

BRCA1 MUTATION – TESTING AND TREATMENT

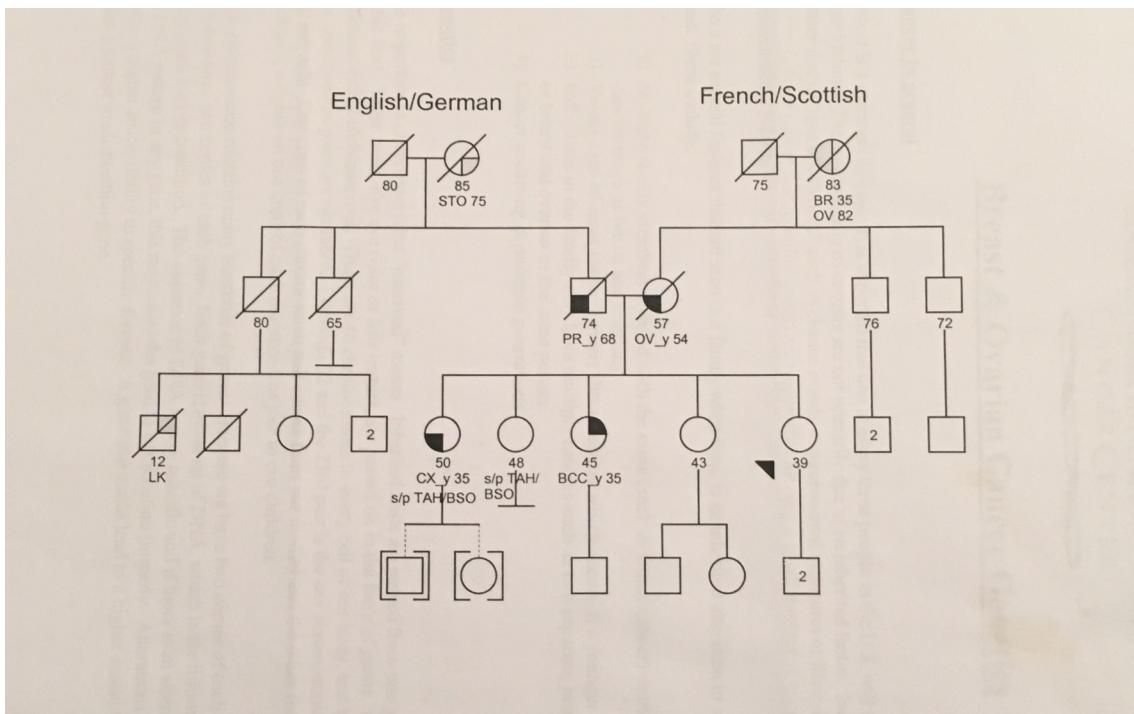


Figure 1: The pedigree above depicts the family history of the Wells family, from two generations prior to the proband, the subject of the pedigree, to one generation ahead. In this case, the proband is Nancy Montanaro. The dashes indicate a deceased family member, and every quarter of the shape filled in with black symbolizes one cancer. Circles and squares indicate females and males respectively. (1)

Michael Montanaro

4/11/17

**AP Biology: Wachtmeister
B Block**

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1. INTRODUCTION

1.1. GENERAL BACKGROUND

On the 21st of February, 2017, Dr. Leif W. Ellisen, professor of medicine at Harvard, prepared for his 8:00 A.M. appointment with one of his patients. Ellisen's laboratory specializes in identifying genetic abnormalities, and practicing genetic counseling. The medical reasoning for this visit was to update Nancy Montanaro on whether or not any of the NCCN Guidelines affected her course of action regarding the monitoring for breast cancer, and the management of the additional risks involved with a BRCA1 gene mutation. In addition to the medical reasoning, Dr. Ellisen kindly stayed behind for over thirty minutes after the appointment in order to allow himself to be interviewed for this paper, answering questions regarding the testing, management, and various treatments that are necessary for individuals carrying or with a high probability of having a mutated BRCA1 gene.

Following the discovery of the chromosome in the late 1800s, the field of genetics spread rapidly, and more information was unearthed with increasing frequency with one piece of data leading to another and so forth. In the early 1900s, scientists linked inherited diseases to chromosomes, opening the door to a whole new field of science: genetic testing. However, this new field did not fully begin until the 1950s when discoveries allowed scientists to develop genetic tests for certain conditions already linked to genetic disorders, such as Down syndrome, cystic fibrosis, Duchenne muscular dystrophy, and phenylketonuria. Nowadays, there are over 2000 conditions for which genetic testing is available. One of the most common reasons for genetic testing today is the BRCA1 gene. (2)

1.2 BRCA1 MUTATION STATISTICS

Testing for the BRCA1 gene searches for known mutations in the nucleotide sequence,

Figure 2 shows the location of the BRCA1 gene on chromosome 17, which shows just how specific these searches must be. The reasoning for the plethora of testing done on individuals suspected of having a mutated BRCA1 gene is due to the inconceivable statistics regarding the relationship between the BRCA1 gene and breast and ovarian cancer. Concerning breast cancer, a mutated BRCA1 gene accounts for 20-25% of breast cancers caused by inherited genes and around 5-10% of breast cancers regardless of cause. These statistics seem improbable seeing as the BRCA1 is just one gene; however, the mutated gene raises the chances of getting breast cancer in women by almost a percentage of 50, going from a 12% chance in the average woman to a 55-65% chance by the age of 70 in women with a mutation in the gene. The data regarding the effects on ovarian cancer are no less grim with the mutation causing around 15% of all ovarian cancers; moreover, the chances for getting ovarian cancer are increased astronomically. Most mutations in the BRCA1 gene increase the chances by almost 40%. What is normally a 1.3% chance in the average woman increases to a 39% chance in women with a BRCA1 mutation. Many BRCA1 gene mutations are also affiliated with an augmented risk for colon, pancreatic, gastric, and fallopian tube cancer; additionally, recent studies have shown a possible correlation between a mutated BRCA1 gene and an increased chance for prostate cancer; however, currently there is not enough data to completely confirm this hypothesis, which leaves only the BRCA2 gene increasing the risk for prostate cancer for now. Patients with a mutated BRCA1 gene are 2 times more likely to get colon cancer, 2-3 times more likely to get pancreatic cancer, and 3-7 times more likely to get gastric cancer. (3, 4)

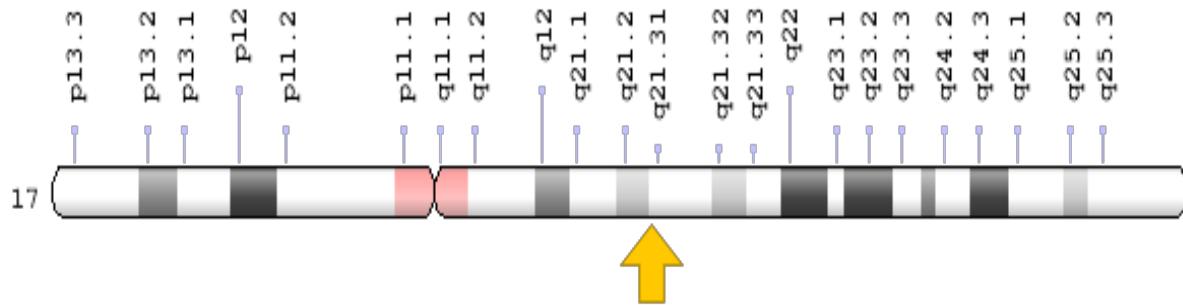


Figure 2: BRCA1 gene is located on the cytogenetic location 17q21.31, which is the long-arm of chromosome 17 at position 21.31. (5)

1.3 ANTI-ONCOGENE AND BRCA1/2 INFORMATION

Before further examining the BRCA1 gene mutation, each aspect that makes up the Breast Cancer 1 gene must be broken down and understood, starting with a gene. Specifically, a gene is a sequence of nucleotides used to translate mRNA which is then used to transcribe a protein that carries out a specific function in the body. In summary, a gene makes a protein do a certain job in the body. The BRCA1 gene is part of a group of genes called anti-oncogenes, which are more commonly known as tumor suppressor genes, and every somatic cell in the human body has a pair of these genes, one from the father and one from the mother. These anti-oncogenes are used to create a growth-inhibiting protein which protects the cell from the uncontrolled growth that causes cancer. The tumor suppressor genes can also repair DNA mistakes, manage the adhesion of cells to the extracellular matrix or to one another, or trigger apoptosis, programmed cell death. Without these growth-inhibiting proteins, cells would be able to multiply unchecked creating a tumor and leading to cancer. Anti-oncogenes, such as BRCA1, are one of the many protectors that the human body has to prevent cancer; however, with these tumor suppressor genes being one of the most important guards, if they are incapable of doing their jobs, the chances for cancer to arise significantly increase due to the fact that, since one of

the two genes in the pair would be mutated, the chances of getting a mutation in the other gene of the pair is far greater than the chances of getting a mutation in both the genes in one cell, depicted in **Figure 3.** (6)

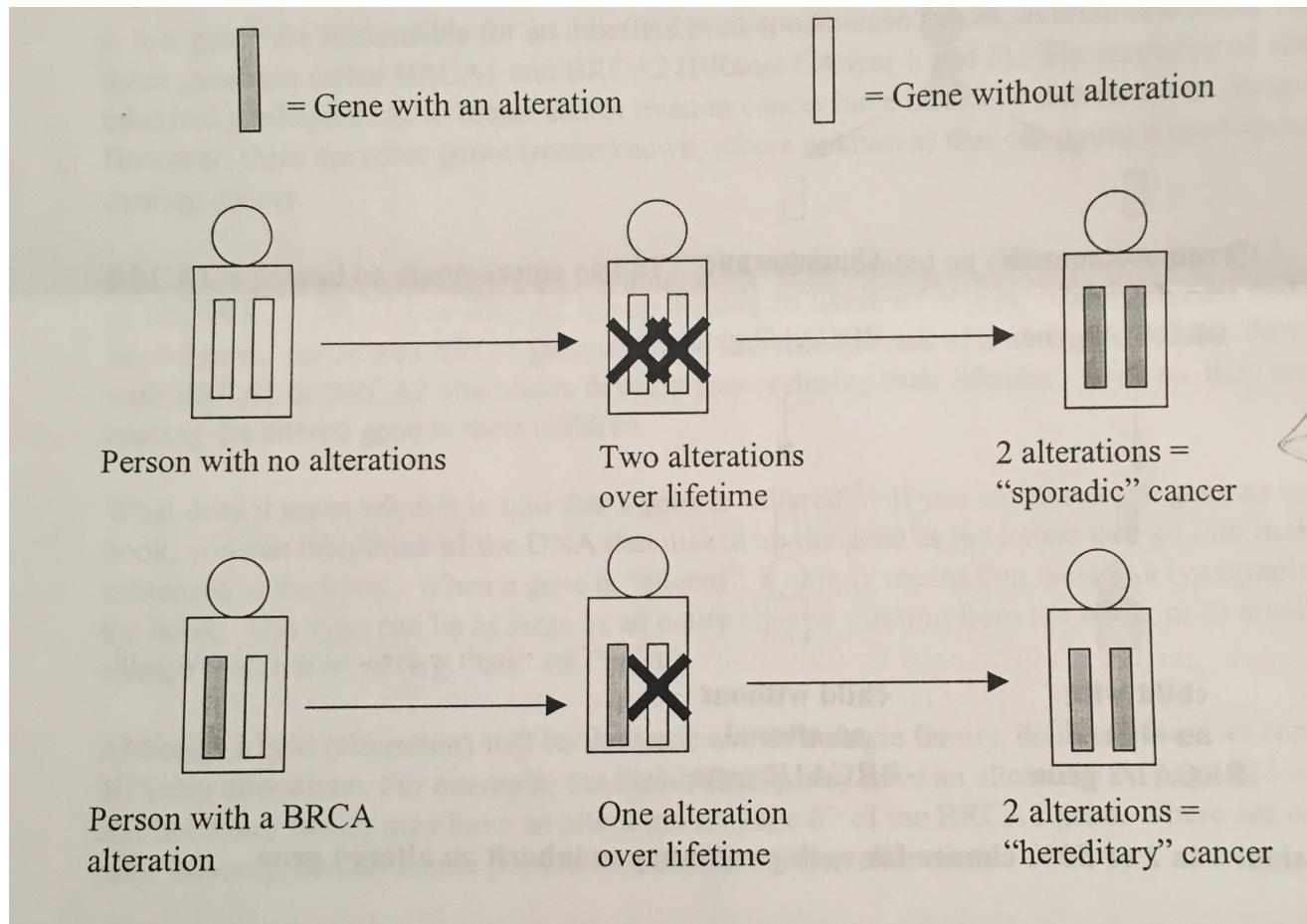


Figure 3: Visual explanation of why the BRCA1 mutation increases the patients risk for cancer. The “X” indicates any variations caused over a lifetime, the black bars indicate a gene with an alteration, and the white bars indicate a gene without a mutation. (1)

Two such anti-oncogenes, that have the greatest negative effect if either gene was to become mutated, are the BRCA1 and BRCA2 genes. Each gene uses more than 20 exons to encode proteins of 1,863, the BRCA1 protein, and 3,350, the BRCA2 protein, amino acids. The only known gene that bears homology with either the BRCA1 or BRCA2 gene is the BRCT, whose domain is in the C-terminus of the BRCA1 gene and will be further analyzed in a later

paragraph. The BRCA1/2 genes are known to mend damaged DNA; however, recent studies have shown that transcription regulation, cell-cycle control, and development may also be roles of the BRCA1 gene. The proteins made by both genes are nuclear proteins, meaning the proteins are found in the nuclei of cells, and the levels of the protein made by the BRCA1 gene are frequently altered by variations in hormone levels; moreover, mutations in these genes have almost always lead to the truncation of their proteins; therefore, scientists believe that the cancer grows when the second copy is lost. Additionally, the rampancy of certain alterations in the BRCA1 and BRCA2 genes varies immensely in certain populations due to the “founder effect” in many subpopulations. The most prevalently known of these subpopulations are those of Ashkenazi Jewish descent, with 90% of the mutations found in members of this group due to two specific mutations in the BRCA1 gene, 185delAG and 5382insC, or to 6174delT in the BRCA2 gene. The lesser known subpopulations with a plethora of BRCA1 or BRCA2 mutations have been found in Russia, France, Belgium, Iceland, Finland, Holland, Sweden, Hungary, Denmark, and Norway; however, most of what is currently known regarding these two genes comes from studies of white citizens in the United States and Europe, meaning there is a lack of data regarding the rates of the mutations in other ethnic or racial backgrounds. (4)

1.4 MUTATIONS

The permanent inactivation of anti-oncogenes occurs due to the most unpredictable aspect of genetics, mutations. These changes in the structure of a gene can be caused by many different factors, which are initiated due to various scenarios including changes and mutagens. The various types of mutations that may change the structure of tumor suppressor genes include missense and nonsense mutations, depicted in **Figures 4 and 5** respectively. Missense mutations

eventuate as a result of one amino acid substituting for another, causing the protein made by the gene to change; however, nonsense mutations do not cause the change of one protein to another, the substitution of amino acids in nonsense mutations creates a stop codon, signaling the cell to stop building the protein. Substitutions are not the only way a mutation can occur, insertions, deletions, and duplications make up the remaining ways a gene can become mutated.

