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# Evaluating Changes in Patient Anxiety Regarding Classic Cancer Genetic Testing Versus Expanded Multiplex Cancer Genetic Testing

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Evaluating Changes in Patient Anxiety Regarding Classic Cancer Genetic Testing  
Versus Expanded Multiplex Cancer Genetic Testing

by

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Bachelor of Science  
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Submitted in Partial Fulfillment of the Requirements

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## **Abstract**

Multiplex cancer genetic testing by next generation sequencing (NGS) offers genetic counselors and patients new options for testing multiple genes beyond *BRCA1* and *BRCA2*, increasing both the yield of positive results and the number of variants of uncertain significance (VUS). This pilot study explored three psychosocial questions related to multiplex cancer genetic testing: 1). Do anxiety levels differ in patients with results of familiar single gene testing versus those with results of multiplex cancer genetic testing of unfamiliar genes? 2). Do different results (i.e., negative, positive, or VUS) affect patient anxiety levels in the post-results period? 3). Is patient anxiety affected by the specific gene in which the mutation or VUS is identified? The study included women diagnosed with breast cancer and considered at high risk for a hereditary cancer syndrome due to age or family history. Participants completed a baseline State-Trait Anxiety (STAI) questionnaire at the pre-test genetic counseling session, and completed the same STAI questionnaire and a Multidimensional Impact of Cancer Risk Assessment (MICRA) questionnaire after the post-results discussion. Twenty individuals participated, of which 17 patients completed the baseline STAI tool. Nine participants completed all questionnaires, yielding five participants with negative results, two with positive results, and two with VUS results. Two participants with negative results showed significant baseline anxiety levels which decreased in the post-results period. The positive and VUS result groups showed non-significantly increased mean anxiety levels by STAI. Differences in anxiety between those with positive results and those with

negative results trended toward significance. Two individuals with positive mutation results in genes other than *BRCA1* and *BRCA2* showed higher post-results anxiety levels on the MICRA scale than did two participants with VUS results. The study was limited by sample size. A larger multi-site study is planned to clarify anxiety, distress, and uncertainty parameters to help guide genetic counselors in their approach to psychosocial aspects of multiplex cancer genetic testing.

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## **Chapter 1: Background**

### **1.1 Cancer and Hereditary Breast and Ovarian Cancer Syndrome**

As described by the National Cancer Institute, cancer is a common condition within the context of all human illness (National Cancer Institute, 2014b). Cancer can affect any person, often in a seemingly random fashion in an isolated individual, and occasionally in a pattern affecting several members across multiple generations of a single family. The development of cancer can be grouped into three classifications by etiology, including sporadic, familial, and hereditary cancers. The majority of cancers (70% to 80%) occur sporadically, and are ultimately due to a combination of environmental exposures and chance cellular events culminating in unchecked cell proliferation through eventual accumulation of one or, more likely, numerous genetic changes (Vogelstein & Kinzler, 2004). Approximately 15-20% of people who develop cancer have a family member with the same cancer, typically at older age of onset, thereby displaying a familial pattern, which could be attributed to shared genetics and other factors like dietary practices and similar environmental exposures. Five to ten percent of breast cancer cases are caused by an inherited germline mutation in a specific gene that confers an increased lifetime susceptibility to cancer development (USPSTF, 2009).

The most common genes currently known to be associated with a significantly increased lifetime risk for breast cancer are the *BRCA1* and *BRCA2* genes. The estimated carrier frequency among women of Northern European descent for deleterious mutations is approximately one in 400. The frequency of pathogenic mutations varies across ethnic

groups, ranging from between one in 250 and one in 840 (Anglian Breast Cancer Study Group, 2000; Antoniou et al., 2002; Narod & Foulkes, 2004). The most frequently affected group is the Ashkenazi Jewish population where three founder mutations identified in this group account for approximately 99% of *BRCA1* and *BRCA2* mutations. Carrier frequency of *BRCA1* or *BRCA2* mutations in women of this ancestry occurs in one out of every forty individuals (Oddoux et al., 1996; Offit et al., 1996).

The effects of various deleterious mutations in these two genes lead to a condition known as Hereditary Breast and Ovarian Cancer syndrome (HBOC). Findings in a family history characteristic of HBOC include early age of onset (diagnosis under age 50), bilateral breast cancer, and possibly multiple primary cancers. Additionally, HBOC can present with a family or personal history of one or more different forms of cancer which may include ovarian cancer, male breast cancer, pancreatic, prostate, fallopian tube, and/or primary peritoneal cancers (National Cancer Institute, 2014a). These genes are considered highly penetrant, as the estimated cumulative lifetime risk for breast cancer is up to 87% for *BRCA1* mutation carriers and up to 84% for *BRCA2* mutation carriers by age 70 (Ford et al., 1998; Ford, Easton, Bishop, Narod, & Goldgar, 1994). Furthermore, the risk for a second primary breast cancer within five years of a first diagnosis is 20% for *BRCA1* mutation carriers and 12% for *BRCA2* mutation carriers (Verhoog et al., 1999). *BRCA1* mutation carriers have also been shown to have up to a 44% risk of developing ovarian cancer (Ford et al., 1994), while *BRCA2* mutations carriers have up to a 27% risk of developing ovarian cancer over their lifetimes (ford et al., 1998). Much progress has been documented in publications about our increasing understanding of the mechanisms by which mutations within the *BRCA1* and *BRCA2* genes predispose an



individual to the development of breast and other cancers (Rebbeck et al., 2015; Konishi et al., 2011; Miki et al., 1994).

The National Cancer Institute has described several models which predict the likelihood of a germline mutation in *BRCA1* or *BRCA2* in an individual or family (National Cancer Institute, 2014a). Two models exist which estimate risk of mutation using Bayesian analysis, employing formulation of a probability estimate based on the incorporation of familial evidence (i.e., family history of cancer or lack thereof), namely BRCAPRO (Parmigiani, Berry, & Aguilar, 1998) and BOADICEA (Antoniou, Pharoah, Smith, & Easton, 2004; Mavaddat, Rebbeck, Lakhani, Easton, & Antoniou, 2010). Both BRCAPRO and BOADICEA have been upgraded since their development to incorporate breast tumor immunohistochemical markers such as hormone receptor status (Biswas et al., 2012; Tai, Chen, Parmigiani, & Klein, 2008). Other models, such as the LAMBDA model for predicting the mutation carrier risk of Ashkenazi Jewish women, the modified Penn model (also known as Couch tables), and the Shattuck-Eidens model rely solely on logistic regression (Apicella et al., 2007; Couch et al., 1997; Evans et al., 2004; Frank et al., 1998, 2002; Shattuck-Eidens et al., 1997).

Some risk models rely on empiric observation, the most well-known of which are the prevalence tables presented by Myriad Genetics Laboratory, which use approximately 169,000 patient results acquired up to 2010 to predict the likelihood of a pathogenic mutation being present given specific conditions in the patient and in her close family members (Malone et al., 2006; Risch et al., 2001; Struewing et al., 1995; Warner et al., 1999). Empiric risk tables have several limitations. Tables often do not capture other cancers which can be associated with *BRCA1* and *BRCA2* mutations, such as pancreatic

and prostate cancer. Additionally, a table may fail to account for risk reduction surgeries such as prophylactic mastectomy and oophorectomy or for limited family history (Katki, 2007; Weitzel et al., 2007). Furthermore, empiric prediction models may underestimate or misrepresent the risk to minority groups such as Asian (Kurian et al., 2008), African-American, and Hispanic populations (Kurian et al., 2009; Vogel et al., 2007).

## **1.2 Other Breast Cancer Predisposition Genes**

Beyond *BRCA1* and *BRCA2*, mutations in several other genes are associated with an increased lifetime risk of breast cancer. Deleterious *TP53* mutations have been identified as the cause for Li-Fraumeni syndrome (LFS), a condition classically associated with an increased lifetime risk for sarcoma, brain tumors, breast cancer, leukemia, and adrenocortical carcinoma, frequently at young ages (Malkin et al., 1990; Nichols, Malkin, Garber, Fraumeni, & Li, 2001). A broader range of LFS associated tumors can include lymphomas, gastrointestinal malignancies, melanoma, and lung cancers, all of which have been documented in families with known *TP53* mutations (Gonzalez, Noltner, et al., 2009). The risk of an individual with a deleterious *TP53* mutation developing cancer by age 45 is up to 84%, with a lifetime risk of at least one type of cancer developing by age 85 at up to 100% (Ruijs et al., 2010). A report by Walsh et al. (2006) found that mutations within the *TP53* gene account for approximately 1% of breast cancers in families with histories consistent with HBOC syndrome that lacked identifiable deleterious mutations in either *BRCA1* or *BRCA2*. Diagnostic criteria for “classic LFS” includes a proband with a sarcoma under the age of 45 with a first-degree relative with cancer prior to age 45, and an additional first- or second-degree relative with cancer diagnosed before age 45, or with a sarcoma at any age (Li et al., 1988). The

Chompret criteria proposed in 2001 includes three possible diagnostic criteria, any one of which were considered sufficiently appropriate to test an individual for a germline mutation in the *TP53* gene:

- 1) a proband diagnosed with a tumor in the LFS tumor spectrum before the age of 46 (these tumors being defined as soft-tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumor, adrenal cortical carcinoma, leukemia, or lung cancer) and one or more first- or second-degree relative with a LFS-related tumor below the age of 56, **or**
- 2) a proband with multiple LFS-related tumors (excluding multiple breast tumors), two of which belong to the LFS tumor spectrum, with the initial cancer occurring before the age of 46, **or**
- 3) a proband diagnosed at any age with adrenal cortical carcinoma or a tumor of the choroid plexus, regardless of family history (Chompret et al., 2001, p. 46).

