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Investigating Hereditary Breast And Ovarian Cancer (hboc) Syndrome In Trinidad And Tobago

Gerneiva Parkinson
Yale University

Investigating Hereditary Breast and Ovarian Cancer (HBOC) Syndrome in Trinidad and Tobago

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Gerneiva Parkinson

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ABSTRACT

Title: Investigating Hereditary Breast and Ovarian Cancer (HBOC) Syndrome in Trinidad and Tobago

Authors: Gerneiva Parkinson, Anees Chagpar, Marcella Nunez-Smith; Kellie Alleyne- Mike, Erin Hofstatter – Internal Medicine, Yale University School of Medicine, New Haven, CT

Trinidad and Tobago (T&T) is a Caribbean island with a population of approximately 1.3 million. T&T has one of the highest breast cancer mortality rates in the region. Notably, a large proportion of breast cancer cases in T&T appear to occur at a young age, as nearly 36% of breast cancers are diagnosed under the age of 50. It is known that a younger age at diagnosis can be associated with Hereditary Breast and Ovarian Cancer syndrome (HBOC). However, the prevalence of HBOC mutations remains unknown in T&T, as accessible health services for genetic counseling and testing in T&T currently are limited. As such, our study aimed to determine the prevalence and spectrum of HBOC mutations among women with breast cancer in T&T who met National Comprehensive Cancer Network (NCCN) criteria for evaluation for HBOC syndrome to determine the need to include genetic counseling and testing in local oncology management in T&T.

At the main oncology unit in T&T, female breast cancer patients who met the NCCN criteria were recruited for this study. We conducted interviews inquiring about their personal breast cancer diagnosis, as well as any relevant family history. This was followed by the collection of saliva samples using Oragene kits, which were then analyzed by Color Genomics Inc. for 30 genes associated with hereditary cancers. Finalized results were returned to patients by genetic counselors from Color Genomics. In total, 58 patients who met NCCN guidelines were sequenced and results were returned. They showed that of 58 samples, 15 patients tested positive for deleterious HBOC germline mutations: 9 - *BRCA*1, 3 - *BRCA*2, 1 – CHEK2, 1- PALB2 and 1 – *PTEN*, with an overall prevalence rate of 25.8%. This prevalence rate is remarkable, given that HBOC mutations among U.S. women with breast cancer are found in only 5-10% of patients. These initial results clearly demonstrate the need to include genetic counseling and testing in the local oncology management in T&T, as the identification of HBOC mutations can influence treatment options, as well as help identify family members who are at high risk for cancer predisposition. Ultimately, this implementation could help alleviate the country's high incidence and mortality rates with respect to breast cancer.

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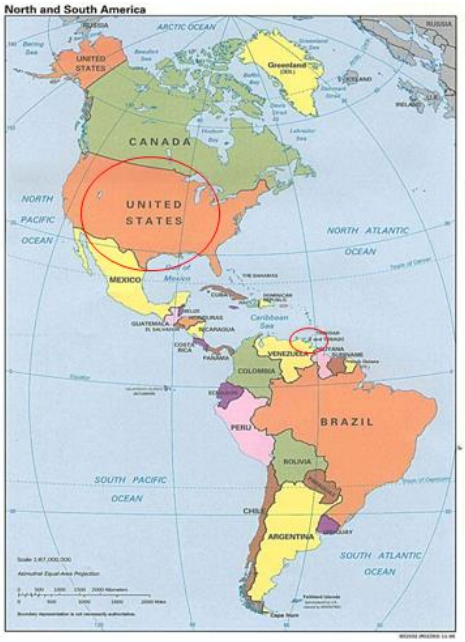
INTRODUCTION AND BACKGROUND

Trinidad and Tobago (T&T) has one of the highest breast cancer rates in the Caribbean region. According to the T&T's National Cancer Registry, breast cancer represents 30% of all new female cancer cases, and 24% of all female-cancer related deaths. In addition, a large proportion of breast cancer cases in T&T appear to occur at a young age. During 1998-2007, 36% of breast cancer diagnoses were under the age of 50, with the rates of early initial diagnosis (<45 years old) being twice as high (20%) than that found in the United States (10%) (1).