Duplications occur when a piece of DNA is copied one or more times abnormally; however, if a specific sequence of DNA is repeated a number of times in a row, the mutation is known as a repeat expansion. Simple insertions, the addition of a piece of DNA, and deletions, the deletion of a piece of DNA, can also cause a gene to become mutated; moreover, if these additions or deletions cause a change in the gene's reading frame, groupings of three nucleotide bases, the mutation is referred to as a frameshift mutation, portrayed in **Figure 6**. Depending on what genes are affected by these mutations and which cells in the body are affected, these unpredictable mutations could be disastrous for future familial generations. Germ cells are any cell holding half the number of chromosomes of a somatic cell and are able to amalgamate with one from the opposite sex to form a gamete, these cells are subject to germline mutations, which are any heritable and identifiable variation in the lineage of germ cells. These mutations have the ability to be transmitted to offspring, causing that individual to also contain the mutation; however, there is only a 50% chance that the mutation will be transferred to the offspring if the germ cell from the opposite sex does not contain the same mutation. These germline mutations have caused many mutated genes to be passed down for centuries, some completely harmless while others are possibly fatal. Although extremely rare in BRCA1 mutations, the spontaneous mutations, discussed above, in the germ cells of the human body do occur, as was the case in the first ever BRCA1 mutation. Once the germline mutation of the BRCA1 gene occurred, the

carrier had a 50% chance to pass it down to his/her offspring, starting the lineage of the familial mutation. The combination of rare spontaneous mutations and the passed down mutations, initially caused by these spontaneous variations, make up the plethora of mutant BRCA1 gene patients in the world today, in fact this duo combines to be 100% of the causes for every gene variant in history. (7)

Missense mutation

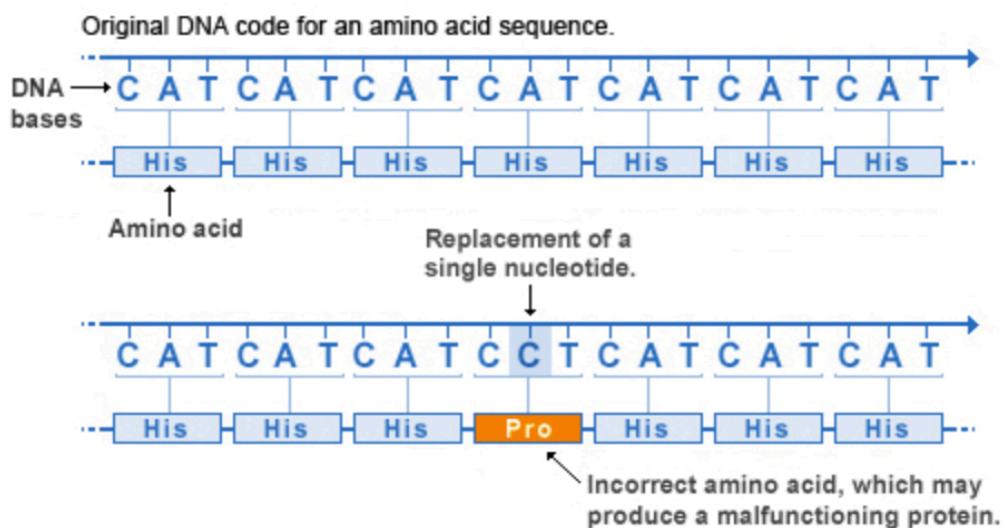


Figure 4: Further explanation of missense mutations and a visual representation of how they occur in amino acid sequence. (8)

Nonsense mutation

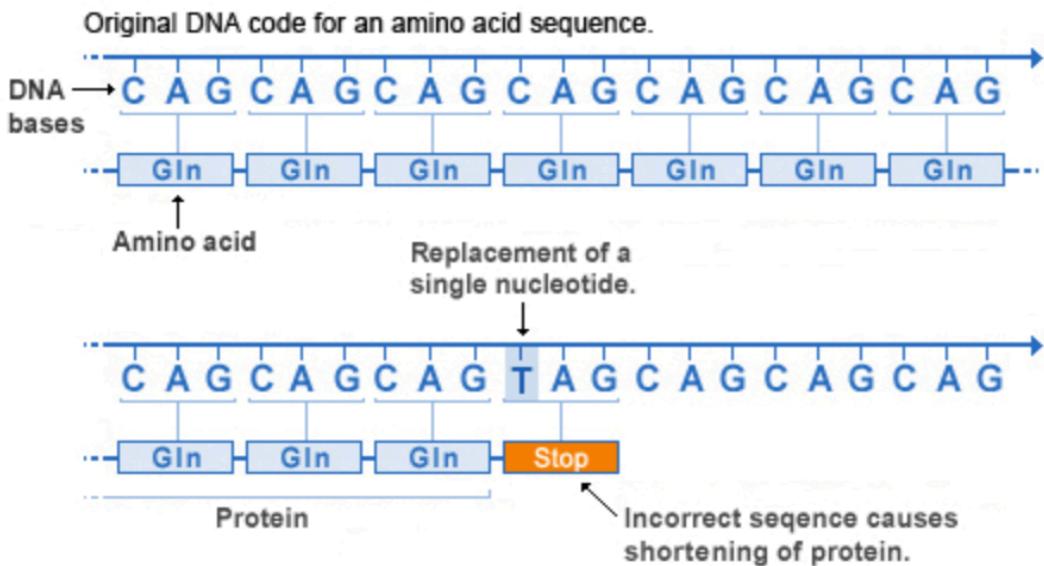


Figure 5: Further explanation of nonsense mutations and a visual representation of how they occur in amino acid sequence. (8)

Frameshift mutation

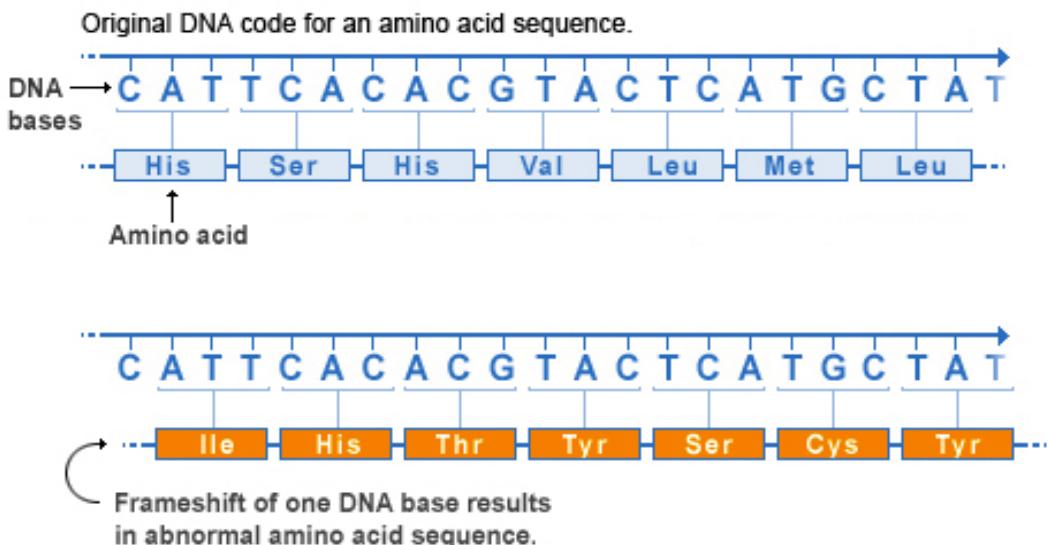


Figure 6: Further explanation of frameshift mutations and a visual representation of how they occur in amino acid sequence. (8)

1.5 COMMON TESTABLE MUTANT ANTI-ONCOGENES

Following the advancements in genetic testing, scientists have been able to formulate a list of breast and/or ovarian causing mutant tumor suppressor genes; however, the testing guidelines are primarily focused on assessments of mutations in the BRCA1/2, TP53, and PTEN genes. Mutations in either the BRCA1 or BRCA2 gene cause the individual to have hereditary breast and ovarian cancer syndrome; moreover, variations in the TP53 gene cause Li-Fraumeni syndrome. Lifetime risk for someone with this syndrome to productize to any type of cancer is 90%, and 50% of these cancers will be diagnosed before age 30. Cowden syndrome, also known as PTEN hamartoma tumor syndrome, is caused by a mutant PTEN gene and increases the lifetime risk for a woman to productize many types of cancer. The risk for breast cancer is around 50-85%, thyroid cancer is between 30-40%, kidney cancer is in the range of 30-35%, and endometrial cancer is around 25-30%. These three mutant genes are the most well-known and increase the likelihood of obtaining breast and ovarian cancer the most; however, there are many other genes that are on the list of testable mutant tumor suppressor genes. (9, 10)

Focusing primarily on those genes that increase the risk for breast and/or ovarian cancer, ATM (ataxia-telangiectasia mutated) holds the most fame in this lesser known mutant tumor suppressor gene list, with a 38% lifetime risk of developing breast cancer, not only is this risk bordering the percentages of PTEN, BRCA1/2, and TP53, but ATM is also associated with the autosomal recessive condition ataxia telangiectasia, which is characterized by escalating difficulty with coordinating physical movements starting in early childhood, the condition also affects the nervous, immune, and other body systems. If the ATM gene is functioning properly, its protein product works together with a protein complex made of the products of the genes: MRE11A, RAD50, and NBN. The ATM protein recognizes the broken strands of DNA and then

coordinates their repair by sending the MRE11A/RAD50/NBN protein complex to repair the strand. The NBN gene that is associated with this process is also a member of the testable mutant anti-oncogenes that augment the risk for breast and/or ovarian cancer. The research for this gene mutation is still young; hence, no exact cancer risk are known. Scientists have been able to link the NBN mutation to an increased risk for breast cancer in women and to an enlarged risk for prostate cancer in men, yet, as stated previously, the exact data is unknown at this epoch. (11, 12, 13)

One anti-oncogene closely related to the TP53 gene and included in the list for testable mutant anti-oncogenes that enlarges the risk for breast and/or ovarian cancer is the CHEK2 gene. When a DNA becomes damaged, the protein product of this gene interacts with tumor protein 53, the protein product of the TP53 gene, in order to halt cell division and determine if the cell will repair the damaged DNA or if apoptosis must be triggered. Mutations in the CHEK2 gene increase the lifetime risk of productizing breast cancer to 28-37%, and the gene was detected in 5% of BRCA-negative patients with breast cancer who have a strong family history of either breast or ovarian cancer. The BRIP1 gene is yet another gene on the list that aids in the job of another on the same list, the BRCA1 gene. The protein complex transcribed using the BRIP1, BRCA1 interaction protein C-terminal helicase 1 gene, helps the BRCA1 protein repair the normal double strand break in DNA. Mutant BRIP1 genes give the individual a 5.8% lifetime risk of developing ovarian cancer by age 80. The BRCA2 gene also receives support from another tumor suppressor gene on the same list as previously stated, the PALB2 gene. The protein encoded by this gene binds to the BRCA2 early onset protein and permits the stable intranuclear localization and accumulation of BRCA2. PALB2 mutations are present in 1-3% of women with breast cancer, and the 10-year survival rate among PALB2 carriers with breast

cancer is only 48% compared to the better known BRCA1 mutation, whose carriers have a 72% 10-year survival rate, and the 75% rate in non-carriers. Additionally, a mutant PALB2 gives a 14% lifetime risk for breast cancer by age 50, and 35% risk by age 70. (13, 14, 15)