Current NCCN guidelines state that in an individual with breast cancer diagnosed at or under the age of 35 years, *TP53* mutation testing can be ordered alone, concurrently with *BRCA1/2* testing and/or other gene testing or as a follow-up test after negative *BRCA1/2* testing. (NCCN, 2015).

Deleterious mutations within the *PTEN* gene have been identified as one cause of Cowden syndrome, although many individuals who meet clinical criteria for Cowden syndrome are not found to carry a deleterious *PTEN* mutation (Pilarski, Stephens, Noss, Fisher, & Prior, 2011). Approximately 85% of patients with a clinical diagnosis of Cowden syndrome are found to carry a deleterious mutation within the *PTEN* gene (Zhou

et al., 2003). Genetic testing for cancer predisposition syndromes, especially with the use of recent multiplex molecular testing, has shown that the phenotype associated with *PTEN* mutations varies greatly. With no truly ubiquitous phenotype, the diagnostic criteria for Cowden syndrome requires amending, should it hope to erase any ambiguity between clinical diagnoses and molecular diagnoses of Cowden syndrome (Pilarski et al., 2013). With the condition's highly variable expression, it is unlikely that any clinical criteria could completely eliminate discrepancies between molecular and clinical diagnoses (R. Pilarski, personal communication, February, 2015). Cowden syndrome has been associated with benign but clinically significant features including oral papillomas, facial trichilemmomas, macrocephaly, uterine fibroids, and fibrocystic breasts (Nelen et al., 1996). Additionally, the condition is often associated with increased lifetime risks for cancers of the breast (25% to 50%), thyroid (10%), and endometrium (5%) (Bubien et al., 2013; Tan et al., 2012). Median onset of *PTEN*-related cancers occurs at 33 years of age, with one-third of all *PTEN*-related breast cancers occurring before age 30 (Birch et al., 1994; Olivier et al., 2003).

Mutations within the *STK11* gene have been identified as a cause of Peutz-Jeghers syndrome. Peutz-Jeghers syndrome is associated with large hamartomatous gastrointestinal polyps, mucocutaneous pigmentation changes, and increased lifetime risk of cancers of the breast (32 to 54%), colon (39%), stomach (29%), pancreas (11 to 36%), lungs (7 to 17%), ovaries (21%), and testicles (9%) (Giardiello et al., 2000; Hearle et al., 2006; McGarrity, Amos, Frazier, & Wei, 2013; van Lier et al., 2010).

Mutations within the *CDHI* gene have been shown to cause hereditary diffuse gastric cancer, which leads to a 70% lifetime risk of gastric cancer and a 60% lifetime

risk of lobular breast cancer (Guilford et al., 1998). Intervention for screening and diagnosis of *CDH1*-related cancers is warranted based on the gene and its significant risk level for developing breast and gastric cancers (NCCN, 2015).

These hereditary cancer syndromes, including HBOC syndrome, are inherited in an autosomal dominant manner, meaning that the inheritance of only one mutated copy of the gene is sufficient to confer a significantly increased susceptibility of developing breast and other cancers across an individual's lifetime. Thus, identifying a deleterious mutation in any of these genes in an individual has important clinical implications for that individual's close relatives regarding genetic testing and possible medical management.

Mutations in other genes have been linked to a genetic predisposition to breast cancer. Some of these genes include at least *ATM*, *CHEK2*, *PALB2*, *STK11*, *NBN*, *BARD1*, *MRE11A*, *RAD51C*, and *BRIP1* genes. *PALB2* has been found to bind directly to *BRCA2*, both genes working in concert within a broader gene complex to repair double stranded DNA breaks (Oliver, Swift, Lord, Ashworth, & Pearl, 2009; Xia et al., 2006). A recent study by Antoniou et al. (2014) showed that breast cancer risk in women with a *PALB2* deleterious mutation is significantly influenced by family history. They reported that the absolute risk of developing breast cancer ranged from 33% by age 70 in an individual with no family history of breast cancer, up to at least 58% by age 70 in a woman who has two or more first-degree relatives with breast cancer diagnosed by age 50 years.

Mutations within the *CHEK2* gene confer an increased lifetime risk of breast cancer of about 23% to 48% based on susceptibility due to the founder mutation, *CHEK2*\*1100del C, in individuals of Northern and Eastern European descent. Due to this

mutation which results in a truncated protein, carriers also have a significantly increased risk of a second breast cancer (Weisher et al., 2012). In their study of over 25,000 women with breast cancer, 1.8% were found to carry this mutation. Additionally, mutations within the *CHEK2* gene confer an increased lifetime risk for colorectal cancer that is estimated at about 7.2% to 9.5% by a meta-analysis reported by Xiang, Geng, Ge, & Li (2011).

Homozygous mutations within the *ATM* gene have previously been associated with ataxia-telangiectasia, an autosomal recessive disorder that presents with childhood onset of progressive neurodegeneration, telangiectasia, immunodeficiency, gonadal atrophy, and a predisposition to malignant tumor growth (Morrell, Cromartie, & Swift, 1986). Heterozygous carriers of mutations within the *ATM* gene have an elevated risk of developing breast cancer, with a lifetime risk of approximately 17% to 52% (Ahmed & Rahman, 2006; Swift, Morrell, Massey, & Chase, 1991; Thompson et al., 2005).

Germline mutations within the *NBN* gene lead to a higher incidence of childhood onset acute lymphoblastic leukemia. When inherited in a homozygous manner, deleterious mutations in both alleles of the *NBN* gene can cause Nijmegen breakage syndrome, a condition which causes microcephaly, short stature, immunodeficiency, and increased chance of malignant tumor growth (Varon et al., 1998; Varon et al., 2001). Other deleterious mutations in the *NBN* gene have been recently reported to cause an increased susceptibility to breast cancer in small numbers of patients (Damiola et al., 2014; Kurian et al., 2014) and individual cases (E. Jordon, personal communication, November, 2014). The lifetime risk in women who carry an *NBN* deleterious mutation is estimated to be up to 30% by age 80, with the most common variant, c.657del15, acting as

a founder mutation in patients of Slavic ancestry. This mutation is known to confer an approximately 30% lifetime risk for breast cancer in the Eastern European population (Steffen et al., 2006; Zhang, Beeghly-Fadiel, Long, & Zheng, 2011). Additionally, individual polymorphisms in the *NBN* gene as, reported by Berardinelli, di Masi, & Antoccia (2013), have been associated with small increased lifetime risks for basal cell carcinoma, leukemia, lymphoma, melanoma, medulloblastoma, and cancers of the bladder, colon, liver, ovaries, ovaries, prostate, kidneys, and lungs . There are no currently widely accepted guidelines for the medical management of individuals with an *NBN* mutation.

Cancer susceptibility conferred by the *BARD1*, *MRE11A*, *RAD50*, *RAD51C*, and *BRIP1* genes is not well defined and relies heavily on case studies or sequencing studies of limited size. Additional segregation studies and collaborative case-control studies are required to further define the associated cancer risks for of these genes (Bartkova et al., 2008; Ellis & Offit, 2012; Rainville & Rana, 2014; Seal et al., 2006; Southey et al., 2013). At this time, intervention for breast cancer screening and diagnosis is not recommended due to insufficient evidence for intervention based on mutations in these genes. However, intervention may still be warranted based on family history or other clinical factors (NCCN, 2015). One example is the *BRIP1* gene, in which mutations were reported to confer a high risk (up to 8.3%) of ovarian cancer (Rafnar et al., 2011). In one family, an unaffected female with family history of two close relatives diagnosed with primary peritoneal cancer was found to carry a deleterious *BRIP1* mutation. She elected prophylactic surgery (bilateral salpingo-oophorectomy) as likely risk-reduction management (P. Walker, personal communication, September, 2014).

### 1.3 Sequencing Options

Since 1977, the gold standard for sequencing genetic information has been Sanger sequencing, a method of DNA sequencing which utilizes chain-terminating inhibitors of DNA polymerase to determine a nucleotide sequence (Sanger, Nicklen, & Coulson, 1977). The capacity of this method is typically up to 96 sequences of approximately 400-500 nucleotides per run (Shendure, Mitra, Varma, & Church, 2004). Historically, DNA sequencing of the *BRCA1* and *BRCA2* gene were completed by Sanger sequencing. This technique requires bases be added one by one, leading to a relatively time-consuming and costly process. For this reason, genetic testing for any of the known hereditary cancer syndromes was most often limited to typically only one or two genes at a time and was available at a high cost when Myriad Genetics Laboratory, first began offering commercial sequencing of *BRCA1* and *BRCA2* in 1996 (Gold & Carbone, 2010).

In 2005, Margulies et al. at 454 Life Sciences Corp introduced a new method of sequencing, which utilized massive parallel sequencing technologies to sequence up to 25 million bases in a single run (Margulies et al., 2005). This method offered an approximately 100-fold increase in throughput over traditional Sanger sequencing methods and laid the framework for multiple contemporary sequencing technologies with competitive throughput, all called next generation sequencing (NGS) (Schuster, 2008; Vogelstein et al., 2013). NGS has demonstrated the advantage of offering much shorter read lengths when compared to Sanger sequencing, and can process far more sequences in a single run, allowing for the processing of several genes or even a patient's entire genome with 90% to 95% coverage (Cirulli et al., 2010; Wheeler et al., 2008). Additionally, turn-around-time is considerably shorter using NGS due to its multiplex



configuration of sequencing reactions. However, one possible limitation to consider is coverage of specific regions by NGS which can be affected by repetitive nucleotide content as well as GC-rich content (Rehm et al., 2013).