Trinidad and Tobago is a twin island nation at the south-eastern area of the Caribbean. It lies about seven miles off the eastern coast of Venezuela, and consists of approximately 5,100 square kilometers. These islands have a population of over 1.3 million citizens with an average life expectancy of 71 years. It is the second largest English speaking country in the Caribbean region, and its population comprises of a heterogeneous ethnic makeup predominantly of African and East Indian descent (2).

According to the Pan American Health Organization (PAHO), cancer incidence and related mortality in the Latin America and the Caribbean (LAC) region have recently been increasing. It is predicted that by 2030, 1.7 million cases of cancer will be diagnosed in this region, with over a million predicted to die each year from the disease. Breast cancer ranks as the most common cancer type for women, both in terms of new cancer cases and deaths. Of note, in LAC, a

greater proportion of breast cancer deaths occur in women under 65 years of age (57%), as compared to North America (41%).



UNITED STATES:
- Population size: approx. 322 million
- Average age of breast cancer diagnosis: 61 years
- BRCA mutation prevalence in breast cancer: 5-10%

TRINIDAD AND TOBAGO:
- Population size: approx. 1.3 million
- Average age of breast cancer diagnosis: 45-50 years
- BRCA/HBOC mutations prevalence in breast cancer: **unknown**

Figure 1: Image showing comparisons of statistics for Trinidad and Tobago versus the United States. Source: http://www.catholiclane.com/250px-nsamerica-pol1/

Hereditary Breast and Ovarian Cancer Syndrome (HBOC), classically characterized by germline mutations in tumor suppressor genes can be associated with a younger age of breast cancer diagnosis. There are several genes that are now recognized to be associated with HBOC, such as *BRCA 1, BRCA 2, TP53, PTEN, STK11*, *CDH11, CHEK2, ATM, RAD50* and *PALB2.*

These genes vary in genetic penetrance, as some lead to a higher estimated lifetime risk than others. For example, *BRCA 1, BRCA 2, TP53, PTEN, STK11* and *CDH11* are all considered high-penetrance breast cancer susceptibility genes. Mutations in these highly penetrant genes carry an approximate 10-fold increase in the estimated lifetime risk of breast cancer as compared to the risk of

an average female. In contrast, genes such as *CHEK2, ATM, RAD50* and *PALB2* can be considered moderate-penetrance breast cancer susceptibility genes, and they are associated with a 2 to 4 fold increased risk. While moderately penetrant genetic mutations carry a lower estimated lifetime risk of developing breast cancer when compared to the highly penetrant genes, they remain clinically relevant and have the potential to alter screening and breast cancer treatment options.

The most well known among the spectrum of recognized HBOC mutations are those which occur in the *BRCA*1 and *BRCA*2 genes. These genes are responsible for tumor suppressor proteins that function in double-strand DNA repair. Therefore, they play a major part in maintaining the stability of cellular genetic material. If a mutation occurs in one of these genes, this can result in the decreased function of the respective protein product, which in turn assists in DNA damage. As a result, this leads to an increased risk of cancer(s) in the breast, ovaries, prostate, pancreas, as well as several other locations (3).

HBOC mutations, including those in *BRCA* 1 and 2, are inherited in an autosomal dominant pattern. As such, a genetic mutation can be inherited via either parent, and in turn each offspring of an affected parent has a 50% chance of acquiring the mutation. The estimated lifetime risk of developing breast cancer for an individual carrying a *BRCA* 1 mutation is 55% to 69% by the age of 70, whereas risks for *BRCA* 2 are slightly lower at 45%. These compare to the estimated lifetime risk of 12% for the general female population. Likewise, for ovarian cancer, the estimated lifetime risk is 46% for *BRCA* 1 mutation carriers and 11-

17% for *BRCA* 2. These again can be compared to the estimated lifetime risk of 1.3% for the general female population (3).

