonald Limited Family(2007):Structure and BRCA Gene Mutation Status in Single Cases of Breast Cancer.Journal of the American Medical Association 297(23): 2587–2595.

[2] Kadouri L, Hubert A, Rotenberg Y, et al. (2007):Cancer risks in carriers of the BRCA1/2 Ashkenazi founder mutations.Journal of Medical Genetics 44(7):467–471

[3] Thompson D, Easton DF, (2002):the Breast Cancer Linkage Consortium.Cancer incidence in BRCA1 mutation carriers.Journal of the National Cancer Institute 94(18):1358–1365

[4] The Breast Cancer Linkage Consortium.Cancer risks in BRCA2 mutation carriers.(1999):journal of the National Cancer Institute 91(15):1310–1316

[5] Yoshida K, Miki Y(2004):Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage.Cancer science 95 (11): 866–71

[6] Friedenson B(2005):BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian 7 (2): 60.

[7] John EM, Miron A, Gong G, et al.(2007):Prevalence of pathogenic BRCA1 mutation carriers in 5 U.S. racial/ethnic groups.Journal of the American Medical Association 298(24):2869–2876

[8] Moslehi, R; Singh, R; Lessner, L; Friedman, JM(2010):Impact of BRCA mutations on female fertility and offspring sex ratio.Am J Hum Biol.22 (2): 201–5

[9] Kotsopoulos J, Lubisnki J, Salmena L, et al(2012): Breastfeeding and the Risk of Breast Cancer in BRCA1 and BRCA2 Mutation Carriers.Breast Cancer Res14 (2): R42

[10] Lim K, Naviaux R, Haas R. (2007): Quantitative DNA mutation analysis by DHPLC.Clin Chem 1046-1052

[11] Palais R, Liew M, Wittwer CT (2005): Quantitative heteroduplex analysis for single nucleotide polymorphism genotyping.Anal Biochem 346:167-175

[12] Verhoog, L. C.; Brekelmans, C. T.; Seynaeve, C.; Van Den Bosch, L. M.; Dahmen, G.; Van Geel, A. N.; Tilanus-Linthorst, M. M.; Bartels, C. C. et al..(1998):Survival and tumour characteristics of breast-cancer patients with germline mutations of BRCA1.Lancet 351 (9099): 316–321

[13] Kurian, AW; Sigal, BM; Plevritis, SK.(Jan 2010): Survival analysis of cancer risk reduction strategies for BRCA1/2 mutation carriers. J Clin Oncol 28 (2): 222–31

[14] Pijpe, A.; Andrieu, N.; Easton, D. F.; Kesminiene, A.; Cardis, E.; Nogues, C.; Gauthier-Villars, M.; Lasset, C. et al (2012): Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations: Retrospective cohort study GENE-RAD-RISK BMJ;345: e5660

[15] Lynch, H. T.; Casey, M. J.; Snyder, C. L.; Bewtra, C.; Lynch, J. F.; Butts, M.; Godwin, A. K. (2009):Hereditary ovarian carcinoma: Heterogeneity, molecular genetics, pathology, and management. Molecular Oncology 3 (2): 97–137

[16] Pruthi, S.; Gostout, B. S.; Lindor, N. M. (2010): Identification and Management of Women with BRCA Mutations or Hereditary Predisposition for Breast and Ovarian Cancer. Mayo Clinic Proceedings 85 (12):1111–1120

[17] Kwon, J. S.; Tinker, A.; Pansegrau, G.; McAlpine, J.; Housty, M.; McCullum, M.; Gilks, C. B. (2013):Prophylactic Salpingectomy and Delayed Oophorectomy as an Alternative for BRCA Mutation Carriers. Obstetrics and gynecology 121 (1): 14–24

[18] Stadler, ZK.; Kauff, ND.(2010):Weighing options for cancer risk reduction in carriers of BRCA1 and BRCA2 mutations. J Clin Oncol 28 (2): 189–91

[19] King, MC; Marks, JH; Mandell, JB.(2003):Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2.Science 302 (5645): 643–6

[20] Friedenson B.(2007):The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. BMC Cancer 7: 15

[21] Jordan VC(2006): Optimising endocrine approaches for the chemoprevention of breast cancer beyond the Study of Tamoxifen and Raloxifene (STAR) trial. Eur J Cancer 42:2909–2913.

[22] Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, et al.(2006):Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial JAMA.:295: 2727–2741.

[23] Jin Y, Desta Z, Stearns V, Ward B, Ho H, et al. (2005): CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 97: 30–39.

[24] Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, et al. (2003):Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 95: 1758–1764.

[25] Pal T, Keefe D, Sun P, Narod SA(2010): Fertility in women with BRCA mutations: a case-control study. Fertil. Steril 93 (6): 1805–8

[26] Oktay, K; Kim, JY; Barad, D; Babayev, SN.(2010): Association of BRCA1 mutations with occult primary ovarian insufficiency: a possible explanation for the link between infertility and breast/ovarian cancer risks.J Clin Oncol 28 (2): 240–4

[27] Moslehi, R; Singh, R; Lessner, L; Friedman, JM. (2010): Impact of BRCA mutations on female fertility and offspring sex ratio. Am J Hum Biol 22 (2): 201–5

[28] Kotsopoulos J, Lubisnki J, Salmena L, et al. (2012): Breastfeeding and the Risk of Breast Cancer in BRCA1 and BRCA2 Mutation Carriers. Breast Cancer Res 14 (2): R42

[29] Cochran, G.; Hardy, J.; Harpending, H.(2006): Natural history of Ashkenazi intelligence. Journal of biosocial science 38 (5): 659–693

[30] Robert Cook-Deegan, MD et al(2010): Impact of Gene Patents and Licensing Practices on Access to Genetic Testing for Inherited Susceptibility to Cancer: Comparing Breast and Ovarian Cancers to Colon Cancers: Patents and Licensing for Breast, Ovarian and Colon Cancer Testing.Genet Med 12(4 Suppl): S15–S38