Other mutant anti-oncogenes that severely augment the risk of productizing breast cancer include CDH1, an anti-oncogene involved in the control of the adhesion of cells to each other. If this cell is not functioning properly, if it is mutated, the gene becomes associated with HDGC and lobular breast cancer, the lifetime risk for breast cancer is intensified to 39-52%. Some other risk-increasing tumor suppressor genes are MLH1, MSH2, MSH6, PMS2, and EPCAM. Any of these five genes, if mutated, causes the Lynch syndrome, increasing the individual's chances of getting endometrial cancer to 60%, ovarian cancer to 24%, and may also increase their risk for getting breast cancer to 18.6%. The remaining anti-oncogenes that are linked to ovarian cancer are the RAD51C and RAD51D genes, which belong to the RAD51 family that is involved in homologous recombination and DNA repair. Those two mutant genes were present in 1.1% of patients with ovarian cancer, which gives them a pretty significant link to ovarian cancer; however, with the data gathered so far, the RAD51C/D genes do not significantly increase the chances of getting ovarian cancer. (13)

1.6 INCREASED RISK CANCERS

1.6.1 BREAST CANCER

Even with these astronomically high lifetime risk for breast cancer, only around 10% of breast cancers are hereditary; however, with 232,340 female and 2,240 male breast cancers per year in the US alone, the 10% is still unpleasantly high. In fact, breast cancer alone accounts for 16% of all female cancer, as well as 22.9% of all invasive cancers in women. **Figure 7** shows the

different types of breast cancers; moreover, the following statistics are talking about all types of breast cancer. With the advancements in technology and screening for breast cancer, doctors have been able to significantly raise the survival ratings for breast cancer patients. If the cancer is situated solely in the breast, the 5-year survival rate is an astounding 99%, but if the cancer has extended to the regional lymph nodes, the rate drops to 85%. The average rate of survival for a patient during the first five years is 89%, the 10-year rate is 83%, and the 15-year rate is 78%; however, more depressingly, if the cancer has expanded to any distant part of the body, the 5-year rate drops drastically to 26%. Thankfully, 61% of cases are diagnosed when the cancer is found only in the breast, which, as previously stated, gives a 99% 5-year survival rate; however, in 2017, an estimated 40,610 women will die from breast cancer. (16, 17)

Types of Breast Cancer

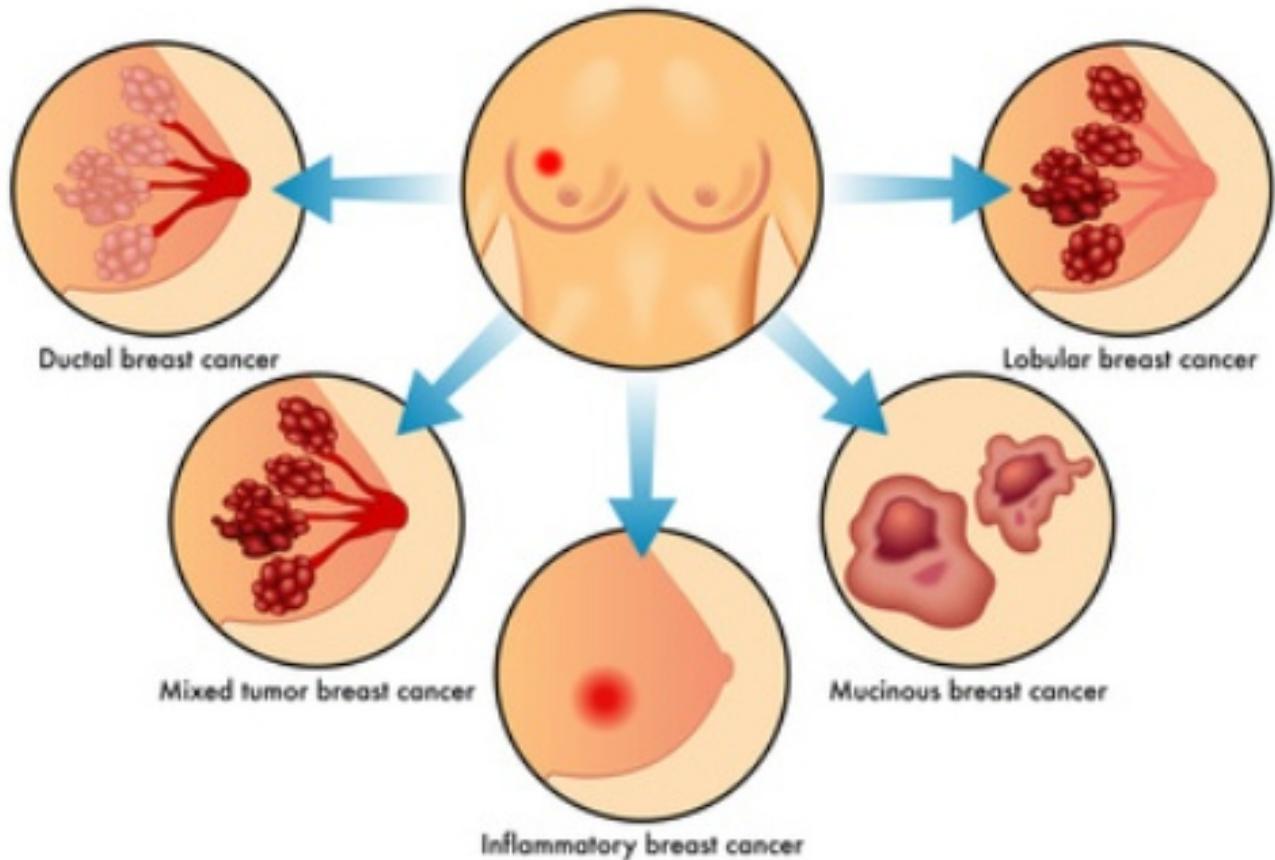


Figure 7: The image depicts the five possible types of breast cancer: ductal, mixed tumor, inflammatory, mucinous, and lobular breast cancer. (18)

1.6.2 OVARIAN CANCER

Although far below the number of individuals diagnosed per year in the US for breast cancer, ovarian cancer is the eighth most common cancer among women in America. Even with this rather low frequency of diagnosis, the cancer has still managed to be the fifth most common cause of cancer deaths in women, showing the lethality of the cancer. In the US alone, ovarian cancer is detected in 22,000 women per year and about 14,000 of these patients will die. All ovarian and fallopian tube cancers have an average rate of survival, during the first five years, of

46%; however, if detected before the cancer has spread outside the ovaries and tubes, the survival rate dramatically increases to 92%, sadly only 15% of women are diagnosed at this stage. The 5-year survival rate if the cancer has expanded to neighboring tissues or organs is 73%; however, if the cancer has stretched out past surrounding organs or tissues to any distant part of the body, the rate decreases to 29%, at this stage, 60% of women are diagnosed. The various stages of ovarian cancer are depicted in **Figure 8**. Depending on the age of the individual, the rate of survival during the first five years of being diagnosed can vary dramatically. In women younger than 45, the average 5-year rate is 77%, yet for women 75 or older, the survival rate drops to 20%. With almost 50% of women diagnosed with ovarian and fallopian tube cancer being 63 years or older, the latter statistic is sadly more common. (19)

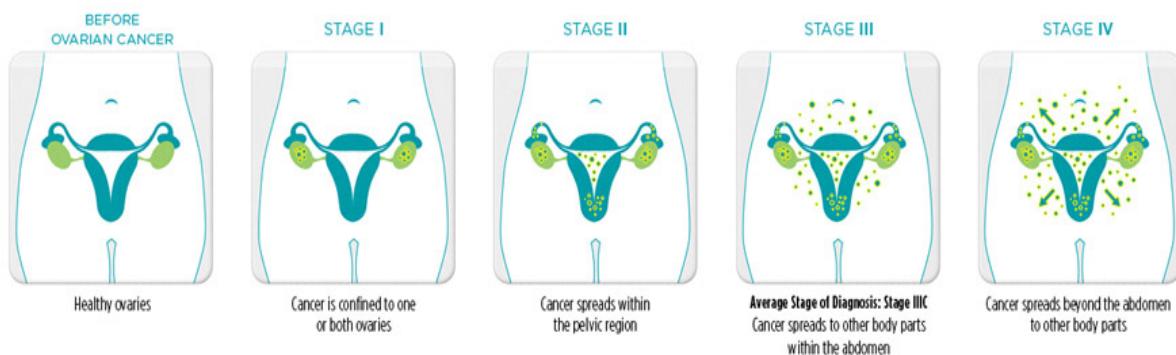


Figure 8: Visual display of the ovaries healthy and at stages I-IV of ovarian cancer. (20)

1.6.3 COLORECTAL CANCER

Additional cancers, other than breast and ovarian cancer, that are at a greater risk for productizing in people with a BRCA1 gene mutation, are stated below; keep in mind, the statistics below are based on patients without a mutated BRCA1 gene. Colorectal cancer will be diagnosed in around 134,490 adults in the United States, 95,270 of which are colon cancer, and the remaining 39,220 cases are with rectal cancer. This inconceivable rate of diagnosis has made

colorectal cancer the fourth most common cancer diagnosed in all adults per year; additionally, this cancer is the third most common in both men and women. The estimated number of fatalities caused by colorectal cancer is no less gruesome, with 26,020 male deaths and 23,170 female deaths adding up to 49,190 total deaths. The 5-year survival rate for these cancers have been steadily increasing since the mid-1980s due to patients being diagnosed earlier and an improvement in treatments. The rate of survival for colorectal cancer during the first five years is 65%, and the average 10-year rate is 58%; however, the rate decreases drastically to 13% if the cancer has expanded to distant parts of the body, this stage is depicted in **Figure 9**. If the cancer is still at the localized stage, the 5-year survival rate is 90%, but if the colorectal cancer has stretched to surrounding regional lymph nodes and/or neighboring tissues or organs, the rate drops to 71%, which is still significantly higher than the average survival rate. (21)

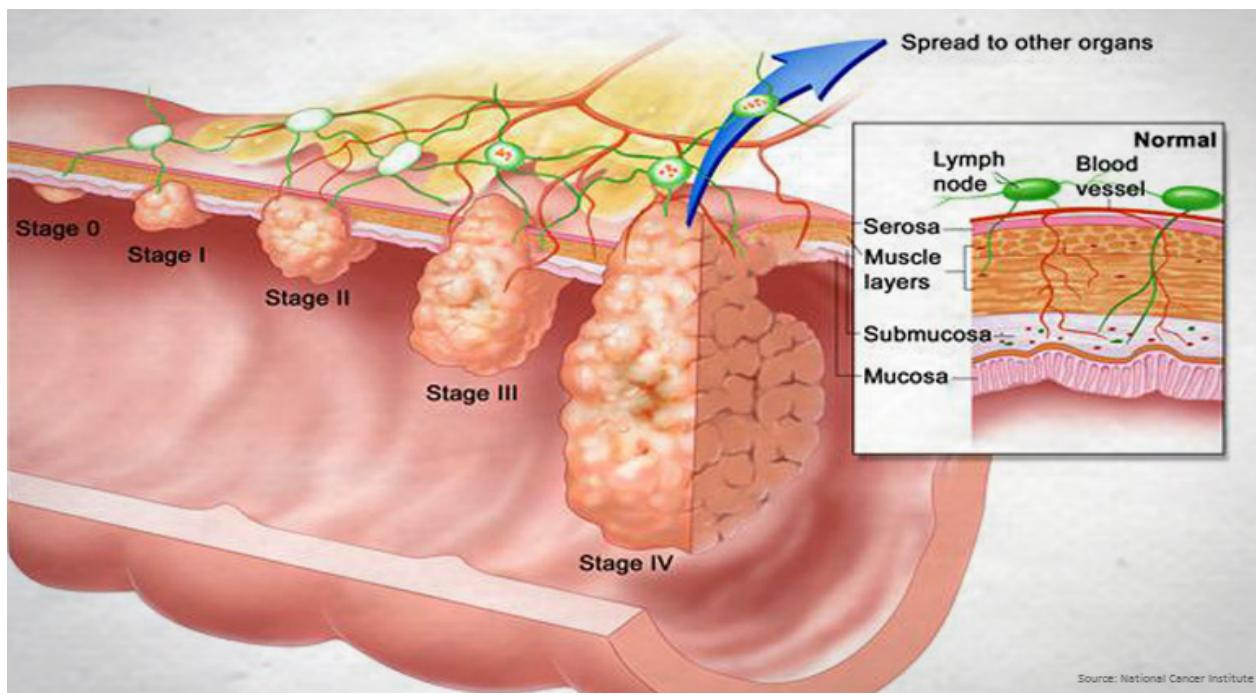
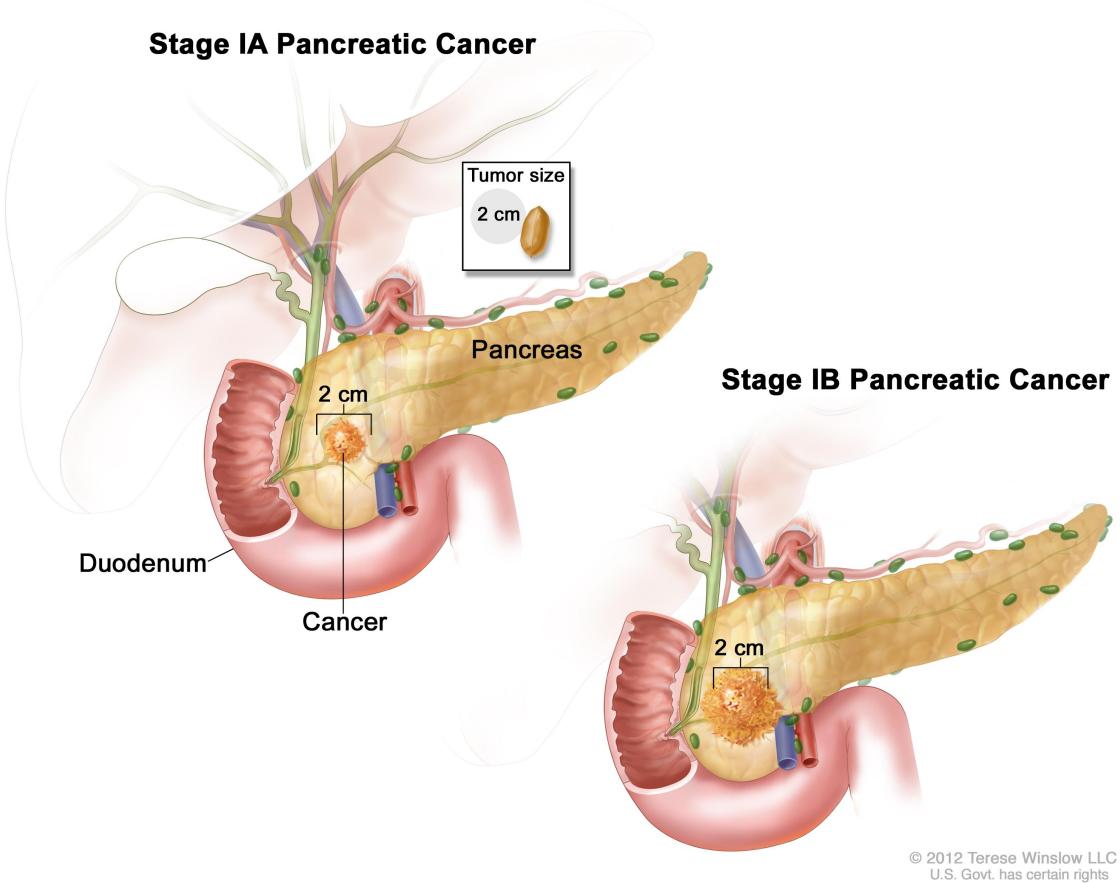