Another HBOC-associated mutation is the alteration in the *PTEN* gene, which is responsible for production of the PTEN protein, which functions in the dephosphorylation process in the AKT signaling pathway. This is responsible for key processes in cell proliferation and regulation, and therefore, provides assistance in tumor suppression. A mutation in this gene results in the increased risk of several cancers including breast, endometrial and thyroid cancers. The increased lifetime risk of developing breast cancer in these mutation carriers is 30-50%. This mutation, also autosomal dominant, is associated with Cowden syndrome, which includes the presence of non-malignant cancers, such as hamartomas, trichilemmomas, and papillomatous papules (4).

In addition to the two genes mentioned above, there are the mutations which occur in *TP53 (*a gene which produces a protein linked to cell apoptosis and genomic stability). This can result in Li-Fraumeni syndrome which increases the risk of breast cancer, soft tissue/bone sarcomas and glioblastomas (5). Carriers of this mutation have 56% chance of developing breast cancer by age 45, with the increased estimated lifetime risk being over 90%. Other highly penetrant mutations include *STK11*, which carries a 32% chance of developing breast cancer by 60, and *CDH1*, which comes with an estimated lifetime risk of 39% for developing lobular breast cancer (6).

With respect to the moderately penetrant breast cancer susceptibility genes,

CHEK2 and *ATM* usually each confer a lifetime risk of at least 20% for breast cancer. *PALB2* mutations bestow an estimated lifetime risk of 35% for breast cancer (7), and *RAD51C, RAD51D* and *BRIP1* each present an estimated lifetime risk of 5-10% for ovarian cancer (6).

As a result of the increased risk associated with these genetic mutations, it is recommended that those who are considered at a higher risk of carrying one of these altered genes be recommended for genetic counseling and testing. In the United States, there are several options for genetic testing, which have progressed significantly over the last twenty years. These genetic tests started from expensive single-panel tests which targeted one gene and have evolved to affordable multi-panel options which incorporate dozens of genes analyzed simultaneously though next-generation sequencing technologies.

At the moment, in the United States, HBOC testing is mostly based on family history and ancestry. Therefore, many medical personnel use various guidelines to determine if an individual is at a high risk for having an HBOC genetic mutation. The most commonly used guidelines are those provided by the National Comprehensive Cancer Network (NCCN) and US Preventative Services Task Force (USPSTF) (8) (9):

These criteria usually include:

- Breast/ovarian cancer diagnosis at a young age (usually before 50 years)
- Multiple primary sites of tumors (such as bilateral breast cancer, or a history of breast cancer as well as ovarian cancer)

- Extensive one-sided family history of breast, ovarian, prostate or pancreatic cancers
- Family history of genetic mutations
- Ancestry inclusive of populations known to have higher rates of HBOC mutations (eg. Ashkenazi Jewish ancestry).

In the United States, *BRCA* 1 and *BRCA* 2 account for about 50% of HBOC, and 5% of all breast cancers. For ovarian cancers, these mutations account for approximately 15% of all ovarian cancer cases. However, it is shown that certain racial, ethnic and geographically-locked populations have a higher prevalence for the *BRCA 1* and *BRCA 2* mutations, such as the descendants of the Ashkenazi Jewish population, and several Scandinavian and Icelandic populations (3). For example, in 2010, a high prevalence of BRCA mutations was found in the Bahamas, which is a small Caribbean nation. These islands, which may have experienced geographic and reproductive isolation until the increased immigration patterns starting in the mid19th century (10), were found to have a high incidence of pre-menopausal women diagnosed with breast cancer. Donenberg and Hurley tested 214 unselected Bahamian women with invasive breast cancer for *BRCA* mutations, and the results showed that 23% of these unselected cases were positive for the *BRCA* mutation. Interestingly, many of the patients who tested positive in this study had one of six distinct *BRCA* 1 mutations, leading to the conclusion that these were most likely founder mutations due to their historically isolated populations which had propagated isolated reproductive patterns (11).