Myriad Genetics Laboratory initially held patents on both *BRCA1* and *BRCA2* which lasted until about 2012. With the 2013 Supreme Court decision (*Association for Molecular Pathology et al. v. Myriad Genetics, Inc.*, 2014) and competing technological advancements, i.e., NGS technology, the possibility of multiplex cancer molecular testing has now become a reasonable clinical tool offered by multiple companies to identify which patients carry a hereditary deleterious mutation in one of numerous genes that can significantly increase their risk of developing breast cancer. The legal decision and this technological shift have enabled a change in in *BRCA1* and *BRCA2* hereditary cancer testing. Patients and healthcare professionals can now consider whether testing only the genes most often associated with an increased lifetime risk for breast cancer (*BRCA1* and *BRCA2*), or testing all clinically actionable genes currently known to be associated with breast cancer predisposition syndromes is more appropriate (Rehm et al., 2013).

Multiplex cancer molecular testing can also include genes associated with cancer development in other parts of the body not associated with HBOC, but with overlapping susceptibilities to common cancers. For example, a colon cancer gene panel for Lynch syndrome can include *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*. The typical phenotype of Lynch syndrome overlaps with HBOC syndrome through a significantly increased lifetime risk of ovarian cancer. Lynch syndrome may also increase the lifetime risk for breast cancer (Buerki et al., 2012; Win, Lindor, & Jenkins, 2013), but that remains ill-defined (Cohen & Leininger, 2014). Current comprehensive multigene cancer genetic

testing offerings for hereditary breast and hereditary ovarian cancers now may include some or all of the genes associated with increased susceptibility of breast and/or ovarian cancer if a deleterious mutation exists.

With the discovery of multiple additional genes associated with inherited cancer predisposition syndromes, various researchers have attempted to estimate the *de novo* mutation rate among these genes. In a single study of 193 patients with sporadic breast cancer, one patient was found to have a single new mutation in the *BRCA1* gene, suggesting from this small study that the *de novo* mutation rate for *BRCA1* and *BRCA2* may be low, likely less than 5% (De Leeneer et al., 2012). Conversely, the *de novo* rates among the *PTEN*, *STK11*, and *TP53* genes are thought to be much higher, approximately within the range of 10% to 30% *de novo* mutation rate (Gonzalez, Buzin, et al., 2009; Schreibman, Baker, Amos, & McGarrity, 2005; Westerman et al., 1999). Through sequencing of one or more genes, *de novo* mutations that cause either a loss of function or gain of function can be detected within the gene that may interfere with its functionality and may explain some apparently isolated cases of early-onset “hereditary” breast cancer in an individual with no family history of the disease.

#### **1.4 Management and Anxiety**

Healthcare providers, particularly genetic counselors, typically understand which patients are the best candidates for genetic testing, given the guidance provided by NCCN and other professional societies which issue their own guidelines for possible genetic testing of women with breast cancer. Healthcare providers who are ordering genetic testing in today’s breast cancer genetic environment must consider the most appropriate testing for each patient. Among populations where the carrier rate for deleterious *BRCA1*

and *BRCA2* mutations is highest, and in which there are known common mutations, such as the Ashkenazi Jewish population, population-based screening for the three most common mutations has been found to be the most cost-effective first step in genetic testing (Manchanda et al., 2015) and is supported by the current NCCN guidelines (NCCN, 2015). In all cases, the patient's personal medical history and a three-generation family history with identification of ancestry are recommended to help guide the healthcare provider in providing an accurate risk assessment for the patient (NCCN, 2015), which then leads to an appropriate decision about testing and which type of testing, e.g., single gene analysis of *BRCA1/2*, or a more comprehensive multiplex analysis of multiple susceptibility genes as described above.

Molecular testing for breast cancer predisposition syndromes often influences healthcare decisions involving risk-reducing surgical measures. Because individuals who carry a pathogenic mutation in either the *BRCA1* or *BRCA2* are at significantly increased risk for developing breast or ovarian cancer, many women opt to undergo risk-reducing bilateral mastectomies and/or risk-reducing bilateral salpingo-oophorectomy (BSO). Prophylactic mastectomies have been shown to reduce the risk of breast cancer by about 90% (Rebbeck et al., 2004), while prophylactic BSO has been shown to reduce the risk for ovarian cancer by up to 96% (Chang-Claude et al., 2007). This surgery has been reported to also reduce the risk for breast cancer by about 50% when performed in premenopausal women (Eisen et al., 2005; Kauff et al., 2008; Rebbeck et al., 1999; Rebbeck, Kauff, & Domchek, 2009). The benefits of prophylactic mastectomy are further evidenced by a reduction in mortality among women with *BRCA1/BRCA2* associated breast cancer (Evans et al., 2013).

In the past decade, uptake of risk-reducing surgical intervention has increased among unaffected *BRCA1/BRCA2* carriers and non-carriers with a strong family history of breast cancer. Community-based physicians have begun incorporating *BRCA1/BRCA2* testing into their practice. In 2008, Keating, Stoeckert, Regan, DiGianni, & Garber reported that geneticists and gynecologists were less likely than medical oncologists and surgeons to recommend prophylactic mastectomy among unaffected women with a known *BRCA1* mutation. Uptake of risk-reducing surgery was reported to be dependent on factors such as lifetime risk, age, and time since testing (Evans et al., 2009), with the strongest influencing factor being a negative or positive genetic test result (Hawley et al., 2014). Another influential factor reported among unaffected carriers was having a family history of death(s) due to cancer. Those who had experienced the death of a relative due to cancer perceived breast cancer as having a “stronger identity” with more “dire consequences” and as being “uncontrollable” when compared to carriers who had not experienced the death of a relative (Samama, Hasson-Ohayon, Perry, Morag, & Goldzweig, 2014).

Differences in uptake between risk-reducing mastectomy and BSO are partially dependent on results of genetic testing, as women who receive an uninformative result from genetic testing were less likely to see the positive aspects of risk-reducing oophorectomy than those who received a positive result (O’Neill et al., 2010). Regardless, a minority of unaffected women who received an uninformative result from *BRCA1/BRCA2* testing still underwent risk-reducing surgery, with 6.8% undergoing risk-reducing mastectomies and 13.3% undergoing risk-reducing BSO (Schwartz et al., 2012). Factors associated with uptake of risk-reducing mastectomy and oophorectomy included

perceived risk of breast and ovarian cancer, education, age, marital status, and perceived risk of carrying a *BRCA1/BRCA2* mutation despite receiving uninformative results. This finding underscores the critical need for comprehensive pre-test and post-test counseling (Tong et al., 2014).

Genetic testing may or may not return a clear answer as to the genetic cause of a patient's hereditary susceptibility to cancer. Possible results can include a negative finding in any gene tested, a known deleterious mutation (positive result), or a variant of uncertain significance (VUS). Negative findings are considered uninformative when the individual tested is the first person in the family to be tested, or when an unaffected person with cancer in the family without previous genetic analysis is tested. While a negative result can be reassuring, limitations exist that do not completely rule out the possibility of a mutation exists in the family but could not be detected. A true negative result is reported only in the circumstance where a deleterious mutation is known to exist within the patient's family, but the specific mutation was not found within the relative who underwent testing specifically for the known familial mutation.

As is typically explained in a genetic counseling session, a negative result does not conclusively rule out the possibility of a hereditary cancer predisposition syndrome for several reasons. Negative testing for a familial mutation does not rule out the possibility of other findings, such as a mutation other than what is being specifically tested for, or a finding in a different gene. Regions exist within every gene that cannot be sequenced, leading to residual risk for deleterious mutations among genes sequenced despite negative results. Negative *BRCA1* and *BRCA2* results do not rule out the possibility of changes within other genes known to cause an increased lifetime risk for

breast cancer. Likewise, negative genetic testing results also do not exclude the possibility of increased risk due to mutations in currently undiscovered genes, low-risk susceptibility genes, or gene-environmental interactions (Mincey, 2003) or possible epigenetic changes to the gene of interest.

The risk of having a VUS result returned is considerably increased when testing multiple genes (Hilbers, Vreeswijk, van Asperen, & Devilee, 2013). The International Agency for Research on Cancer (IARC) supports a common classification system which would facilitate standardized categorization of VUS results by statistical models of pathogenicity (Goldgar et al., 2008; Thompson et al., 2014). Five classes of possible findings exist as follows:

Class 1: negative;

Class 2: likely not pathogenic (i.e., likely benign);

Class 3: uncertain pathogenicity, i.e., variant of uncertain significance (VUS);

Class 4: likely pathogenic; or

Class 5: positive (i.e., pathogenic).

Classification of a finding into one of the above categories often requires statistical analysis by activity models (Guidugli et al., 2014). Reclassification of a VUS may be simplified by an accurate and detailed family history if several affected relatives are found to have the same variant. While counseling regarding clinical management for a known deleterious mutation is typically well-defined for the most well understood cancer predisposition genes, guidelines for clinical management for VUS results are not available and medical management is advised to be based on the individual's personal and family history without consideration of the VUS report. In these situations,

information regarding penetrance is limited, sometimes solely to the individual's family history. Counseling regarding screening and risk reduction must be individualized to the patient in these situations (Plon et al., 2008).