Figure 9: The image depicts the colon cancer at stages 0-IV, and the spread of the cancer to other organs at stage IV. (22)

1.6.4 PANCREATIC CANCER

Although not as prevalent as colorectal cancer, pancreatic cancer is extremely efficient, killing almost the same amount per year as colorectal cancer, but with almost one third of the patients. This year, pancreatic cancer is estimated to take the lives of 43,090, of which 22,300 are men and the remaining 20,790 are women; additionally, 53,670 adults will be diagnosed with the cancer in America alone, making the number the death rate just below the rate of diagnosis. Pancreatic cancer's deadly efficiency makes it the fourth leading cause of cancer fatalities in women and men, as well as the ninth most common cancer in women. The 5-year survival rates show why this cancer kills almost as many patients as is diagnosed, starting with the average survival rate being only 8%; however, detection at the earliest stage increases the survival rate to 29%, but this is only because doctors are able to surgically remove the tumor at these early stages, shown in **Figure 10**. Although the 29% survival rate may seem optimistic, the cancer is only diagnosed this early 9% of the time, leaving the rest of the patients at a stage where the cancer has either extended to neighboring tissues or organs and/or regional lymph nodes, or to any distant portions of the body. The former situation brings with it a rate of survival, in the first five years, of 11%, but the combination of the earliest stages and this stage still does not make up the majority of the stages at which patients are diagnosed. The most advanced stage, when the cancer has expanded to a distant portion of the body, is also the point at which more than half the patients are diagnosed, and with a fatality rate of 97% within the first five years of being diagnosed, this stage is also the deadliest. (23)



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Figure 10: Image displays the growth of the pancreatic tumor from stage IA to IB. (24)

1.6.5 GASTRIC CANCER

Although the yearly rate of diagnosis in America pales in comparison to cancers previously described, gastric cancer is still the fifth most prevalent cancer in the world, with 952,000 new worldwide cases during the year 2012; additionally, the cancer is almost two times more likely to develop in men than in women. The rate of diagnosis in America is around 26,370 people diagnosed per year, 16,480 of which are men, and the remaining 9,890 are women. Gastric cancer is estimated to take the lives of 6,540 men and 4,190 women per year in the US, totaling to an estimated 10,730 yearly deaths. Taking into consideration that the majority of patients are diagnosed once the cancer has spread to various parts of the body, the average rate of survival is a measly 29%; however, if diagnosed at the early stages of the gastric cancer, before

the expansion of the cancer had begun, the survival rate increases to 65%. **Figure 11** gives a visual representation of the severity of the tumors at each stage. This rate rapidly decreases to 30% if the cancer has stretched out to regional lymph nodes and/or neighboring tissues or organs; withal, the rate further decreases to 5% if the gastric cancer has expanded to distant parts of the body, signifying one of the most advanced stages of the cancer. (25, 26)

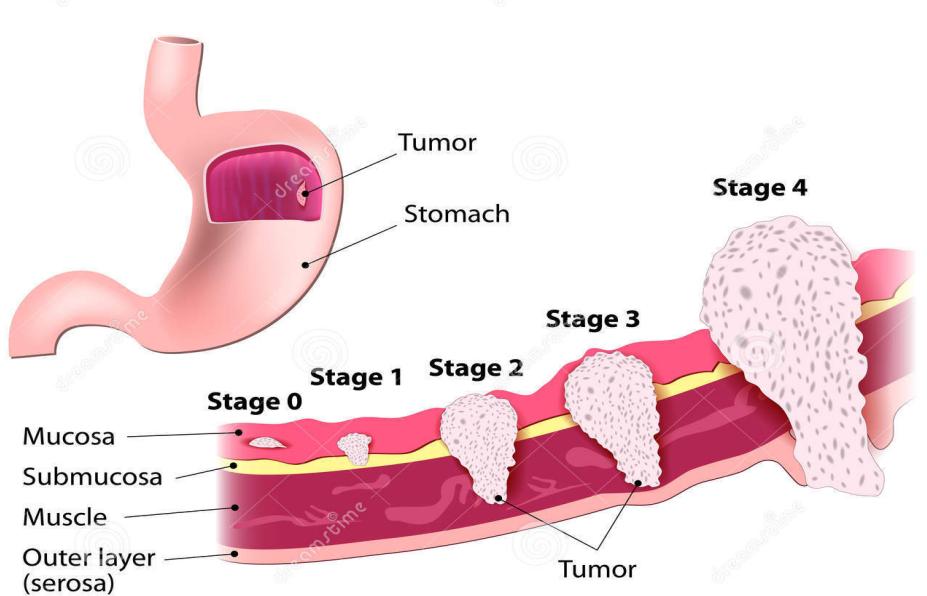


Figure 11: The image depicts the various layers punctured by the different stages of the gastric cancer, until the stage 4 tumor completely raptures the serosa. (27)

1.7 BRCA1 TESTING CRITERIA

In order to help with the detection and prevention of many of these cancers above, genetic testing has become crucial to the field of oncology. Physicians, specifically genetic counselors, recommend various screening and treatment options based on the clinical practice guidelines in the field of oncology which are updated yearly by the NCCN, the National Comprehensive Cancer Network. These regulations, known as the NCCN Guidelines, encompass not only all the different types of cancers, but also the factors that cause a predisposition to

getting cancer, including the BRCA1 and BRCA2 gene mutations. Specifically, for those two mutated genes, the NCCN guidelines lay out the testing criteria that each patient must meet in order to be recommended for genetic testing by their physician. Many of the criteria involve knowledge of the various definitions of relatives, specifically first, second, and third relatives. The guidelines give a pedigree displaying the family chain, shown in **Figure 12**. If one or more of the following criteria, referred to as the testing criteria, are met by an individual, that patient is highly recommended to pursue specialized risk assessment, further genetic counseling, and almost always genetic testing and treatment. The most obvious principle that must be considered initially is if the patient's lineage has a known BRCA1 mutation, meaning that there is a history of genetic variations in the BRCA1 gene in his/her family. As is the case with almost all of these principles, knowledge of a familial BRCA1 gene mutation does not immediately subject the patient to genetic testing. One situation in which genetic testing is most likely not necessary is if the individual's father and/or mother have previously been tested for their own familial gene variations, due to a known familial BRCA1 mutation, and have themselves tested to be true-negative. Due to the true-negative result in the individual's parent(s) with a known familial variation, the parents would only be able to pass on functioning genes, meaning the patient would have no chance of obtaining the mutation from them. In the cases that do have a parent with the BRCA1 variation or a parent with familial history of a mutation has not been tested, the procedure is most likely going to lead the individual to genetic testing. (13)

PEDIGREE: FIRST-, SECOND-, AND THIRD-DEGREE RELATIVES OF PROBAND^a

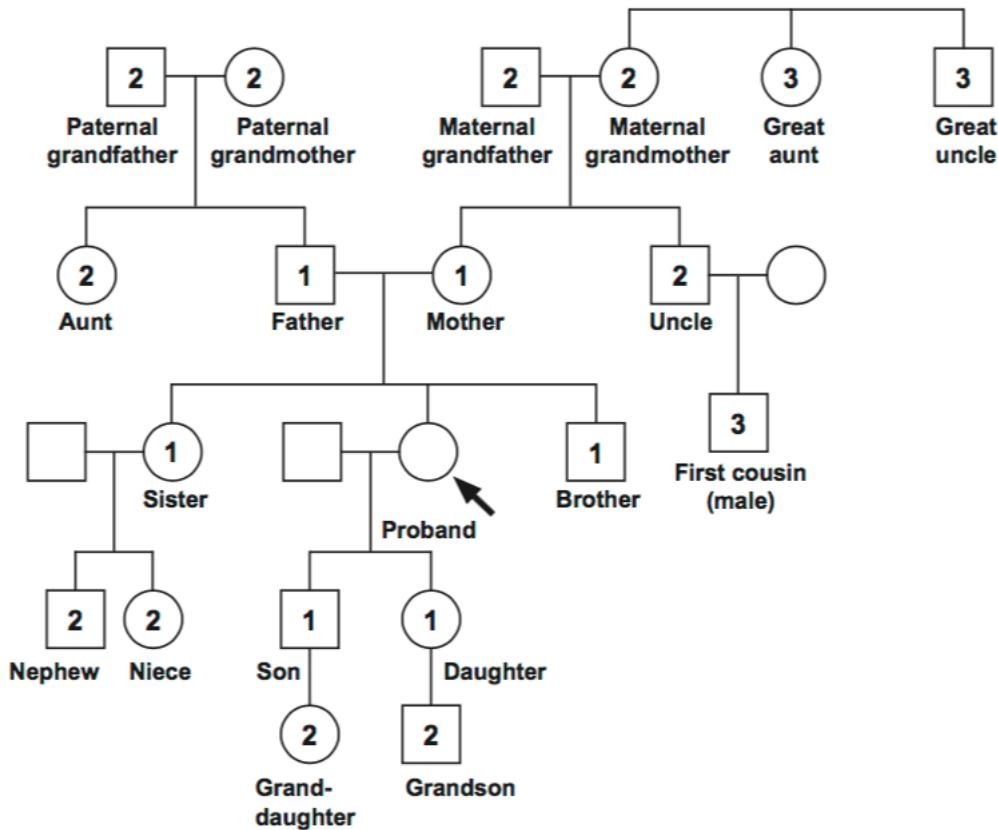


Figure 12: The pedigree gives a visual explanation of what first, second, and third degree relatives are in relation to an individual. Squares are males and circles are females. (13)

Another possible situation in which specialized risk assessment and genetic testing is highly recommended is if the patient has a personal history female of breast cancer; however, this alone does not constitute the recommendation, one of the following situations must have occurred for the possibility of a BRCA1 genetic disorder. These situations depend on the age of diagnoses of the patient, starting with being diagnosed at or before age 45, which automatically meets one of the testing criteria; however, most physicians will not immediately recommend genetic testing if this is the only criterion met. The remaining two specific diagnosis ages will most likely cause the individual's genetic counselor to suggest testing for a mutated BRCA1 gene. In order for a woman, diagnosed at most at age 50, to meet this testing criterion is if at

least one of these five familial conditions are met. The first of these conditions is if the patient has had any additional primary breast cancer, which is if the cancer has not spread to beyond the breast. Having at least one close blood relative (see **Figure 12** for 1st, 2nd, and 3rd degree relatives) with breast cancer also, combined with a diagnosis at or prior to age 50, meets another one of the testing criteria. Using the same age restriction as stated, if one or more close relatives, not solely confined to close blood relatives, has pancreatic cancer, the situation would also complete the criterion. Growing more broad in terms of familial history, if one or more relative, not even just a close relative, productized prostate cancer with a Gleason score of seven or more, the woman with a personal history of breast cancer diagnosed during or prior to her 50th year would qualify for that one criterion for the testing of the BRCA1 mutation. Getting slightly off track, the Gleason score for rating prostate cancer is a grading system on how the cancer looks under a microscope, the lower the score the more similar the cancer tissue is to the normal tissue, thus lowering the risk for the tumor to spread; contrastingly, a high score signifies an extreme difference from the normal and tumor tissues, meaning the tumor has a very high likelihood of spreading. The grade given is actually the sum of the grades given for two observations, one of which is the visual similarities between the cancerous and normal tissue, and the other being the severity of the abnormal growth patterns of the cancer cells. The last situation with which being diagnosed at or before the age 50 completes the parameters to meet the criterion is if there the patient also has a limited or unknown family history because the lack of information may result in the likelihood of detection of a familial mutation to be underestimated. Being diagnosed at age 61 or less has a lot less conditions that must be filled for the criterion, in fact there is only one condition: the breast cancer must be a triple negative breast cancer. BRCA1 gene mutations causes the greatest increase in risk for the triple negative cancer than any other type of breast

cancer, this specific type of breast cancer is when the estrogen receptor, the progesterone receptor and the HER2 receptor are missing. Set universal conditions are also present for a woman diagnosed at any age, meaning that if a patient with breast cancer matches any of the following situations, she would meet one of the testing criteria. Due to the extreme infrequency of this situation, if an individual has any close male blood relative with breast cancer as well as breast cancer themselves, the criterion is met. Completion also occurs if the patient has one of the following: one or more close blood relatives diagnosed before the age 50 with breast cancer, at least one close blood relative with ovarian carcinoma, or two or more close blood relatives with pancreatic, breast, or prostate cancer (Gleason score ≥ 7) at any age. The last of the possible universal requirements with a diagnosis at any age is if the patient's ethnicity is commonly associated with a higher mutation frequency, such as Ashkenazi Jewish; if the patient matches this situation, additional family history may not be required. (13)