As many Caribbean islands share the same cultural, geographical and immigration history as the Bahamian islands, one may wonder whether islands that experience younger ages of breast cancer diagnoses would have similarly high hereditary genetic attributions to their breast cancer cases, and if there is a resulting need for accessible genetic counseling and testing throughout the Caribbean region. Trinidad and Tobago, has reported a young average age of breast cancer diagnoses. However, the prevalence and spectrum of the HBOC mutations in the country are unknown.

One of the barriers to understanding the contribution of HBOC mutations in the breast cancer population in T&T is the limited access to genetic counseling and testing for the local population. The structure of Trinidad and Tobago's health system is mainly divided into two segments: public (which is free to the citizens) and private (which is paid out of pocket by the citizens). However, currently genetic testing is only available through the private sector of the health care system at a cost of$1500 - $2000 USD. Therefore, there are no options for this test in the public sector, and as a result, many patients and their families are not able to afford the genetic test.

This is in stark contrast to the services provided in the United States and other developed countries who provide genetic consultation for high risk patients, followed by genetic testing, which is either covered by insurance or can be directly purchased at a reasonable price (approx. $250 - $500USD). Genetic testing has been proven useful in the United States, as the identification of HBOC mutations can influence treatment options in cancer patients (12), as well

as help identify family members who are at higher risk for having them. These can lead to prophylactic interventions which have been proven to reduce the risk of developing future cancer(s) should they test positive for a harmful mutation (13) (14).

Therefore, with Trinidad and Tobago's high breast cancer mortality and average young age of breast cancer diagnosis, our main aim was to determine whether these services, which have been beneficial in developed countries, should be implemented in this country.

STATEMENT OF PURPOSE

With the young age of breast cancer diagnosis in Trinidad and Tobago coupled with the limited access to genetic testing, this project sought to determine if screening for HBOC mutations should be made more accessible to the population of Trinidad and Tobago.

This main purpose was accomplished by completing the following two aims:

1. Investigate the prevalence and spectrum of HBOC mutations in T&T's breast cancer population.
2. Determine the proportion of the general female population who met criteria for having a high risk of carrying a *BRCA* mutation, and therefore would potentially benefit from a referral for further genetic counseling and testing according to U.S. national guidelines.

Through these aims, we examined the need to include genetic testing infrastructure into the local oncology management in T&T.

METHODS

AIM ONE: Investigate the prevalence and spectrum of HBOC mutations in T&T's breast cancer population.

This portion of the project was conducted in the main cancer institution of the public health sector - the National Radiotherapy Center of Trinidad and Tobago. Recruitment focused on female breast cancer patients over 18 years, who met the National Comprehensive Cancer Network (NCCN) criteria for genetic counseling and testing. The patients were recruited either through doctor referrals or chart reviews.

The NCCN criteria are stated below (15):

- Diagnosed ≤45 years
- Diagnosed ≤50 years with:
    - An additional breast cancer primary – (this can either be bilateral or a separate ipsilateral breast cancer)
    - ≥1 close blood relative (ie. first, second or third degree) with breast cancer at any age
    - ≥1 close relative with pancreatic cancer
    - ≥1 close relative with prostate cancer (Gleason score ≥7)
- Diagnosed ≤60 years with a:
    - Triple negative breast cancer
- Diagnosed at any age with:
    - ≥1 close blood relative with breast cancer diagnosed ≤50 years

- ≥2 close blood relatives with breast cancer at any age
- ≥1 close blood relative with ovarian cancer at any age
- ≥2 close blood relatives with pancreatic cancer and/or prostate cancer (Gleason score ≥7) at any age
- A close male blood relative with breast cancer
- Personal history of ovarian cancer

We conducted interviews inquiring about their personal breast cancer diagnosis, and collected relevant family history.