Another step in the process of genetic counseling is to assess the level of anxiety in patients and assist in their understanding and adaptation to their genetic test results (Resta et al., 2006). Negative test results have been shown to significantly reduce perceived risk and distress when compared to those patients who receive a positive mutation result (Croyle, Smith, Botkin, Baty, & Nash, 1997; Schwartz et al., 2002). Patients who receive a positive result typically experience heightened distress after disclosure concerning their risk for cancer, while non-carriers have been shown to have decreased psychological distress (Meiser et al., 2002; van Roosmalen et al., 2004). Prolonged intervention rather than a single informational session is a better strategy for reducing distress and meeting information needs in the case of a positive result (White et al., 2014). Patients with an uncertain result, defined as -a VUS, have been shown to have increased anxiety related to testing (O'Neill et al., 2009), but markedly less than those who receive a positive result (Smith et al., 2008). Additionally, patients who receive uncertain results have been shown to experience decisional conflicts regarding prophylactic measures for risk reduction (Rini et al., 2009). While patients with a VUS result are more likely to overestimate their associated cancer risk than patients who receive a positive result, they are also more likely to have infrequent screening compared to those who receive a positive result (Vos et al., 2012).

As discussed previously, a positive finding or a VUS can be detected in any gene being sequenced and can often influence patient misunderstandings of the results, causing

anxiety and distress. These results may complicate medical recommendations as VUS results do not influence medical recommendations for management. As Domchek, Bradbury, Garber, Offit, & Robson (2013) stated, the likelihood of detecting a VUS is directly related to the number of genes being sequenced. Therefore, multiplex testing presents a significant risk of yielding uncertain results as multigene testing can currently include up to 40 genes depending on the test provider. VUS results are more likely to generate inappropriate recommendations regarding patient care by the physician (Plon et al., 2011). Richter et al. therefore recommend that the field would benefit from additional education for referring physicians to make them aware of the possibility of VUS results and the clinical uncertainty they can present (2013). Furthermore, formal risk assessments should be based on the counselee's personal and family cancer history and should not be affected by VUS results, as uncertain results do not significantly decrease the probability of cancer within families with a significant history of cancer (Gadzicki et al., 2011; Plon et al., 2011). Healthcare providers should work to establish VUS-related guidelines for disclosure, management, and follow-up, as no clear risk guidelines presently exist (Gadzicki et al., 2011; Richter et al., 2013).

This pilot study explored the differences between anxiety levels for patients who participated in classic single-gene genetic testing for HBOC due to *BRCA1* and *BRCA2* mutations associated with breast cancer, compared with those who chose cancer genetic testing by multigene panels for inherited breast cancer predisposition syndromes. The study explored three areas: the overall anxiety changes associated with classic genetic testing versus expanded panel testing; how negative, positive, or VUS results can differentially affect anxiety; and how patient anxiety is affected by the gene in which a



genetic change was found. We hypothesized that a positive or VUS finding within a gene other than *BRCA1* or *BRCA2* will induce increased anxiety over anxiety measured due to a positive or VUS finding in *BRCA1* or *BRCA2*, as these are the classically “expected” genes for breast cancer predisposition syndromes. If we find that anxiety levels differ significantly depending upon which gene gives a negative, positive, or VUS result, this psychosocial information could be of value in influencing how genetic counselors and other medical professionals discuss genetic testing options. The information gained by this pilot study is expected to be beneficial for healthcare providers and their patients, if proven in an upcoming multisite study that we anticipate will demonstrate significantly different levels of anxiety dependent on the above factors. Gaining a better understanding of how multiplex genetic test results can influence levels of anxiety will benefit genetic counselors and their patients, as genetic counselors can then better address patient’s concerns and anxiety more specifically in future sessions.

## Chapter 2: Manuscript

### Evaluating Changes in Patient Anxiety Regarding Classic Cancer Genetic Testing Versus Expanded Multiplex Cancer Genetic Testing<sup>1</sup>

#### 2.1 Abstract

Multiplex cancer genetic testing by next generation sequencing (NGS) offers genetic counselors and patients new options for testing multiple genes beyond *BRCA1* and *BRCA2*, increasing both the yield of positive results and the number of variants of uncertain significance (VUS). This pilot study explored three psychosocial questions related to multiplex cancer genetic testing: 1). Do anxiety levels differ in patients with results of familiar single gene testing versus those with results of multiplex cancer genetic testing of unfamiliar genes? 2). Do different results (i.e., negative, positive, or VUS) affect patient anxiety levels in the post-results period? 3). Is patient anxiety affected by the specific gene in which the mutation or VUS is identified? The study included women diagnosed with breast cancer and considered at high risk for a hereditary cancer syndrome due to age or family history. Participants completed a baseline State-Trait Anxiety (STAI) questionnaire at the pre-test genetic counseling session, and completed the same STAI questionnaire and a Multidimensional Impact of Cancer Risk Assessment (MICRA) questionnaire after the post-results discussion. Twenty individuals participated, of which 17 patients completed the baseline STAI tool. Nine participants completed all questionnaires, yielding five participants with negative results, two with

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<sup>1</sup>Alfonso, A., Walker, P., Chapman, C., & Dobek, W. To be submitted to *Journal of Genetic Counseling*

positive results, and two with VUS results. Two participants with negative results showed significant baseline anxiety levels which decreased in the post-results period. The positive and VUS result groups showed non-significantly increased mean anxiety levels by STAI. Differences in anxiety between those with positive results and those with negative results trended toward significance. Two individuals with positive mutation results in genes other than *BRCA1* and *BRCA2* showed higher post-results anxiety levels on the MICRA scale than did two participants with VUS results. The study was limited by sample size. A larger multi-site study is planned to clarify anxiety, distress, and uncertainty parameters to help guide genetic counselors in their approach to psychosocial aspects of multiplex cancer genetic testing.

## **2.2 Introduction**

Five to ten percent of breast cancer cases are caused by an inherited germline mutation that confers an increased lifetime susceptibility to breast cancer development and may be accompanied by increased susceptibility to ovarian, prostate, pancreatic, and other specific cancers (USPSTF, 2009). The most common genes currently known to be associated with an increased lifetime risk for breast cancer are the *BRCA1* and *BRCA2* genes. The effects of various deleterious mutations in these two genes lead to a condition known as Hereditary Breast and Ovarian Cancer syndrome (HBOC). Other genes have been reported which have been shown to be associated with cancer predisposition syndromes in which a high risk for breast cancer is present. Some genes have clear associated lifetime risks, as well as guidelines for screening, including *TP53*, *PTEN*, *CDH1*, and *STK11*. Mutations in *PALB2*, *CHEK2*, *ATM*, *NBN*, *BARD1*, *MRE11A*, *RAD50*, *RAD51C*, and *BRIP1* genes have been linked to a genetic predisposition to breast

cancer, but do not have well defined screening guidelines. Molecular testing for breast cancer predisposition syndromes often influences healthcare decisions involving risk reducing surgical measures. Prophylactic mastectomies have been shown to reduce the risk of breast cancer by about 90% (Rebbeck et al., 2004). Prophylactic oophorectomies have been shown to reduce the risk for ovarian cancer by up to 96% (Chang-Claude et al., 2007), and to reduce the risk for breast cancer by about 50% when performed in premenopausal women (Eisen et al., 2005; Kauff et al., 2008; Rebbeck et al., 1999, 2009). Uptake of risk-reducing surgery is a complex decision based on clinical factors such as lifetime risk, age, and genetic test results, and often times on emotional factors such as the death of a relative due to cancer and time since genetic testing (Evans et al., 2009; Samama et al., 2014). Hawley et al. stated that the strongest influencing factor in this decision is clinical genetic test results (2014).

Possible genetic test results include a negative result, a known deleterious mutation (positive result), or a variant of uncertain significance (VUS) result. Several studies have attempted to explore the psychological impacts of genetic test results. Negative test results from *BRCA1* and *BRCA2* genetic testing have been shown to significantly reduce perceived risk and distress when compared to those patients who received a positive mutation result (Croyle et al., 1997; Schwartz et al., 2002). Furthermore, patients who received a positive result typically experience heightened distress after disclosure about their risk for cancer, while non-carriers have shown decreased psychological distress (Meiser et al., 2002; van Roosmalen et al., 2004). Rini et al. reported that patients who received VUS results have experienced decisional conflicts regarding prophylactic measures for risk reduction (2009). While patients with a VUS result were more likely to

overestimate their associated cancer risk than patients who received a positive result, those receiving a VUS results were also more likely to have infrequent screening than those who received a positive result (Vos et al., 2012). VUS results were more likely to generate inappropriate recommendations regarding patient care by the physician (Plon et al., 2011). Richter et al. recommended that the field would benefit from additional education for referring physicians to make them aware of the possibility of VUS results as well as the associated clinical uncertainty (2013).

We explored the differences between anxiety levels for patients who participated in classic cancer genetic testing for a single hereditary breast cancer syndrome to those who chose cancer genetic testing by multiplex cancer genetic testing for multiple hereditary breast cancer predisposition syndromes. This study explored three areas: the overall anxiety changes associated with classic genetic testing versus anxiety associated with multiplex cancer genetic testing; how different results can affect levels of anxiety; and if patient anxiety is affected by the gene in which a genetic change was found.

## **2.3 Materials and Methods**

**2.3.1 Participants.** This study targeted adult women and men at high risk for a hereditary breast cancer syndrome based on personal and family history. Participants were invited to participate in the study if they met the following criteria:

- Women or men who had been clinically diagnosed with breast cancer and who qualified for genetic testing related to HBOC due to personal and/or family history of cancer, based on the most recent guidelines from the National Comprehensive Cancer Network (NCCN, 2014),
- Participants who were fluent in English,

- Participants with recognized competence to read and understand the written material.