Keeping in mind the previous criterion was female specific, any patient with a personal history of male breast cancer automatically fills one of the testing criteria. Additional personal histories of cancer that meet a criterion are ovarian carcinoma, prostate cancer, with some specific parameters, pancreatic cancer, also with additional parameters; withal, any genetic testing, on a tumor of one of these cancers, that shed light on a BRCA1 variation causes a recommendation for further genetic testing. Patients with a personal history of prostate cancer (Gleason score of ≥ 7) can be diagnosed at any age in order to meet the testing criterion; however, they must have at least one close blood relative with breast cancer at or before age 50, ovarian carcinoma at any age, or two relatives with breast, pancreatic, or prostate cancer, always with a Gleason score of at least 7, at any age. The specific parameters that must accompany the personal history of pancreatic cancer are exactly the same as those with prostate cancer, described above;

however, if the patient is of Ashkenazi Jewish descent, the pancreatic cancer is all that is needed in order to receive the recommendation for genetic testing. (13)

In some cases, family history is all that is needed to meet the testing criteria; however, the genetic counselor must discuss the limitations involved with interpreting test results of unaffected, non-true-positive, individuals because of the possibility of an indeterminate or inconclusive result that would only bring an increased stress level to the patient. The two familial situations which would constitute the recommendation from a genetic counselor are the following: a first-degree or second-degree blood relative that meets any of the criteria listed in the two paragraphs above, and a third-degree blood relative with breast and/or ovarian cancer who also has at least 2 close blood relatives with ovarian and/or breast cancer; it must be noted that at least one of the close blood relatives with breast cancer must have been diagnosed at or before age 50. In matching with at least one of the above testing criteria, the individual is strongly advised to seek personalized genetic counseling and, following the counseling, testing for any genetic mutations, which then obligates the patient to have more genetic counseling for the suggested course of action once the results return. (13)

1.8 TESTING PROCESS AND RESULTS

Once at least one of the above testing criteria are met, the patient must then endure risk assessment and counseling, which is part of the pre-testing procedure mandatory for all genetic counselors. The process begins with a round of questioning meant as a psychosocial assessment of the individual, seeing the amount of support present for each patient allows the counselor to better understand how his/her patient will handle the stress and anxiety involved with the testing and possible treatments. The counselor must then use known familial cancer and/or mutation

history through the generations to create a BRCA1 pedigree, as shown in **Figure 13**. The pedigree can then be used by the counselor to determine the patient's risk for obtaining the a mutated BRCA1 gene, not only is the pedigree a great tool for determining the mutation risk, but the simplicity of the figure allows for patients to see and understand the information. Based on the testing criteria that had to have been met prior to the risk counseling, the probability of a patient having no risk for a mutation would be close to zero; however, since it may occur from time to time, depending on the patient's results from the pedigree, the genetic counselor must then educate the individual, to the specificity level desired by the patient, on the increased cancer risks of a mutated BRCA1 gene. Once the patient is copacetic with the specificity of the information, a discussion of genetic testing must take place, focusing on the clarification of the possible test results and the implications of each of the results, and the testing procedure. The patient then must consent to the genetic testing, as long as he/she feels enough information was given to make an informed decision. The final step before the actual testing is the family status check, which is simply whether or not there is a known deleterious familial BRCA1 mutation in the patient's family. One method in which genetic counselors may be able to get a more educated answer to this question involves the use of "tumor banks". Throughout the country, there are facilities, known as "tumor banks", that store and preserve the tumors of cancer patients, past and present, meaning, if the patient had any deceased relatives who had breast or ovarian cancer, their tumors could be tested. These tumors, if tested, would shed light on whether or not there was a familial gene mutation and, more importantly, what and where exactly that mutation would be, if it were to have been passed down to the patient. (13, 28)

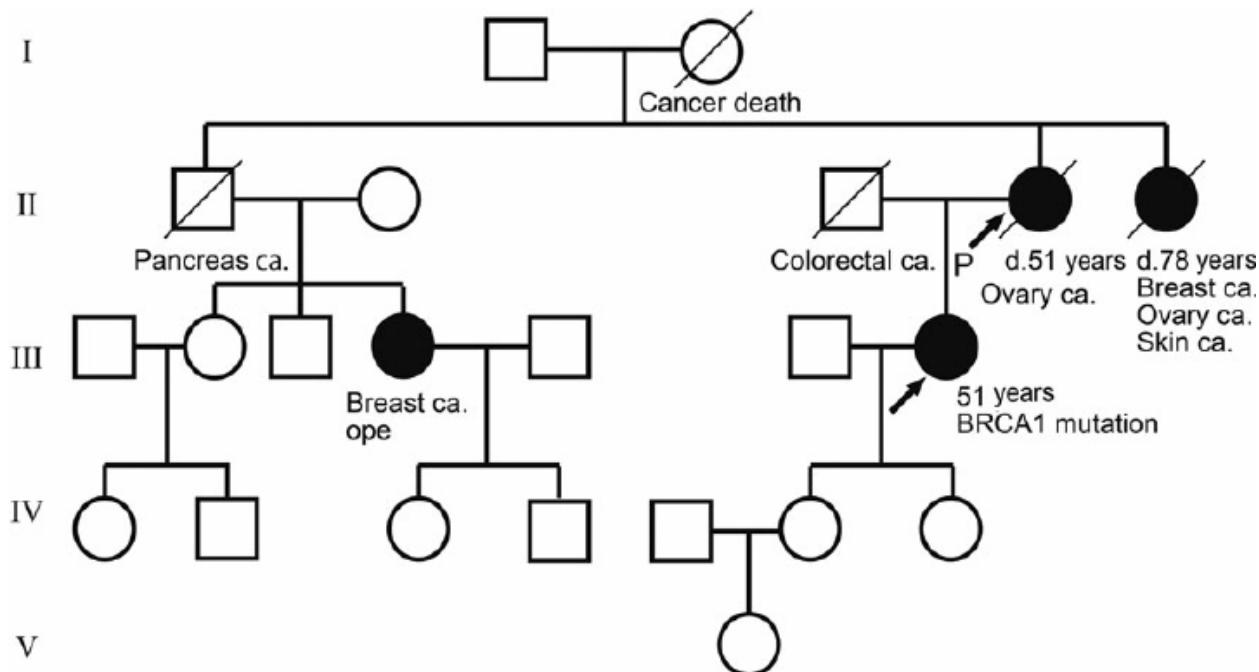


Figure 13: An example of a family pedigree with members that are positive for a BRCA1 mutation. As was the case with the pedigree above, squares are males and circles are females; Additionally, if the shape has a dash through it, the individual is deceased, and all the filled in shapes mutated BRCA1 patients. (29)

If this ancestral tumor testing does not show a familial mutation, the genetic counselor would be able to, with little doubt, recommend the patient for a less specific genetic testing. The initial nomination is a comprehensive testing of the BRCA1 gene or if unaffected, the counselor encourages testing of a family member with highest likelihood of a mutation; withal, if the patient is Ashkenazi Jewish, the counselor must make sure to check for those founder mutations in BRCA1: 185delAG, 5382insC. In some cases, the patient may be recommended for multi-gene testing; however, since this method is quite expensive and new, this nomination is rarely given. Knowing of a familial BRCA1 mutation ensues only one logical option, testing for the specific familial mutation. (14)

With the advancements in genetic testing, scientists are now able to use various methods in order to further analyze different aspects of the human genome. The broadest form of testing

begins with the chromosomes, packages of genetic information made up of DNA, histones, and nucleosomes. Human beings have a chromosome count of 46, which is a combination of two sets of 23 chromosomes, one set formed from the genetic information of the gamete of the father and the other set from the gamete of the mother. However, occasionally, this number may change. A common example of this change is in the disease known as Down Syndrome, which is caused by an extra copy of chromosome 21, making there be three chromosomes there instead of the normal two. Chromosomal genetic testing is used to detect large genetic changes such as is in Down Syndrome; more specifically put, these tests allow for the analysis of whole chromosomes or long lengths of DNA, showing the “big picture” view of the human genome. Moving away from the whole genome, and even whole chromosomes, molecular genetic testing allows for a far more specified search area and testing results. These tests, also known as gene tests, investigate short lengths of DNA or specific genes, allowing for the identification of mutations/variations in the nucleotide sequence that may lead to genetic disorder, such as a predisposition to developing various forms of cancer. The next step in the specificity of the genetic testing comes to the protein products of the genes that make up the chromosomes that combined are the genome of the human body. The analysis of the quantity and/or activity level of the proteins comes from the results of biochemical genetic tests. By studying the protein products of genes, irregularities from either area of analysis, quantity or activity, can portray alterations to the DNA, specifically the genes that code for the proteins, that result in a genetic disorder. As can be easily anticipated already, the molecular genetic tests, gene tests, would be used to analyze the BRCA1 gene, giving one of four results: true positive, true negative, indeterminate, or inconclusive. Analysis of these test results will occur in later paragraphs. The patient’s job in the testing process, once the individual receives basic genetic counseling from their doctor, is simply to give a sample of

blood or saliva to their doctor, who then sends it off to a commercial laboratory or research testing facility; the majority of patients send their samples to commercial laboratories such as Myriad Genetic Laboratories, GeneDx, and Ambry Genetics. The cost of this genetic testing ranges from \$300-\$5,000 depending on the size of the area being tested and insurance. Once sent to the laboratory, the patient must wait on average 2-4 weeks for the retrieval of the test results, which can then be analyzed by a genetic counselor and the path goes from there. (7, 30, 31)

The testing outcome also depends on whether or not the patient has a known familial BRCA1 mutation, due to the different levels of negative results that could result from either option. Beginning with the option that causes the simplest outcomes, when there is a known familial BRCA1 mutation, the results can either be true-positive for the mutation or true-negative for the familial mutation, but another circumstance would be if the testing was not performed in the first place. Testing done on patients with no known familial variation ensues the outcomes that genetic counselors try to avoid due to their tendency to dramatically increase stress and anxiety in the patients. The two simplest situations are if a variation of known cancerous significance is found, or if the individual was not tested. The remaining two possible outcomes fill the place of the true-negative outcome because there is no true negative outcome in patients with no known familial variation. If no mutation is found during the testing, the outcome is referred to as indeterminate or uninformative due to the inability to completely guarantee a true-negative result. This inability to guarantee is due to the possibility of the familial mutation being a variation not yet identified; however, if a variation were found yet the significance was unknown at the time, the results would be known as inconclusive. Geneticists cannot immediately combine a new mutation with a predisposition for cancer, in fact, everyone has polymorphisms, which are natural mutations in DNA, that have no effect on their health. (13, 32)

1.9 MANAGEMENT METHODS

The testing results then lead to possible management methods, such as screening recommendations, to increase the chances of detecting cancer in the early stages of development. In women with true-positive test results for a mutated BRCA1 gene or if testing was not performed on them, the self-management tactic is to teach the patient to have a high breast awareness, using breast self-examinations to check for irregular bumps, starting when the patient reaches 18 years of age. Additionally, the counselor recommends routine clinical, non-screening, examinations, starting at 25 years old and occurring every six to twelve months. For the most precise breast examination, between 25 and 29 years of age, annual breast MRI screenings with contrast, or mammogram screening if the MRI is unavailable. Additionally, if a breast cancer diagnosis before age 30 is present in family history, mammogram screening is preferred over MRI. When deciding between MRI and mammogram, the counselor must consider the fact that false negative results are frequent of mammography in younger women with a BRCA1 mutation and high breast tissue density. Additionally, MRI screening in high risk women had a 77%-94% sensitivity rate, compared to mammography, with a rate of 33%-59% in detecting breast cancer. In between ages 30 and 75, when chances of developing breast cancer severely increase, annual screening of both breast MRI with contrast and mammogram screening is highly encouraged. In patients over the age of 75, screening methods must be examined individually, the counselor most likely would like to undergo another psychosocial analysis of the patient. It must be understood that the meaning of breast MRI screening used throughout this paragraph refers to the ideal MRI screening for breast cancer. In order to achieve a high-quality screening, the MRI must consist of a dedicated breast coil, an ability to perform biopsy under MRI guidance,

experienced radiologists in breast MRI screening, and regional availability. Taking into the expenses of the extensive screenings, women are encouraged to consider entering clinical trials, which are at no financial expense to the patient, in which she would be part of investigational imaging and screening studies. (13)

Currently, there is no effective detection method for ovarian cancer, meaning none that significantly increases the likelihood of detecting the cancer in the early stages. For those women who desire ovarian screening, the most common method is a transvaginal ultrasound, pictured in **Figure 14**. Women begin to consider the ultrasound between the ages 30 and 35. During the testing, an ultrasound probe, known as a transducer, is placed into the vagina covered with gel and a condom. The probe then emits sound waves, which reflect off body structures. These waves are then collected by a computer and used to create a picture, which is then used by the doctor to maneuver the probe around to see the pelvic organs and check for tumors.