The specific questions were as follows:

GENERAL QUESTIONS

1. Name
2. Current Age
3. Age of Diagnosis of breast cancer
4. Self-reported Race/Ethnicity

FAMILY HISTORY:

1. Family history of breast cancer (include age of diagnosis)
2. Family history of breast cancer before 50 (include age of diagnosis)
3. Family history of bilateral breast cancer (include age of diagnosis for each)
4. Family history of ovarian cancer (include age of diagnosis)
5. Family history of multiple primary cancer diagnoses (include age of diagnosis for each)
6. Family History of any other cancer not mentioned above (include age of diagnosis)

Each patient was given pre-test genetic counseling to ensure that they understood the entire genetic testing process, as well as the benefits and risk of the test. Participants each signed a consent form (details below in ethical considerations), and were assured that they could withdraw from the study at any point, which would not affect their current care at NRC.

Next, each patient gave a saliva sample using the Oragene DNA sample collection kit (OG-250 format, DNA Genotek, Kanata, ON, Canada). This sample was then sent to the Color Genomics lab in California, United States for genetic sequencing, using their standard 30 gene- panel which covers most clinically relevant hereditary cancer syndromes and includes the major HBOC-associated genes. The genes investigated were *APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A (p14ARF), CDKN2A (p16INK4a), CHEK2, EPCAM, GREM1, MITF, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53.* Full methods can be found in Appendix 1.

To ensure that results were returned in a safe and informative manner, post-test counseling provided by a Color Genomics genetic counselor either by phone or in person. The patients were given copies of their results to be shared with their personal physicians, and they received written educational material summarizing the topics discussed in the genetic counseling meeting. Patients were

encouraged to bring family members to the sessions, and first degree relatives of positive patients were offered genetic testing at a reduced cost.

AIM TWO: Determine the proportion of the general population, who met the criteria for having a high risk of carrying the *BRCA* mutation.

In order to estimate the proportion of the general female population without a cancer diagnosis at a high risk of having the *BRCA* mutation, we used data from the T&T's Cancer Society. Paper medical records, were obtained for 1807 women over the age of 18 years, without a personal history of breast cancer, who came into this center between 2010-2013 for preventative screenings such as mammograms and pap smears. Family history criteria which were associated with an increased likelihood of having a *BRCA* mutation were extracted. These were taken from the U.S. National Institutes of Health/National Cancer Institute (NIH/NCI), which were guided by the US Preventative Services Task Force, as a screening method to capture those who should be evaluated by a genetic counselor (3). These included:

- Three or more first or second degree relatives diagnosed with breast cancer or ovarian cancer
- Two first degree relatives diagnosed with breast cancer, with one below age 50
- An immediate family member with bilateral breast cancer
- A relative diagnosed with ovarian cancer (regardless of age at diagnosis)
- A combination of two or more first- or second-degree relatives diagnosed with both breast and ovarian cancer (regardless of age at diagnosis)
- Breast cancer diagnosed in a male relative

ETHICAL CONSIDERATIONS

Ethics committee approvals were sought by the Human Investigation Committee (HIC) at Yale, as well as the local ethics committee in Trinidad and Tobago at the Northwest Regional Health Authority.

For this study, the potential risk was minimal in terms of sample acquisition and data collection. Saliva collection posed minimal risk to participants, and medical data was kept in secured areas. All patients were required to sign informed consent which reviewed the personal (and for their family members) benefits, risks and limitations of undergoing genetic testing provided through Color Genomics. Color Genomics is compliant with Health Insurance Portability and Accountability Act of 1996.