Participants were excluded from the study if any of the following criteria were met:

- Individuals who did not have a clinical diagnosis of breast cancer,
- Individuals undergoing targeted testing for a known familial mutation,
- Individuals who did not qualify for genetic testing, based on the most recent Guidelines from NCCN, 2015,
- Participants whose reading comprehension was judged insufficient to understand the questionnaire information,
- Individuals not fluent in English.

**2.3.3 Study Measures.** Demographic questions collected information regarding the participants' gender, ethnicity, highest level of education achieved, and number and gender of biological children were collected.

**2.3.3.1 State Trait Anxiety Inventory.** The 20-item State-Trait Anxiety Inventory (STAI) questionnaire (See Appendix B) was used to establish a baseline anxiety level among participants at the time of genetic testing, following pre-test genetic counseling. The post-results STAI questionnaire was provided approximately one week following the discussion of the patient's genetic test results. These paired surveys evaluated possible changes in levels of anxiety experienced before and after genetic test results were reported. In this pilot study, STAI responses were obtained over the phone by the principal investigator. The statements on the STAI questionnaire were read to the participants, and they were asked to choose which Likert scale response best matched

their current emotional state. A weighted STAI value of 47 or greater was indicative of statistically significant anxiety in either instance.

**2.3.3.2 *Multidimensional Impact of Cancer Risk Assessment.*** The Multidimensional Impact of Cancer Risk Assessment (MICRA) Scale was used to examine levels of distress among participants following their genetic test result disclosure (Cella et al., 2002). Permission to use the MICRA scale for this study was granted by the questionnaire's lead author. The MICRA questionnaire was provided to participants whose genetic test results included a positive result or VUS finding, but not to patients with a negative result. The statements as found on the MICRA questionnaire (see Appendix C) were read to the participants over the phone by the study's principal investigator approximately one week following results disclosure. Participants were asked to choose which Likert scale response best matched their current emotional state regarding their genetic test results. We calculated the mean and standard deviation of the four MICRA score values of participants with positive or VUS results. Typical range of statistical significance would be plus or minus 0.5 standard deviation of the mean in a large group of participants who received negative results (D. Cella, personal communication, March, 2015).

A brief section of Likert scale questions assessing the patient's understanding of their results, as well as how they felt their results would influence their healthcare management was included. These Likert scale questions used a 1 to 4 scale of Strongly Disagree to Strongly Agree. These questions also assessed with whom, if anyone, the patient intended to share her results.

**2.3.3 Study Methods.** Patients who underwent initial genetic counseling and who qualified for genetic testing for one or more hereditary cancer syndromes were invited to participate in the study. At the end of the initial genetic counseling session, and prior to genetic testing, eligible patients were informed of the study and then given the letter of study introduction by their genetic counselor. At that time, participants were given a print copy of the baseline State-Trait Anxiety Inventory (STAI) to be completed in the waiting room or to be taken home and returned to our center via a self-addressed postage-paid envelope. Blood was drawn for genetic testing typically on the same day and shipped to the appropriate laboratory. Results of genetic testing were provided to the participants by telephone. Patients with a VUS or positive result were encouraged to return for a post-results counseling session. At least one week following the date on which the participant's genetic test results were discussed, the participant was called by the principal investigator to schedule a time when the two follow-up questionnaires (the post-result STAI and the MICRA Scale) could be administered over the telephone as explained above.

Each participant was assigned a unique alpha-numeric identifier (e.g., A101, A102, A103, etc.) that was coded on each of the three questionnaires for confidential comparison of anxiety experienced by the participant at each stage of the study. The genetic counselor was provided a preconfigured electronic spreadsheet for recording patient information, genetic test selected, and test results. Personal identifying information and genetic test results were removed from the study spreadsheet by the genetic counselor before contact information was provided to the principal investigator. The patient information given to the principal investigator for administering the



questionnaires included only the patient's phone number and surname. Personal information was destroyed by shredding after phone calls were completed. This research study was approved by the Institutional Review Board, Office of Research Compliance, of the University of South Carolina, Columbia, SC, in September, 2014.

**2.3.4 Data Analysis.** Data from patient demographic questions were analyzed using descriptive analysis, including percentages, proportions, means, and ranges of values. See Table 2.1 below for specific demographic information.

The results of the baseline STAI questionnaire for anxiety levels were scored according to instructions provided with that tool, resulting in a State Anxiety Score for each participant. Anxiety scores were analyzed by results grouping and compared by paired sample t-test for possibly significant differences in anxiety state before genetic testing and after results were reported to them to ascertain possible differences in anxiety levels due to the type of result received.

The results of the MICRA scale were analyzed according to the instructions provided with that tool. The scale results in three subscales including Distress, Uncertainty, and Positive Experiences. The individual composite scores were used in the following quantitative analyses where appropriate and were analyzed by descriptive statistics.

In addition, a chi-square goodness-of-fit test was used to analyze the scores from the MICRA questionnaire using gene identity and positive or VUS results as variables.

Mann-Whitney U-test was conducted on this nonparametric data set to determine if there were differences in anxiety scores measured by the MICRA questionnaire between the negative ( $n = 5$ ), positive ( $n = 2$ ), and VUS ( $n = 2$ ) subgroups.

**Table 2.1 Participant Demographics and Anxiety Levels (N = 17)**

Variable	n	Response	(%)
Diagnosed in past 5 years	10	Yes	(59%)
	7	No	(41%)
Age	2	30-39	(12%)
	4	40-49	(24%)
	5	50-59	(28%)
	4	60-69	(24%)
	2	70-79	(12%)
Gender	17	Female	(100%)
Ethnicity	13	Caucasian	(76%)
	3	Black	(18%)
	1	Hispanic	(6%)
Education	2	High School (diploma or GED)	(12%)
	5	Some College	(29%)
	3	Associate's Degree	(18%)
	5	Bachelor's Degree	(29%)
	2	Beyond Bachelor's Degree	(12%)
Marital Status	13	Married	(77%)
	3	Divorced	(18%)
	1	Remarried	(6%)
Children	2	Daughters only	(12%)
	4	Sons only	(24%)
	11	Both	(65%)

Kruskal-Wallis analysis was conducted to determine if there were differences in anxiety amongst the three groups of participants (negative, positive, and VUS findings by genetic testing) as assessed by the MICRA questionnaire.

## 2.4 Results

Seventeen participants completed the baseline STAI questionnaire and were enrolled in the study. Table 2.1 provides the sociodemographics of the 17 individuals who

completed the baseline questionnaire. All participants received the post-result questionnaire packet, which included the post-result STAI and the MICRA questionnaires, to be completed at home and returned to our center by pre-paid envelope. Nine participants completed all questionnaires, yielding a 53% response rate. Of these nine participants, all had undergone multiplex cancer genetic testing. Five (56%) received negative results (no mutation and no VUS), two (22%) received positive results of pathogenic mutations, and two (22%) received VUS results.

The mean weighted score of the post-result STAI questionnaire was 37 ( $SD = 9.6$ ) across all groups. Among participants who received a negative result ( $n = 5$ ), the mean weighted score of the baseline STAI questionnaire was 39 ( $SD = 11$ , range = 28 to 50) and the mean weighted score of the post-result STAI questionnaire was 34 ( $SD = 10$ , range = 24 to 44). Among participants who received a negative result, paired sample t-test showed a mean decrease in anxiety score of 4.8 ( $SD = 13.85$ ,  $p = .808$ ). Two of the five participants with negative results displayed statistically significant anxiety by baseline STAI (47 and 53, respectively). Both showed decreased anxiety scores on the post-results STAI (43 and 31, respectively) following a negative result.

The two participants who received a positive result differed in the specific genes found to have deleterious mutations. The individual with the *ATM* mutation was also found to have a VUS in the *BMPRIA* gene. This individual's baseline anxiety score was 25 and increased to 53 in the post-results period, which is considered statistically significant. The second participant, who received a positive mutation result in the *TP53* gene, had weighted anxiety scores of 34 (baseline) and increased to 40 (post-results), which did not reach statistical significance for anxiety. Among the two participants who

received a positive mutation result, paired sample t-test showed a mean increase in anxiety score of 17 ( $SD = 15.56, p = .366$ ).

For the two participants who received a VUS result, the weighted scores of the initial STAI questionnaire were 20 and 27. Weighted scores of the STAI post-results questionnaire were 20 and 40 respectively, showing an increase in anxiety in one of two participants and no change in the other. For these two participants, paired sample t-test showed a mean increase in anxiety score of 6.5 ( $SD = 9.19, p = .000$ ), which was statistically significant; these results are shown in Figure 2.1.