Occasionally, a saline infusion sonography, a special method of transvaginal ultrasound, may be necessary in order to clearly view the uterus. New methods for detection are surfacing rapidly, but the process that stands out among the rest is known as Serum CA-125. The caveats for this procedure are similar to the transvaginal ultrasound; however, unlike the ultrasound, Serum CA-125 does not involve the observation of the physical uterus, ovaries, cervix, tubes, and pelvic area, instead a blood sample is tested for large amounts of CA-125, which is known as a tumor marker, or biomarker, and is frequently found in greater concentrations in ovarian cancer cells than in non-mutated cells. One of the major setbacks of this process is the fact that by the time a sufficient amount of CA-125 is able to be detected, the cancer would have already been past the early developmental stage, rendering the testing almost pointless unless caught at the perfect time. (13, 33, 34)

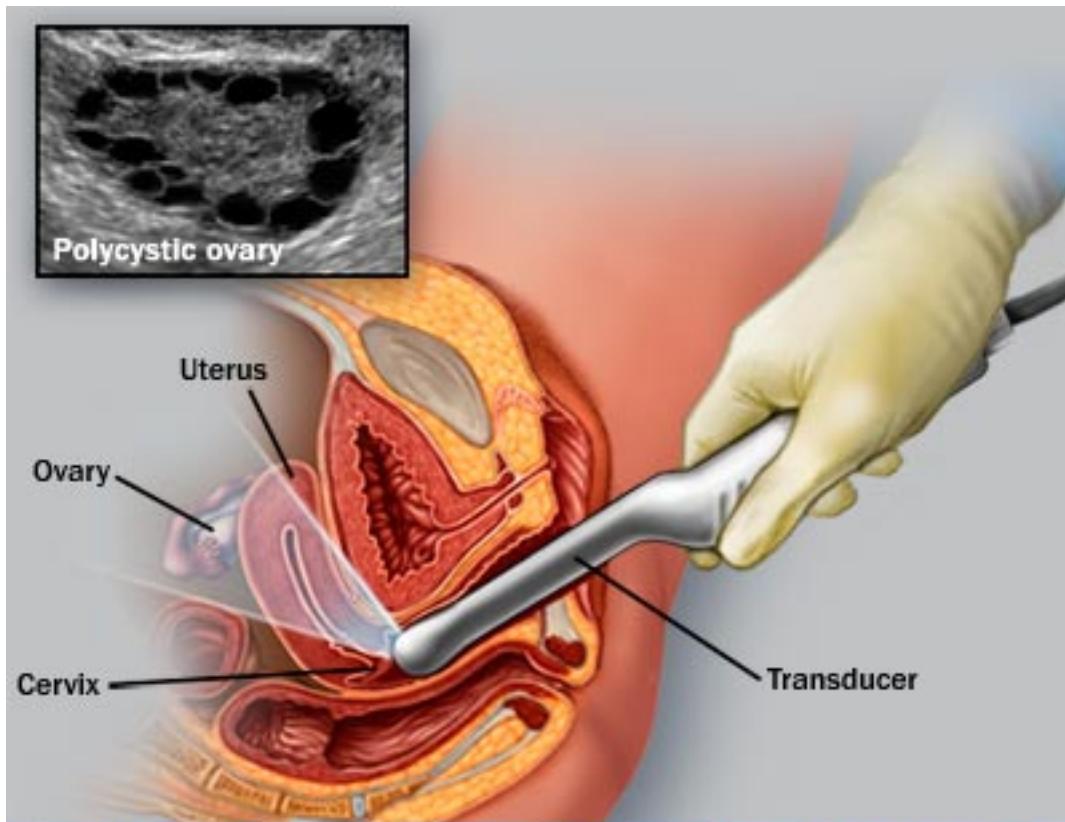


Figure 14: Visual representation of the inside of the body during the transvaginal ultrasound. The image in the top left depicts polycystic ovaries, small cysts form on the outer edges of the ovaries. (35)

Patients have a choice of avoiding the screening process all together by doing two prophylactic surgeries or doing a combination of the screening and prophylactic surgery. The risk-reducing surgery for breast cancer is known as a mastectomy. Patients considering the prophylactic breast cancer surgery must engage in a discussion with their genetic counselor regarding the degree of protection against the cancer, reconstruction options, and risks involved with the surgery. Further information on this subject matter will occur in later paragraphs regarding the decision making process for the management of a high-risk for breast and ovarian cancer; however, in order to give a better understanding of the advantages of prophylactic mastectomy, women carrying a BRCA1 gene variation may reduce their breast cancer risk by

95%, and a 90% decrease in risk for women with a strong family history of breast cancer. (13, 36)

Due to the ineffectiveness of the ovarian cancer screening methods, many BRCA1 positive patients will undergo prophylactic salpingo-oophorectomy (RRSO), which is the removal of the ovaries and fallopian tubes, between the ages of 35 and 40, upon completion of childbearing, due to the inability to reproduce once the removal has transpired. As with the prophylactic mastectomy, and almost every decision in the testing and treatment process, the counselor must engage in a discussion regarding the decision, specifically regarding the reproductive desires of the patient, the extent of cancer risk, degree of protection for ovarian cancer, possible short-term hormone replacement therapy, management of menopausal symptoms, and other related medical issues. Explained in the paragraph regarding prophylactic mastectomy, the specific information and stats needed to make this decision will be discussed in a future paragraph discussing the decisions for treatments. As a brief summary of the benefits of RRSO, the surgery prevents an estimated 80% of ovarian and fallopian tube cancer. If a BRCA1 positive patient is considering either of the preventative surgeries, the counselor must address the psychosocial, social, and quality of life aspects of undergoing either surgery, in order to insure the mental safety of the patient, because of the risk of the surgeries and the potential life-changing consequences, many patients experience feelings of extreme stress and anxiety. (13, 37)

The treatment possibilities for BRCA1 positive men or high-risk men who did not undergo genetic testing are nowhere near the complexity of the management for BRCA1 positive women. Men alone have only three recommended treatments. Similar to that of the treatment for women, breast education and self-examination for men is encouraged starting at age 35, which is

also when yearly clinical breast exams are recommended for the men. Additionally, due to the increased risk for prostate cancer in BRCA1 carriers, screening, such as MRI and mammograms, for prostate cancer is encouraged starting at the age of 45. There are also universal management options for male and female BRCA1 carriers and high-risk patients. Education is the first priority of the genetic counselor, learning the signs and symptoms of the cancers for which a BRCA1 mutation causes a carrier to have a predisposition, as well as advise about a possible inherited cancer risk, is the most important step in diagnosing cancer at the earliest stage possible.

Individual screening based on cancer observed in the family is also a possibility for patients wanting to feel more security; withal, further genetic counseling and testing is also available, although the option of more testing is not usually backed by the genetic counselor. One of last few possible testing outcomes is if the patient is negative for familial BRCA1 mutation, cancer screening may be recommended based on further analysis of family history. Individualized recommendations for managements and further research according to personal and family history is the outcome of patients who did not have genetic testing performed, or had no mutation found or uninformative results in the testing. (13)

2. OBSERVATIONS

2.1 DR. ELLISEN INTERVIEW

While interviewing Dr. Leif W. Ellisen, the professor of medicine at Harvard touched on three main topics: the path he took to become a genetic specialist, the possible future use of CRISPR in fixing heritable mutations, and lastly the indeterminate or inconclusive genetic testing results. Regarding the educational route that he took in order to become a genetic specialist, Dr. Ellisen first explains the new field that is unfolding, known as genetic counselors.

These counselors speak to individuals about their family history, cancers in the family, and they can assess the likelihood of having a hereditary predispositions and whether or not that individual should undergo genetic testing. Prior to the genetic counselors, non-specialized doctors would do a less comprehensive job; however, nowadays doctors pay much closer attention to family history of cancer. In fact, in Massachusetts, it is mandated by law that an individual sees a genetic counselor about family history before receiving genetic testing. Dr. Ellisen diverged into this slight overview of genetic counselors because, after college, there are two choices to become more specialized in genetics, the first of which is genetic counseling, a master's program to be a licensed practitioner. The other route, which Dr. Ellisen took, is the medical route, through being a physician, M.D., or a Ph.D. in genetics, which is a doctoral program in genetics, part of which is cancer genetics. Specifically, Dr. Ellisen is an M.D. in oncology, and he specialized in genetics during his oncology training. The specialization on genetics was after college, after medical school, and after oncology fellowship. As Dr. Ellisen described, "In an institution like [MGH], everyone is a super specialist. There are not many super specialists around here. Mass General Hospital has one of the largest genetics programs in the northeast, it covers New Hampshire, Vermont, and even Maine. Our genetic counselors drive all over and do teleconsulting because they are very specialized." Due to the relative youth of the new field of genetics, as Dr. Ellisen said, there are not many doctors that are specifically in genetics alone. (28)

The professor then gives an explanation of the possible future uses of CRISPR; however, he begins with a brief overview of what CRISPR is. There are two parts to the revolutionary new method of gene editing. Before understanding CRISPR, it is necessary to know a summary of restriction enzymes. These enzymes both cut and paste pieces of DNA in a precise way,

commonly known as recombinant DNA. Dr. Ellisen explained that “CRISPR is exactly analogous to restriction enzymes because they both were derived from bacteria; however, CRISPR cuts the DNA at a precise site but does not care about putting the DNA back together because it is used by bacteria as a defensive mechanism against viruses that put their DNA in the DNA of the bacteria.” The term CRISPR is used to describe both the cutting and fixing of the DNA when talking about gene editing; however, CRISPR is only involved with the cutting, and there are several different enzymes involved with making sure the DNA is repaired without a mutation, shown in **Figure 15**. The CRISPR cast is the first time that scientists can target one site in the entire genome, then the CAS9 enzyme of CRISPR cuts the specific location. One of the only ways to completely un-mutate every BRCA1 gene in the body is to use CRISPR while the patient was an embryo, the point at which cell differentiation had not occurred. During *in vitro* fertilization, many people will do embryo selection, which would allow for the testing of certain common genetic mutations, such as the BRCA1 mutation, and the parents then could choose the embryo that was negative for the mutations. CRISPR could also be used to fix the mutation in the specific cells of a tumor, which would re-activate the BRCA1 gene, a tumor-suppressor gene, and inhibit further tumor growth. (28)

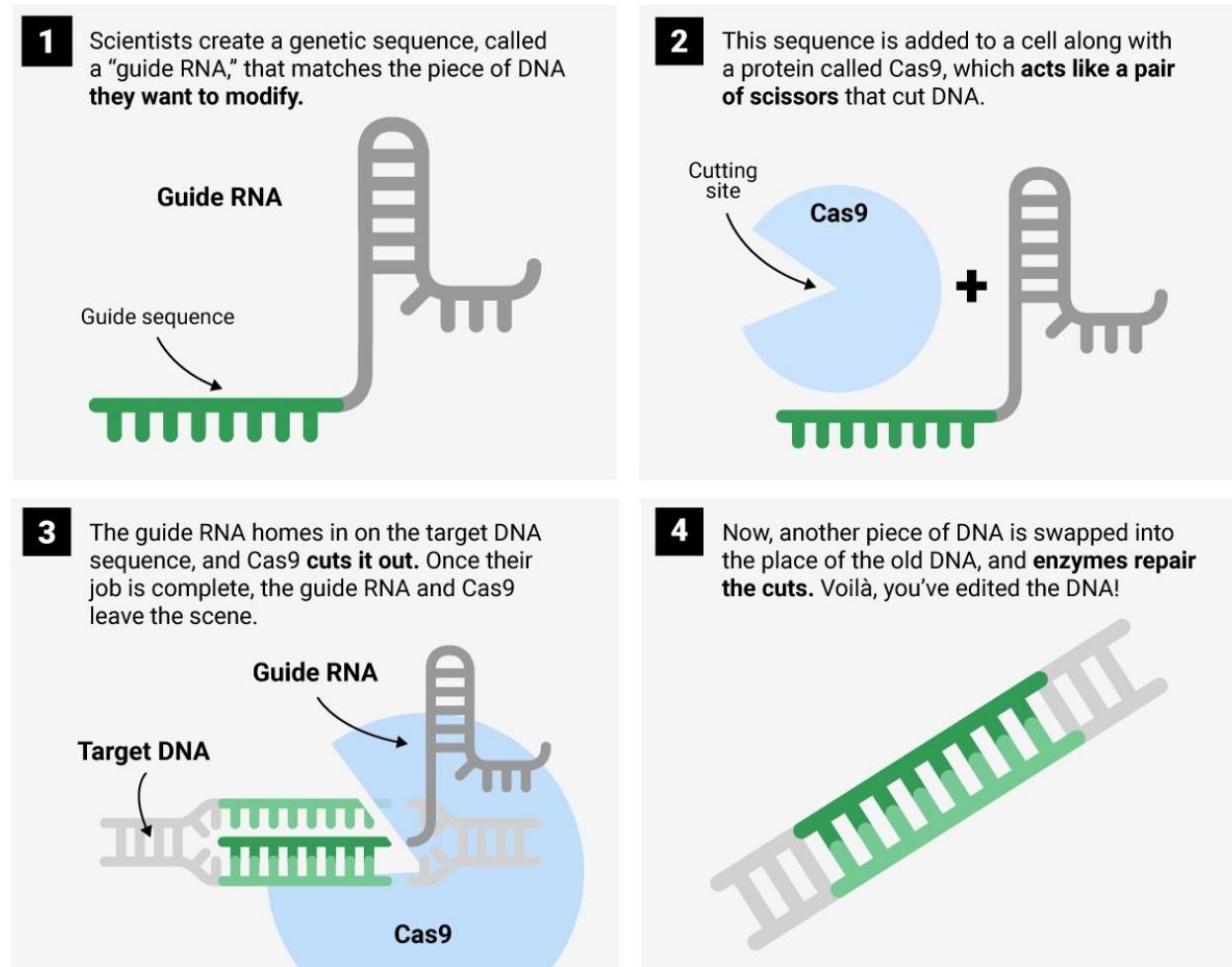


Figure 15: The diagram further explains the CRISP/CAS9 editing technique. As can be seen in step 4, neither CRISPR nor CAS9 is involved with the actual repair of the DNA. Instead, there are specific enzymes for different amino acid sequences for the mending of the DNA strand. (38)