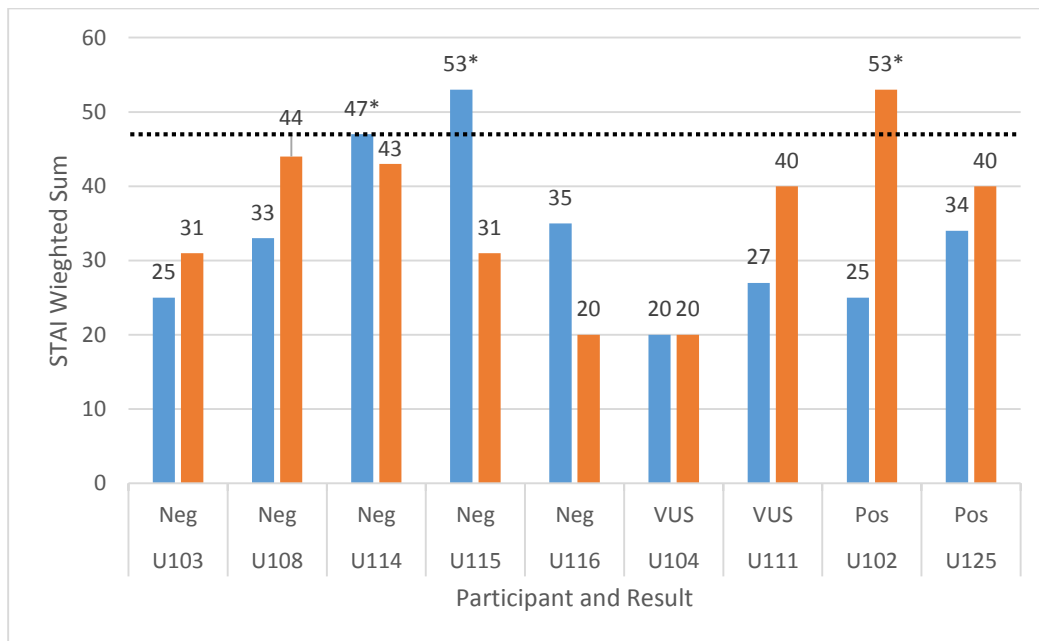


Figure 2.1 Weighted Baseline and Post-Results STAI Scores

Note: Values of 47 or greater are considered a statistically significant level of anxiety.

Using the Mann-Whitney U-test, comparison of the median MICRA scale scores from the positive mutation group (mean rank = 84.5) and the VUS group (mean rank =

34.5) did not differ significantly,  $U = .000$ ,  $z = -1.549$ ,  $p = .121$ . Comparison of the median values of the negative mutation group (mean rank = 23.8) and the VUS group did not differ significantly,  $U = 3.00$ ,  $z = -.782$ ,  $p = .434$ . Comparison of the median values of the positive mutation group and the negative group trended toward significance,  $U = .000$ ,  $z = -1.954$ ,  $p = .051$ .

Using Kruskal-Wallis analysis, anxiety scores from the MICRA questionnaire increased from the negative result group (Median = 19), to the VUS group (Mean = 34.5), to the positive group (Mean = 84.5). When cross-tabulated by chi-squared analysis, differences between MICRA scores of the three groups were not significant, but appeared to be trending toward significance,  $\chi^2(9) = 4.612$ ,  $p = .100$ . Comparison of baseline STAI and post-result STAI scores among the three groups did not demonstrate significantly different associations of anxiety levels based on test results,  $\chi^2(9) = 2.968$ ,  $p = .227$  and  $\chi^2(9) = 2.062$ ,  $p = .357$  respectively.

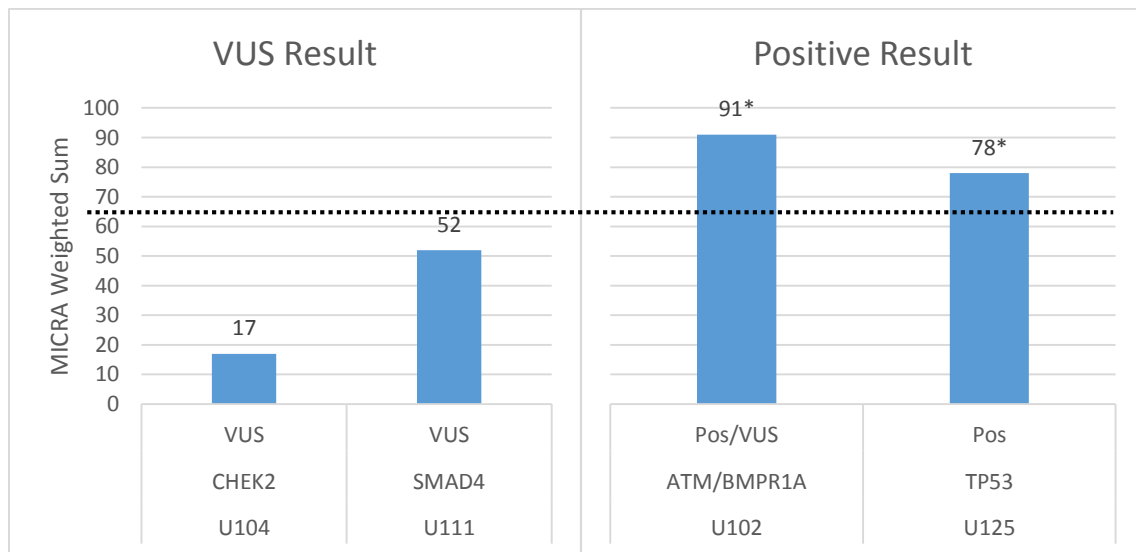


Figure 2.2 A Pilot study Example of MICRA Scale Results

Note: Statistically significant levels could not be calculated due to small sample size.

Knowledge questions regarding how the participants planned to use their results were asked of those participants who received a VUS or a positive mutation result. The responses available to each question were, Strongly Disagree; Somewhat Disagree; Somewhat Agree; and Strongly Agree. Only one participant with a VUS result responded. Table 2.2 shows the responses from this participant, and Table 2.3 shows the responses from the two participants with positive mutation results.

**Table 2.2 Participant Intention to Use VUS results**

Statement	Responses from Participants with VUS Result
I felt well-prepared from my first counseling session about the possibility of getting a VUS result.	Somewhat agree
When I got the VUS result, I felt more anxious about the result than I thought I would.	Somewhat agree
I expect that my doctors will help me make healthcare decisions based on my VUS result.	Strongly agree
I intend to share my VUS results with my children so that they can be tested.	Strongly agree
I intend to share my VUS results with my other family members (parents, sisters, brothers, aunts/uncles/ cousins) so that they can be tested.	Strongly agree
Why or why not would you share your VUS result?	“I want my other family members to have the choice to know it that are positive or negative and to be as proactive as possible in making decisions about their lives based on the results [ <i>sic</i> ]”

**Table 2.3 Participants' Intention to use Positive Mutation Results**

Statement	Response from Participants with Positive Mutation Result
I felt well-prepared from my first counseling session about the possibility of getting a positive mutation result.	1) Somewhat agree 2) Somewhat agree
When I got the positive mutation result, I felt more anxious about the result than I thought I would.	1) Strongly agree 2) Strongly agree
I expect that my doctors will help me make healthcare decisions based on my positive mutation result.	1) Strongly agree 2) Strongly agree
I intend to share my positive mutation results with my children so that they can be tested.	1) Strongly agree 2) Strongly agree
I intend to share my positive mutation results with my other family members (parents, sisters, brothers, aunts/uncles/ cousins) so that they can be tested.	1) Strongly agree 2) Strongly agree
Why or why not would you share your positive mutation result?	1) "I will share my result so my family can be informed and watch for warning signs" 2) No response

## 2.5 Discussion

Data collected for analysis during this pilot study often did not meet statistical significance by analytical methods primarily due to sample size. We note that we are reporting VUS and mutation results in genes other than *BRCA1* and *BRCA2* in this pilot study. A full study is planned and will be expanded to multiple sites with a greater number of participants anticipated. We expect that increased participation will help to clarify answers to the goals set forth in this study. Initial data on this small sample supports previous studies conducted by Croyle, Smith, Botkin, Baty, & Nash (1997) and Schwartz et al. (2002) which found that negative genetic test results lead to significantly

reduced perceived risk and distress when compared to those who received a positive mutation result from *BRCA1* or *BRCA2* genetic testing. However, our participants underwent multiplex cancer genetic testing of 21 to 29 genes for each individual. Analysis of data collected during our pilot study showed that the changes in the levels of anxiety experienced by the negative group and the mutation positive group trended toward significance, suggestive of a similar association as reported by the above authors when their participants receive a positive test result. Additionally, analysis of differences in distress and anxiety between the negative, positive, and VUS result groups, as measured by the MICRA questionnaire, trended toward significance.

O'Neill et al. (2009) previously reported that patients who receive an uncertain result (i.e., VUS) have been shown to have increased anxiety. A single participant from our initial data displayed a similar association, as self-reported anxiety for this individual was elevated between baseline and post-result STAI. The second VUS participant had no reported increase in self-reported anxiety between initial and post-result STAI. However, neither participant receiving a VUS result had clinically significant anxiety levels. Data is also supportive of previous findings by Smith et al. (2009) which reported that patients who were found to have a VUS had increased anxiety but markedly less so than those who received a positive result. In our larger study, we hope to have sufficient participation to analyze subgroups of the MICRA questionnaire, which include distress, uncertainty, and positive value of cancer genetic counseling, with enough participants to find statistical significance of responses using the subgroup scores.

Baseline anxiety scores ranged dramatically among participants, even in the group with negative results, and scores were sometimes higher at the time of pre-test genetic



counseling than after results had been reported. Two participants displayed statistically significant anxiety levels immediately following genetic testing. Interestingly, these participants received negative results and displayed a decrease in anxiety between baseline and post-result STAI questionnaires. We suggest that these initially high anxiety reports from patients at their first genetic counseling session may be due to a variety of factors. In our clinic, most participants were seen within two weeks of their initial diagnosis of breast cancer and had not yet established a treatment plan with their surgeon and/or oncologist. This time-related factor, could likely influence both distress and anxiety. Some participants verbalized that the speed with which they were scheduled for various appointments was too rapid to adequately process all of the information, while others remarked that they just wanted to move on to surgery and have the cancer removed as quickly as possible (P. Walker, personal communication, March, 2015). Likewise, some patients may have been very anxious about many other factors during this period that are unrelated to their genetic testing but may accompany a cancer diagnosis. Examples from patients in general include anxiety about their daughter's genetic status, insurance coverage, financial hardship, and lack of family or social support.

Among participants who completed all parts of the survey, insufficient data exists to analyze how changes in anxiety are affected by the gene in which a finding is made. The participant with a deleterious mutation in the *TP53* gene was the only participant to reach statistically significant anxiety on the post-result STAI questionnaire. We note that clear guidelines and clinical management recommendations exist for post-results genetic counseling are available for *TP53* mutation carriers. This participant displayed a smaller increase in anxiety than the participant with both a positive result in the *ATM* gene and a

VUS result in the *BMPRIA* gene. It is interesting to note that the participant with both a positive mutation result and a VUS result additionally had a higher composite score related to genetic test results as measured by the MICRA questionnaire. The lack of clearly defined risks of a second breast cancer and lack of screening guidelines for mutation carriers of the *ATM* gene may have added to the patient's anxiety, as well as the uncertainty around a VUS result. In some individuals, the finding of two results simultaneously could be unsettling and increase their uncertainty and anxiety. We note that both patients receiving positive results appeared to have significantly higher MICRA scores than those who received VUS results (see Figure 2.2), although we could not calculate a cut-off for statistically significant levels of anxiety on the MICRA score due to small sample size. In the upcoming study, we expect that we will see sufficient examples of positive results and VUS results to support our hypothesis and to compare anxiety, distress, uncertainty, and positive value scores with those received from individuals found to have *BRCA1* and *BRCA2* positive and VUS results.

It is interesting to note in tables 2.2 and 2.3 that statements of the two participants who received positive results and the participant with a VUS result were similar for the most part. These participants reported feeling somewhat, but not entirely, prepared for receiving their respective results after their first genetic counseling session. This retrospective perception of being somewhat unprepared for a positive result in patients who receive positive or VUS results would likely be realistic to each person, as typically patients expect to get negative results. These few results are anecdotal in this small study, and we will depend on our larger study to help us clarify information in this area.

Participants stated that they felt strongly that their doctors would use their results to help make healthcare decisions. This statement by the VUS group is interesting as VUS findings, by virtue of their “uncertain significance,” have no recommendations for changes to medical management. The statement in Table 2.2 “I want my other family members to have the choice to know it that are positive or negative... [sic]” may simply require further clarification, but it may suggest that patients do not fully or accurately comprehend the implications of a VUS result regarding their own risk and/or healthcare management. This finding is supported by the findings reported by Richter et al., (2013), in which they found that incorrect risk recall was higher in patients who had received VUS results than in patients with either negative or positive genetic test results.

One factor not explored that may affect changes in anxiety might be educational material regarding the gene in which a change is discovered. Materials provided by the health care professional or obtained by the patient through independent research, may help to ease, in some patients, feelings of anxiety and distress associated with a VUS or positive result. However, other patients may increase their own anxiety by searching for too much information which is not generally available or may be anecdotal and yet to be understood about many of the genes included in multiplex cancer genetic testing. Clearly, new educational material for patients needs to be developed.

Further investigation, both in our own full study and by additional studies, is crucial to explore the factors associated with increased anxiety related to genetic test results. Given the changing landscape of genetic testing related to hereditary cancer syndromes and the trend toward large multiplex cancer genetic testing, it is important to know how different results might affect a patient’s psychological well-being.

Clarification regarding additional factors affecting levels of anxiety may help develop a more complete pre-test counseling conversation or a more targeted conversation for results discussion. Information gained by the full study is expected to be beneficial to genetic counselors or other healthcare professionals to inform them how best to counsel patients who choose testing from among multiple genetic test options. These findings are likely to be significant to broader applications, as the field approaches whole genome sequencing (WGS) and/or whole exome sequencing (WES) in the context of care for individuals predisposed to developing breast cancer and cancers involved in other hereditary cancer syndromes.

#### *Study Limitations*

Time constraints limited data collection to a single site, limiting both population size and, theoretically, variation in population race and ethnicity. We were unable to analyze differences in anxiety between those who chose *BRCA1* and *BRCA2* testing versus those who chose multiplex cancer genetic testing. Small population size limited statistical power. There was not an even distribution of participants in the negative, positive, and VUS result categories, which is to be expected as negative results are more common. Men undergoing genetic testing for hereditary breast cancer predisposition syndromes are more uncommon than women, but an absence of male participants makes results less generalizable. Obviously, the results from this pilot study are not generalizable to the general population of breast cancer patients.

### *Future Study*

Moving forward from the pilot study will be critical towards fulfilling our research goals about patient anxiety levels and genetic test results. We have enlisted two additional centers for data collection, both larger than our own. A larger sample size should enable us to answer the questions proposed by our study and may help to clarify what factors are associated with increased anxiety in the context of breast cancer genetic testing. It may be beneficial to consider additional questions to the initial questionnaire in order to further search out extrinsic sources of anxiety that are not associated with their test results, such as job stress, marital stress, or family demands.. These additional factors not previously considered may help explain variation in initial anxiety scores. We also look forward to the larger population to assess for accurate understanding of how patients understand their VUS results, rather than speculating on anecdotal data.

### *Future Research*

Qualitative studies would be of great value to explore the individual factors which lead to an increased feeling of anxiety, as reported by participants. We theorize that factors such as a recent diagnosis, unclear plan for treatment, financial concerns and information/educational materials presented by the healthcare professional providing pretest counseling may be contributing factors to heightened anxiety at the time of testing. Additionally, exploration of specific factors which play a significant role in causing heightened anxiety in the event of a positive or VUS finding is an area for further investigations. It is possible that a state of heightened anxiety could be a driving force behind compliance for recommended breast surveillance in mutation carriers, whereas individuals with low anxiety levels may be lacking the motivation for frequent exams to .

Further investigation regarding anxiety and adherence to recommended screening and/or uptake of prophylactic surgical intervention could be of benefit to clinicians, as they develop individual management plans to meet the needs of their patients.

## **2.6 Conclusions**

This study has interesting implications for the field of cancer genetic counseling, as well as for other healthcare providers involved in ordering, reporting, and counseling patients regarding genetic testing for hereditary cancer predisposition. We were unable to explore the difference in anxiety experienced by those who undergo classic *BRCA1/BRCA2* testing versus those who pursue multiplex cancer gene testing, as all participants in our study chose multiplex testing. Similar to findings by previous studies, participants who received a negative result were found to have a decreased overall anxiety, while those who received a VUS or positive result were found to have an increased overall anxiety. The levels of changes in anxiety experienced by the negative result group and the positive result group approached statistical significance, even at small sample size. The gene in which a mutation or VUS was found also appears to affect overall changes in anxiety, although it is unclear at this time what specific factors are associated with this anxiety-causing effect (e.g., a lack of management guidelines for some genes or varying associated lifetime risks). Further study using a larger clinical population is expected to help further clarify the differences in anxiety experienced between those who undergo classic *BRCA1* and *BRCA2* testing and those who undergo multiplex testing. Results from a larger population may help us better understand what specific factors influence changes in anxiety between patients with negative, positive, or VUS test results. If significant differences are found in our larger study, the presentation of information pertaining to

genetic testing options would ideally be tailored by genetic counselors for optimal psychological patient support.

### Chapter 3: Conclusions

This study has interesting implications for the field of genetic counseling, as well as for other professions involved in ordering, reporting, and counseling patients regarding genetic testing for hereditary cancer predisposition. We were unable to explore the difference in anxiety experienced by those who undergo classic *BRCA1/BRCA2* testing versus those who pursue multiplex cancer gene testing as all participants in our study chose multiplex testing. Similar to findings by previous studies, participants who received a negative result were found to have a decreased overall anxiety, while those who received a VUS or positive result were found to have an increased overall anxiety. The difference in changes in anxiety experienced by the negative result group and the positive result group approached statistical significance, even at small sample size. The gene in which a mutation or VUS was found also appears to affect overall changes in anxiety, although it is unclear at this time what specific factors are associated with this anxiety causing effect (e.g. a lack of management guidelines for some genes or varying associated lifetime risks). Further study using a larger clinical cohort may help to further clarify the differences in anxiety experienced between those who undergo classic *BRCA1* and *BRCA2* testing and those who undergo multiplex testing. Results from a larger population may help us better understand what specific factors influence changes in anxiety between patients with negative, positive, or VUS test results. If significant differences are found in our larger study, the presentation of information pertaining to



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## **Appendix A: Participant Introductory Letter**

### **University of South Carolina School of Medicine USC Genetic Counseling Program**

Dear Potential Participant:

You are invited to participate in a graduate research study focusing on patient distress and uncertainty regarding genetic testing for a personal history of breast cancer. I am a graduate student in the genetic counseling program at the University of South Carolina School of Medicine. My research investigates patient distress and uncertainty regarding the possible results from genetic testing for hereditary cancer predisposition syndromes. The research involves completing one survey during your initial counseling session when genetic testing is completed and possibly a follow-up survey at a future time regarding your test results.

The surveys attempt to interpret your current emotional state, in regards to distress and uncertainty, at the time of undergoing genetic testing and again at the time of results disclosure. The surveys will include questions regarding your current emotional state as well as opinion questions regarding genetic testing and results. Please answer all questions truthfully as there are no right or wrong answers.

All responses gathered from the surveys will be kept anonymous and confidential. The results of this study might be published or presented at academic meetings; however, participants will not be identified.

Participants who include contact information will also be entered into a raffle to win a \$25 Visa gift card. If you are chosen, this prize will be sent to you at a later date, after having collected all data. Your contact information will not be used for any other purposes beyond sending the raffle prize if you have won.

Your participation in this research is voluntary. By completing the survey, you are consenting that you have read and understand this information. At any time, you may withdraw from the study by not completing the survey.