Beginning the discussion of inconclusive and indeterminate test results, Dr. Ellisen drew a small pedigree, depicting a mother that developed breast cancer at 38 years old and is currently 58, and her daughter that is 35 years old and wants to receive genetic testing. In the past, and in many genetic clinics today, the daughter would be sent to get the testing, which could result in an inconclusive test result. This results means that either the mother had a mutation and the daughter did not get it, or it is not a known mutation but there could still be an inherited risk. The testing should always initially be done on the informative person or the person most-likely to

have a mutation because most genetic tests are negative due to the plethora of unknown mutations. Going back to the diagram, if the mother had been tested first and no mutation was found, then the genetic testing will not help her with her risk, making the daughter no longer need to undergo the testing. Once a gene mutation is found in the family, then the testing results of the family members are informative, either true-negative or -positive. (28)

2.2 THE WELLS SISTERS INTERVIEWS AND COUNSELING LETTERS

In interviews with Nancy Montanaro, Kathie Stayton, Ellen Matiasevich, Ruth Griffin, and Margery Piercey, all the aspects and steps of the five sisters' genetic testing process were attempted to be uncovered; however, the complete unearthing of their experience was not fully attainable without the help of the notes of the initial genetic counseling with Nancy, and the testing results for Nancy and Margery. In response to a question regarding how she was initially recommended for genetic counseling, Nancy explained, "My primary care physician, who has known me forever and for whom my mother was the office manager, knew that my mother died of ovarian cancer and asked me about other familial cancers. When he found out about my maternal grandmother as well, he suggested that all my sisters receive genetic testing. Once we looked into the testing, we learned that the first step was to go to genetic counseling, at which we would be guided through the process." Four of the sisters then proceeded to have an appointment at the Breast and Ovarian Cancer Genetics and Risk Assessment Clinic on January 30, 2006. (39)

2.2.1 PRE-TEST GENETIC COUNSELING

During this visit, Dr. Paula D. Ryan and Genetic Counselor Jessie Hastings reviewed both the personal cancer history of each sister as well as the family history. The interview of

Nancy Montanaro uncovered no personal information that would point toward a possible genetic predisposition for cancer. At the age of 39, she had no personal history of cancer and was of English, German, French and Scottish ancestry, only one of which, French, is a member of the “founders effect” subpopulations with a high frequency of BRCA1 mutations. While reviewing her family history of cancer, the real reason for the high possibility for a genetic mutation becomes understood; the following list is the occurrences of cancer in the Wells family:

- Oldest sister, Ruth, diagnosed with cervical cancer at the age of 35
- Another sister, Margery, was diagnosed with basal cell skin cancer in her 30s
- Mother was diagnosed with ovarian cancer at 54 and passed away at the age of 57
- Maternal grandmother was diagnosed with breast cancer in her 30s and later was diagnosed with ovarian cancer in her early 80s, she passed away at 83 years old
- Father was diagnosed with prostate cancer in his late 60s and passed away at 74
- Paternal first cousin was diagnosed with leukemia and passed away at the age of 12
- Paternal grandmother was diagnosed with stomach cancer in her 70s and passed away in her 80s

This information is condensed into **Figure 16**, a pedigree created by their genetic counselor, Jessie Hastings. Jessie Hastings then continues by writing, “based on the family history [Nancy] reported, it is possible that the cancer in [her] family is due to an inherited cancer predisposition gene.” Once Nancy learned of her possible genetic variation, she explained the steps her doctor and she took prior to the genetic testing: “I had a meeting with a genetic counselor during which the main focus was, of course, the hypotheticals and whether or not I was certain that I wanted to do the testing and know the answer. In order to make that decision, I needed to know what a positive would mean and what could be done about it. After being informed of the increased

likelihood of breast and ovarian cancer if diagnosed with the BRCA1 mutation, and the fact that there were steps that could be taken to protect against and/or detect both the breast and the ovarian cancer, I decided to move forward with the testing.” This genetic counseling was not the case for second-eldest sister, Ellen, who lived in California at the time, she explained that “the east coast sisters had the counseling and I then got the information and benefits from them. I then talked to one of the oncologists that I knew from the hospital at which I worked. (1, 39)

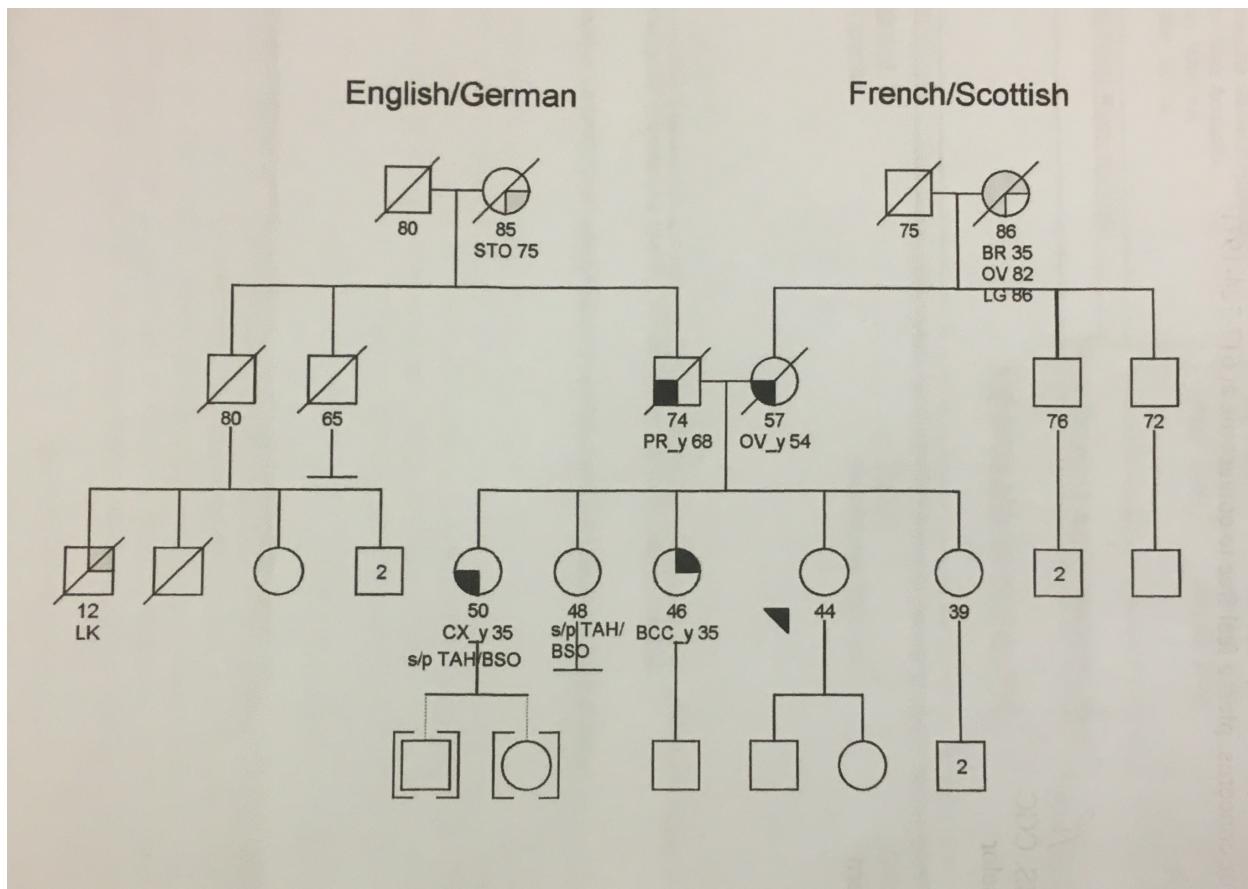


Figure 16: The pedigree above depicts the family history of the Wells family, from two generations prior to one generation ahead of the proband, Margery Piercy. The interpretation of this pedigree is the same as the one for Figure 1. (40)

Part of this genetic counseling, due to the inability to do genetic testing on a living family member with cancer, included the discussion of the emotional consequences for the person being

tested as well as the threat of genetic discrimination, the use of genetic information by health insurers, employers, life insurers, and disability insurers. In the notes of their genetic counselor giving the sisters more information regarding genetic testing, the counselor writes “individuals may feel anxiety and or depression as they await their result, and may experience many different emotions once they receive their results.” Not only does this affect the individual receiving the genetic testing, but it may have implications spreading to many other family members, such as future generations and close relatives. In explanation of genetic discrimination, Kathie was told by her genetic counselor to “make sure [she] had the life insurance [she] wanted because life insurance would be more difficult and expensive to get if [she] tested positive for the mutation. The possibility of the genetic variation also affected three of the five sisters as biological mothers due to having to possibly worry about their children inheriting their mutation if they get a true-positive test result. Ellen did not have to take this possible anxiety-causing consequence into her decision due to a hysterectomy three years following the death of her mother due to non-cancer related uterine and or ovarian pain for several years. Ruth, the oldest sister, had also had a hysterectomy, due to earlier cervical cancer, so the possibility of passing down a mutation to her children, since they were both adopted, did not play a part in her decision making as well.

Attempting to shed light on the motherly thought processes of the three biological mothers, Nancy summarizes, “Kathie had only one child at the time, a boy, so that was something that entered into her thought process - the ability to have more children. Margery already had her two children, one of which was a girl. The idea of having passed it on to her daughter, Natalie, was what seemed to make her most uneasy. I already had my two sons and, having seen my mother and my father suffer through cancer, I wanted to do everything in my power to keep my children from having to go through that.” (39)

In Massachusetts General Hospital at the time of their testing, the BRCA1 and BRCA2 alteration testing involved two visits with both a genetic counselor and nurse practitioner: one pre-test education session and a result disclosure session. The last of the considerations discussed in the pre-test education session was the financial aspect of the testing. The cost of a full gene testing was \$2975, which may have been covered depending on health insurance plans; if an alteration had already been identified, the cost of subsequent testing for relatives was \$350 because the testing was only for the specific family gene variation. Due to the three alterations common in individuals of Ashkenazi Jewish descent, the cost for testing of the three gene variations was only \$415. In the case of the Wells, who were not of Ashkenazi Jewish descent, the initial fee of \$2975 would be charged for each sister until one of the sisters receives a true-positive result, meaning a family mutation would have been identified, and the subsequent sisters would only have a fee of \$315 to search for the known gene variation. The first sister to get tested, Kathie Stayton, received a true-positive test result, which allowed the remaining four sisters to no longer have to undergo the full gene testing and only pay \$315 to search for the mutation found in Kathie, which turned out to be the BRCA1 mutation 5256delG. (1, 39)

2.2.2 TESTING PROCESS

Before divulging into the specific implications of the germline BRCA1 mutation 5256delG, the testing process, having already obtained an informed consent, must be described and understood. In response to a question asking for a detailed experience of their testing, Nancy answers, “It was like any other blood test: a quick appointment and then the sample was sent off to some far-away lab. The waiting process was long and we were all constantly checking in with each other. We all did it within six months of each other.” The place to which the blood samples

were sent was called Myriad Genetic Laboratories, founded in Salt Lake City, Utah. For the four sisters following Kathie's testing results, the lab only had to search for one mutation, 5256delG, because a positive test result uncovered their family genetic mutation and a full gene search was no longer necessary. Margery described a feeling of anxiety during the waiting period and Kathie recalled wanting to get the answers quickly because she "didn't really like waiting." Devanshi Patel, another genetic counselor, explained in a letter that "Myriad laboratory performed the test at least twice making it a highly accurate result." By performing the test more than once, the laboratory reduced the risk of error which could have changed a true-negative result to true-positive. (39)

2.2.3 TESTING RESULTS

The first of the sisters to both receive genetic testing and obtain the results was Kathie Stayton. The testing uncovered a true-positive result for her; however, Kathie had not only felt ready for the results, she also had expected them: "I truly believed I had the mutation before I got the result, I really hoped I was wrong. I remember being disappointed, but felt I was prepared to deal with it based upon all of the time I had to discuss with sisters, doctors, my husband, friends and counsellors." The next to get tested was Margery, the second youngest sister. The results came back as negative, meaning she could not have passed down the mutation to her daughter, Natalie, which was her biggest fear at first and turned into her biggest relief. Margery also explained that "[her] regret was having told Natalie that I was having the test. [She] would definitely want a redo on that. Because if [Margery] had [the mutation], [she] would not want [Natalie] to have known that at her age!" The fear of passing down the variation to future generations resonated through all the sisters that were biological mothers. The eldest sister, Ruth,

received the testing third, and she learned of her positive test result for the familial BRCA1 mutation, 5256delG. Nancy, the youngest, was the fourth to receive her results, which, along with Ruth and Kathie, were true-positive. The last to receive their results was Ellen, and since she was living in California, she received her results from the oncology nurse at the hospital at which she worked. Ellen explained her emotions once she received her true-negative results: "I was very relieved. I also felt guilty that I was negative and 3 of my sisters were positive." The guilt that Ellen felt is just one of the many psychological responses that can arise from any BRCA1 testing. The common responses for patients with a variation are anxiety, sleeplessness, and/or depression. (39, 40)