Thank you for your time and consideration to participate in this survey. Your responses may help genetic counselors better serve patients who undergo similar genetic testing as you in the future. If you have any questions regarding this research, you may contact either myself or my faculty advisor, Peggy Walker, MS, CGC, using the contact information below. If you have any questions about your rights as a research participant, you may contact the Office of Research Compliance at the University of South Carolina at (803) 777-7095.



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## Appendix B: 20-Item State Trait Anxiety Inventory

### Self-Evaluation Questionnaire

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate value to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to best describe your present feelings.

- |     |  |            |          |               |              |
|-----|--|------------|----------|---------------|--------------|
| 1.  | I feel calm.                                       | Not at All | Somewhat | Moderately So | Very Much So |
| 2.  | I feel secure.                                     | Not at All | Somewhat | Moderately So | Very Much So |
| 3.  | I am tense.  | Not at All | Somewhat | Moderately So | Very Much So |
| 4.  | I am strained.                                     | Not at All | Somewhat | Moderately So | Very Much So |
| 5.  | I feel at ease.                                    | Not at All | Somewhat | Moderately So | Very Much So |
| 6.  | I feel upset.                                      | Not at All | Somewhat | Moderately So | Very Much So |
| 7.  | I am presently worrying over possible misfortunes. | Not at All | Somewhat | Moderately So | Very Much So |
| 8.  | I feel satisfied.                                  | Not at All | Somewhat | Moderately So | Very Much So |
| 9.  | I feel frightened.                                 | Not at All | Somewhat | Moderately So | Very Much So |
| 10. | I feel comfortable.                                | Not at All | Somewhat | Moderately So | Very Much So |
| 11. | I feel self-confident                              | Not at All | Somewhat | Moderately So | Very Much So |

12.	I feel nervous.	Not at All	Somewhat	Moderately So	Very Much So
13.	I feel jittery.	Not at All	Somewhat	Moderately So	Very Much So
14.	I feel indecisive.	Not at All	Somewhat	Moderately So	Very Much So
15.	I feel relaxed.	Not at All	Somewhat	Moderately So	Very Much So
16.	I feel content.	Not at All	Somewhat	Moderately So	Very Much So
17.	I am worried.	Not at All	Somewhat	Moderately So	Very Much So
18.	I feel confused.	Not at All	Somewhat	Moderately So	Very Much So
19.	I feel steady.	Not at All	Somewhat	Moderately So	Very Much So
20.	I feel pleasant.	Not at All	Somewhat	Moderately So	Very Much So

**General Information About You**

My Breast Cancer Diagnosis was made within the past 5 years: Yes No (please circle one)

What is your age? \_\_\_\_\_

What is your gender? Male Female (please circle one)

What is your ethnicity?

- a. Caucasian
- b. African-American
- c. Hispanic
- d. Native American
- e. Asian
- f. Other: \_\_\_\_\_

What is your highest level of education?

- a. Did not complete high school
- b. Completed high school (diploma of GED)
- c. Some college
- d. Associate's degree
- e. Bachelor's degree
- f. Beyond bachelor's degree

Marital Status:

- a. Single and never married
- b. Married
- c. Widowed
- d. Separated
- e. Divorced
- f. With partner
- g. Remarried

Do you have children?      Yes      No      (please circle one)

If you have young or adult children, please list the age of each child and their relationship to you.

Age \_\_\_\_\_ Daughter      Son      (please circle one)

Age \_\_\_\_\_ Daughter      Son      (please circle one)

Age \_\_\_\_\_ Daughter      Son      (please circle one)

Age \_\_\_\_\_ Daughter      Son      (please circle one)

Age \_\_\_\_\_ Daughter      Son      (please circle one)

Age \_\_\_\_\_ Daughter      Son      (please circle one)

## Appendix C: Multidimensional Impact of Cancer Risk Assessment (MICRA)

### MICRA Scale about Cancer Risk Assessment

The questions below are about some specific responses you may have had after receiving your genetic test results. Please answer every question in Section 1, regardless of whether you were given a positive or negative test result. Please indicate whether you have experienced each statement *never, rarely, sometimes, or often* in the past week, by circling the corresponding number.

#### Section 1.

- |    |  |       |        |           |       |
|----|--|-------|--------|-----------|-------|
| 1. | Feeling upset about my test result.                      | Never | Rarely | Sometimes | Often |
| 2. | Feeling sad about my test result.                        | Never | Rarely | Sometimes | Often |
| 3. | Feeling anxious or nervous about my test result.         | Never | Rarely | Sometimes | Often |
| 4. | Feeling guilty about my test result                      | Never | Rarely | Sometimes | Often |
| 5. | Feeling relieved about my test result                    | Never | Rarely | Sometimes | Often |
| 6. | Feeling happy about my test result                       | Never | Rarely | Sometimes | Often |
| 7. | Feeling a loss of control.                               | Never | Rarely | Sometimes | Often |
| 8. | Having problems enjoying life because of my test result. | Never | Rarely | Sometimes | Often |

9.	Worrying about my risk of getting cancer (or getting cancer again if you have ever been diagnosed with cancer).	Never	Rarely	Sometimes	Often
10.	Being uncertain about what my test result means about my cancer risk.	Never	Rarely	Sometimes	Often
11.	Being uncertain about what my test result means for my child(ren) and/or family's cancer risk.	Never	Rarely	Sometimes	Often
12.	Having difficulty making decisions about cancer screening or prevention (e.g., having preventive surgery or getting medical tests done).	Never	Rarely	Sometimes	Often
13.	Understanding clearly my choices for cancer prevention or early detection	Never	Rarely	Sometimes	Often
14.	Feeling frustrated that there are no definite cancer prevention guidelines for me.	Never	Rarely	Sometimes	Often
15.	Thinking about my test results has affected my work or family life.	Never	Rarely	Sometimes	Often

**Remember, we are talking about the past seven days.**

	<b>Never</b>	<b>Rarely</b>	<b>Sometimes</b>	<b>Often</b>
16. Feeling concerned about how my test results will affect my insurance status.	Never	Rarely	Sometimes	Often
17. Having difficulty talking about my test results with family members.	Never	Rarely	Sometimes	Often
18. Feeling that my family has been supportive during the genetic counseling and testing process.	Never	Rarely	Sometimes	Often
19. Feeling satisfied with family communication about my genetic test result.	Never	Rarely	Sometimes	Often
20. Worrying that the genetic counseling and testing process has brought about conflict within my family.	Never	Rarely	Sometimes	Often
21. Feeling regret about getting my test results.	Never	Rarely	Sometimes	Often

**Section 2.** *If you have children, regardless of your test result, please answer questions # 22 and 23. Otherwise, please go to Section 3.*

22. Worrying about the possibility of my children getting cancer.	Never	Rarely	Sometimes	Often
23. Feeling guilty about possibly passing on the disease risk to my child(ren).	Never	Rarely	Sometimes	Often

**Section 3.** *If you currently have cancer, or have had it in the past, please answer questions # 24 and 25. Otherwise, please check this box : Please go on to the next page, Section 4.*

- |     |   |       |        |           |       |
|-----|---|-------|--------|-----------|-------|
| 24. | Feeling that the genetic test result has made it harder to cope with my cancer. | Never | Rarely | Sometimes | Often |
| 25. | Feeling that the genetic test result has made it easier to cope with my cancer. | Never | Rarely | Sometimes | Often |

**Please continue the survey on page 3.**



**Section 4.** *After this genetic counseling session, how do you think you will use these genetic results that have been discussed with the genetic counselor?*

**A1. If you have received a POSITIVE result, please circle the best answer that shows your opinion about how you feel now about getting this POSITIVE result. (If you received a VUS result, please skip this question and answer Questions in B1 instead – See Below.)**

1. I felt well-prepared from my first counseling session about the possibility of getting a positive result.

Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
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2. When I got the positive result, I felt more anxious about the result than I thought I would.

Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
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3. I expect that my doctors will help me make healthcare decisions based on my positive result.

Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
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4. I intend to share my positive result with my children so that they can be tested.

Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
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5. I intend to share my positive result with my other family members (parents, sisters, brothers, aunts/uncles/cousins) so that they can be tested.

Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
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6. Why or why not would you share your POSITIVE result?

**If you have received a POSITIVE result, the survey is now complete. Please skip the following questions in B1, found on page four, and proceed directly to our offer to participate in a random drawing for a \$25 gift card. Please fill-out the attached sheet regarding your contact information. If you would not like to participate in the random drawing, please disregard the final sheet.**

**B1. If you have received a VUS result, please circle the best answer that shows your opinion about how you feel now about getting this VUS result.**

7. I felt well-prepared from my first counseling session about the possibility of getting a VUS result.

Strongly	Somewhat	Somewhat	Strongly
Disagree	Disagree	Agree	Agree

8. When I got the VUS result, I felt more anxious about the result than I thought I would.

Strongly	Somewhat	Somewhat	Strongly
Disagree	Disagree	Agree	Agree

9. I expect that my doctors will help me make healthcare decisions based on my VUS result.

Strongly	Somewhat	Somewhat	Strongly
Disagree	Disagree	Agree	Agree

10. I intend to share my VUS result with my children so that they can be tested.

Strongly	Somewhat	Somewhat	Strongly
Disagree	Disagree	Agree	Agree

11. I intend to share my VUS result with my other family members (parents, sisters, brothers, aunts/uncles/cousins) so that they can be tested.

Strongly  
Disagree

Somewhat  
Disagree

Somewhat  
Agree

Strongly  
Agree

12. Why or why not would you share your VUS result?

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