2.2.4 POST-TEST GENETIC COUNSELING

The three 5256delG positive sisters were then instructed to schedule an appointment with Dr. Paula Ryan, the director of the Breast and Ovarian Cancer Genetics Program at Mass General Hospital at the time. The appointment would be to further discuss the results, the specific cancer risks involved with 5256delG, and the various breast and ovarian cancer reduction options. Once the three sisters entered the office, the meeting began with the specifics of their mutation, 5256delG, starting with the variation being a nonsense mutation. As was explained in a previous paragraph, the nonsense mutation results in a stop codon. The resulting stop codon of 5256delG is at amino acid position 1713 of the BRCA1 protein. The exact risk of cancer conferred by the mutation was and still is not determined; however, studies had narrowed down the percentages to around 87% risk of breast cancer and 44% risk of ovarian cancer by age 70. Additionally, there is a 20% risk of developing a second breast cancer within five years of

the first and an increase of almost ten times the risk of subsequent ovarian cancer in women with the 5256delG mutation. (39)

2.2.5 DECISION OF MANAGEMENT OPTIONS

Using the specific cancer risk statistics of 5256delG, the three women were then able to make informed decisions on their choice of management and detection processes; however, this new information did not affect Kathie's decisions at all. In fact, she had decided her course of action before she even received her results: "I had already decided what I would do with this result and didn't have any change of heart once I actually was positive. I was ready to move forward with the surgeries. I scheduled the oophorectomy quickly and waited for the mastectomy until after I got back from China." As soon as Kathie learned of her possible genetic predisposition for breast and ovarian cancer, she dove into research, finding out as much as was known at the time about a BRCA1 mutation, she even joined a BRCA support group. She explained her reasoning for waiting for her mastectomy until later: "I didn't think I should have two major surgeries with 6 months of adopting my daughter and I wasn't sure how the surgical menopause symptoms might impact me." The choice for the total hysterectomy with bilateral salpingo-oophorectomy for both Kathie and Nancy, Ruth had gotten the total hysterectomy prior to the genetic testing, was an easy one in the sense that both were done having kids; however, for Nancy, there was a different and much larger risk for her surgery than for any of the other sisters. Kathie was able to further explain this risk by stating, "The risk of surgical menopause impacting our life was greatest for Nancy since she was the youngest. There was a much higher risk of difficult menopausal symptoms for her being younger because it was a drastic change in estrogen levels. As you get older, and are pre-menopausal, your estrogen goes down and that decrease in

estrogen is more gradual and symptoms from that are easier to manage.” The increased risk did not stop Nancy from having the hysterectomy. She had finished having children and did not want the possibility of developing ovarian cancer in the back of her mind forever: “ I know how hard ovarian cancer is to detect and I did not want to risk going through what my mom had been through – or putting my family through that.” Another reason for Nancy’s decision to do the hysterectomy was due to the fact that the operation was much simpler than the mastectomy, only one surgery was required and the recovery time was about a month, which fit into her life schedule. She was able to do the surgery at the beginning of March, 2007 and missed only a two weeks of school and spring break before returning to work. Nancy opted for annual MRI and mammograms because she knew breast cancer was very detectable and treatable. The mastectomy, on the other hand, was a two-step process at the time, which would have been extremely complicated to fit into her schedule. The first surgery of the mastectomy process was simply the removal of the breast, and the second surgery was the reconstruction of the breast. Nowadays, however, surgeons are able to fit both steps into one, doing both the removal and reconstruction in one surgery. Ruth, having already undergone a hysterectomy, simply opted for the regular breast cancer screenings for the same reasons as Nancy. In summary, all three 5256delG positive sisters opted to do the surgeries and screening methods that best suited their life schedule, Kathie with two surgeries, Nancy with one and screening, and Ruth with just screening; moreover, the Wells sister example shows the importance of having more than one course of action once a true-positive test result is received. Patients must be able to customize their management in order to suit their schedule and their personality, otherwise they may not be able or choose to move forward wisely. (39)

3. CONCLUSION

In conclusion, when considering genetic testing, the main focus of the patients is to consider the dangers of having a mutation; however, the job of genetic counselors is to analyze all other aspects involved with and or affected by the testing. The counselors will understand their patient's psychosocial and psychological state before the genetic testing and directly following the discussion of the results, specifically if the results are not true-negative. The potential risk for a BRCA1 mutation, and the identification of which relative is best suited to undergo the genetic testing first are two other key roles of the counselors. By testing the patient with the highest potential of having the variation, the chances of receiving an indeterminate or inconclusive test result is minimized, reducing the potential anxiety and paranoia included with those test results. Once a genetic counselor has guided the patient into the management methods that best suit the individual's life, meetings with an M.D. in genetics, such as Dr. Ellisen, are then scheduled at the convenience of the patient, preferably annually, in order to discuss the most recent changes to the NCCN BRCA1 mutation guidelines. For this project, six individuals connected to the BRCA1 mutation, Dr. Ellisen and the five Wells sisters, were interviewed. However, if the project were to be redone, a shadowing of Dr. Ellisen or a genetic counselor during the initial meeting prior to the testing or the meeting immediately following the genetic testing would have aided in the research. The two meetings would have given insight to the specific questions asked by the genetic counselor in order to gain information about the following: family history, psychosocial and psychological state, and management options. Additionally, a viewing of the actual testing process would have been an extraordinary resource to collect information; however, this option was not available at the time of the research for this project. The care and patience that Dr. Ellisen showed to his patients and during the interview

and the fact that he gave thirty minutes of his time in between appointments to answer my questions, was absolutely astounding. Moreover, this project and the research necessary for its completion have peaked my interest in genetic engineering, which is now my preferred field of engineering, surpassing the previously favored electrical engineering. I would immensely enjoy joining in on this rapidly growing and tremendously important study of genes, and the revolution of gene editing that has arisen with the unearthing of CRISPR. Lastly, understanding this aspect of my mother's and, thus, my own genetic history is something that, although somewhat frightening, will remain with me for the rest of my life. (28, 39)

References

1. Hastings, J., & Ryan, P. D. (2006). Breast and ovarian cancer genetics and risk assessment clinic information summary. In *Massachusetts general hospital cancer center clinic email to Nancy Montanaro* (pp. 1-6).
2. (n.d.). NIH Fact Sheets - Genetic Testing: How it is Used for Healthcare. Retrieved March 28, 2017, from <https://report.nih.gov/NIHfactsheets/ViewFactSheet.aspx?csid=43>
3. (n.d.). BRCA1 and BRCA2: Cancer Risk and Genetic Testing Fact Sheet Retrieved March 28, 2017, from <https://www.cancer.gov/about-cancer/causes-prevention/genetics/breca-fact-sheet>
4. (n.d.). Genetic Basis of Cancer Syndromes - Holland-Frei Cancer ... - NCBI. Retrieved March 30, 2017, from <https://www.ncbi.nlm.nih.gov/books/NBK12959/>
5. (2017, April 4). BRCA1 gene - Genetics Home Reference - NIH. Retrieved April 9, 2017, from <https://ghr.nlm.nih.gov/gene/BRCA1>
6. (2014, June 25). Oncogenes and tumor suppressor genes | American Cancer Society. Retrieved March 28, 2017, from <https://www.cancer.org/cancer/cancer-causes/genetics/genes-and-cancer/oncogenes-tumor-suppressor-genes.html>
7. Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., Jackson, R. B., Reece, J. B., & Campbell, N. A. (2014). *Biology in focus* (B. Wilbur, Ed.). Pearson Education.
8. (2017, April 4). What kinds of gene mutations are possible? - Genetics Home Reference. Retrieved April 9, 2017, from <https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations>
9. (n.d.). Li-Fraumeni Syndrome | Cancer.Net. Retrieved March 28, 2017, from <http://www.cancer.net/cancer-types/li-fraumeni-syndrome>
10. (n.d.). Cowden Syndrome | Cancer.Net. Retrieved March 28, 2017, from <http://www.cancer.net/cancer-types/cowden-syndrome>
11. (2017, March 21). ataxia-telangiectasia - Genetics Home Reference. Retrieved March 28, 2017, from <https://ghr.nlm.nih.gov/condition/ataxia-telangiectasia>
12. (2017, March 21). NBN gene - Genetics Home Reference. Retrieved March 28, 2017, from <https://ghr.nlm.nih.gov/gene/NBN>
13. (n.d.). Genetic/Familial High-Risk Assessment: Breast and Ovarian ... - NCCN. Retrieved March 28, 2017, from https://www.nccn.org/professionals/physician_gls/PDF/genetics_screening-japanese.pdf

14. (2017, March 21). CHEK2 gene - Genetics Home Reference. Retrieved March 28, 2017, from <https://ghr.nlm.nih.gov/gene/CHEK2>
15. (2017, March 21). PALB2 gene - Genetics Home Reference. Retrieved March 28, 2017, from <https://ghr.nlm.nih.gov/gene/PALB2>
16. (2016, May 5). Breast Cancer - Medical News Today. Retrieved March 28, 2017, from <http://www.medicalnewstoday.com/articles/37136.php>
17. (n.d.). Breast Cancer: Statistics | Cancer.Net. Retrieved March 28, 2017, from <http://www.cancer.net/cancer-types/breast-cancer/statistics>
18. (2016, December 2). Why Does Breast Cancer Attack Men When They Have No Breast Retrieved April 9, 2017, from <https://sites.psu.edu/siowfa16/2016/12/02/why-does-breast-cancer-attack-men-when-they-have-no-breast/>
19. (n.d.). Ovarian, Fallopian Tube, and Peritoneal Cancer: Statistics | Cancer.Net. Retrieved March 28, 2017, from <http://www.cancer.net/cancer-types/ovarian-fallopian-tube-and-peritoneal-cancer/statistics>
20. (n.d.). Types & Stages - National Ovarian Cancer Coalition. Retrieved April 9, 2017, from <http://ovarian.org/about-ovarian-cancer/what-is-ovarian-cancer/types-a-stages>
21. (n.d.). Colorectal Cancer - Statistics | Cancer.Net. Retrieved March 30, 2017, from <http://www.cancer.net/cancer-types/colorectal-cancer/statistics>
22. (n.d.). Bowel Cancer Staging - Bowel Cancer Australia. Retrieved April 9, 2017, from <https://www.bowelcanceraustralia.org/bowel-cancer-staging>
23. (n.d.). Pancreatic Cancer: Statistics | Cancer.Net. Retrieved March 30, 2017, from <http://www.cancer.net/cancer-types/pancreatic-cancer/statistics>
24. (2016, December 23). Pancreatic Cancer Treatment (PDQ®)—Patient Version - National Retrieved April 9, 2017, from <https://www.cancer.gov/types/pancreatic/patient/pancreatic-treatment-pdq>
25. (n.d.). Stomach Cancer: Statistics | Cancer.Net. Retrieved March 30, 2017, from <http://www.cancer.net/cancer-types/stomach-cancer/statistics>
26. (n.d.). Stomach cancer statistics | World Cancer Research Fund International. Retrieved March 30, 2017, from <http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/stomach-cancer-statistics>
27. (n.d.). 1000+ ideas about Stomach Cancer on Pinterest | Bone cancer Retrieved April 9, 2017, from <https://www.pinterest.com/explore/stomach-cancer/>

28. Ellisen, L. (2017, February 21). Personal Interview with N. Montanaro.
29. (n.d.). Figure 1. Pedigree of a proband individual with a BRCA1 mutation Retrieved April 9, 2017, from https://www.researchgate.net/figure/261512187_fig1_Figure-1-Pedigree-of-a-proband-individual-with-a-BRCA1-mutation-The-arrow-indicates
30. (2016, June 23). Genetic Testing Facilities and Cost - Breastcancer.org. Retrieved April 2, 2017, from http://www.breastcancer.org/symptoms/testing/genetic/facility_cost
31. (2017, March 28). What is genetic testing? - Genetics Home Reference. Retrieved April 2, 2017, from <https://ghr.nlm.nih.gov/primer/testing/genetictesting>
32. (2017, April 4). What do the results of genetic tests mean? - Genetics Home Reference. Retrieved April 9, 2017, from <https://ghr.nlm.nih.gov/primer/testing/interpretingresults>
33. (2015, September 26). Transvaginal Ultrasound: Purpose, Procedure & Results - Healthline. Retrieved April 6, 2017, from <http://www.healthline.com/health/transvaginal-ultrasound>
34. (2016, July 26). CA 125 Blood Test, Normal Ranges, and Results - MedicineNet. Retrieved April 6, 2017, from http://www.medicinenet.com/ca_125/article.htm
35. (n.d.). Transvaginal ultrasound - Mayo Clinic. Retrieved April 10, 2017, from <http://www.mayoclinic.org/diseases-conditions/pcos/multimedia/transvaginal-ultrasound/img-20007770>
36. (2017, February 14). Prophylactic Mastectomy | Breastcancer.org. Retrieved April 6, 2017, from http://www.breastcancer.org/treatment/surgery/prophylactic_mast
37. (n.d.). Case 3: Risk-reducing salpingo-oophorectomy - SGO. Retrieved April 6, 2017, from <https://www.sgo.org/wp-content/uploads/2016/08/2016-SGO-Genetics-Toolkit-Case-3.pdf>
38. (2015, December 21). How CRISPR is revolutionizing biology - Business Insider. Retrieved April 10, 2017, from <http://www.businessinsider.com/how-crispr-is-revolutionizing-biology-2015-10>
39. Montanaro, N., Piercy, M., Matiasevich, E., Griffin, R., Kathie, S. (2017, April 4). Personal Interview.
40. Hastings, J., & Ryan, P. D. (2006). Breast and ovarian cancer genetics and risk assessment clinic information summary. In *Massachusetts general hospital cancer center clinic email to Margery Piercy* (pp. 1-7).