Participants were asked to acknowledge and consent to the following statements:

- I am the individual providing the sample and I am at least 18 years of age
- I understand that I have the choice to store my genetic sample with Color
- I understand that I should not use Color if I have an allogenic bone marrow transplant (bone marrow from a donor), a blood transfusion within 7 days prior to providing a sample, or have an active hematologic malignancy such as leukemia, lymphoma or multiple myeloma.
- I understand that these test results will not tell me whether or not I have or will get disease in the future. These results will only tell me about my hereditary risk related to disease or other genetic traits.

- I understand that I should not make any medical decisions based on these results by myself, and that Color recommends that I consult with my healthcare provider to create a personalized screening and prevention plan.
- Genetic counseling services are available to me via Color at no additional charge.
- Color will donate my de-identified variants found to public databases like NCB's ClinVar, where anonymous genetic information will be accessible to researchers to better understand the connection between genetics and disease.

Participants were given the opportunity to ask questions, as well as withdraw at any time if they felt uncomfortable or uncertain about taking the genetic test.

RESULTS

AIM ONE: Investigate the prevalence and spectrum of HBOC mutations in Trinidad and Tobago's breast cancer population.

At the National Radiotherapy Center, 150 female breast cancer patients were initially approached for participation in the study based on meeting NCCN criteria for further genetic counseling and testing. Of those, 60 patients were ultimately enrolled into the study for genetic sequencing and interviewing based on their interest and availability to participate. At Color Genomics, 58 patient samples were completely processed and analyzed, but two did not pass quality control and therefore were not completed. Table 1 below shows characteristics of the patients tested, and Table 2 stratifies the patients based on test results.

This study found a prevalence rate of 25.8 % for the HBOC mutations tested in the multi-gene panel. Out of the 15/58 positive results, there were 9 *BRCA*1 mutations, 3 *BRCA2*, 1 *PTEN*, 1 *PALB2* and 1 *CHEK2* mutations (Figure 2), distributed throughout the islands as shown in Figure 3. .

Our positive patients are further described below (Table 3) showing their respective self-reported ethnicities, personal cancer histories, genetic sequence details, as well as their associated family history. Of note, in our 15 positive patients, only two were related (Proband 1-1 and 1-2). They were a mother/daughter pair with extensive family history.

Table 1: Characteristics of the patients tested for HBOC genetic mutations

Characteristics	**N**	**Proportion**
Mean Age (Range)	47 (19-68)	
Ages		
<19	1	0.02
20-29	2	0.03
30-39	14	0.24
40-49	15	0.26
50-59	18	0.31
≥60	8	0.14
Ethnicity		
Afro-Caribbean	17	0.29
East Indian	17	0.29
Chinese	2	0.03
Mixed/Other	22	0.39
Family History		
Yes	35	0.60
No	23	0.40

Table 2: Further classification of patients enrolled in the study according to self-identified ethnicity and age.

GENETIC TEST RESULT	POSITIVE	VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS)	NEGATIVE
Total	15	16	27
ANCESTRY (Self – Identified)			
African	6 (0.35)	3 (0.12)	8 (0.53)
East Indian	5 (0.29)	6 (0.35)	6 (0.35)
Mixed	4 (0.17)	7 (0.29)	13 (0.54)
AGE			
<20	0 (0.00)	0 (0.00)	1 (0.04)
20-29	2 (0.13)	0 (0.00)	0 (0.00)
30-39	3 (0.20)	5 (0.31)	8 (0.30)
40-49	4 (0.27)	8 (0.50)	6 (0.22)
50-59	5 (0.33)	3 (0.19)	9 (0.33)
>60	1 (0.07)	0 (0.25)	3 (0.11)

Table 3: Characteristics of the enrolled patients who tested positive for HBOC genetic mutations

P = paternal Br.Ca = breast cancer Thy = Thyroid cancer Endo = endometrial cancer

M= maternal CRC = colorectal cancer

*Proband ID 1-1 and 1-2 is a mother/daughter pair (the only related participants)

+ c.HGVS – Human Genome Variation Society mutation description on the chromosomal level

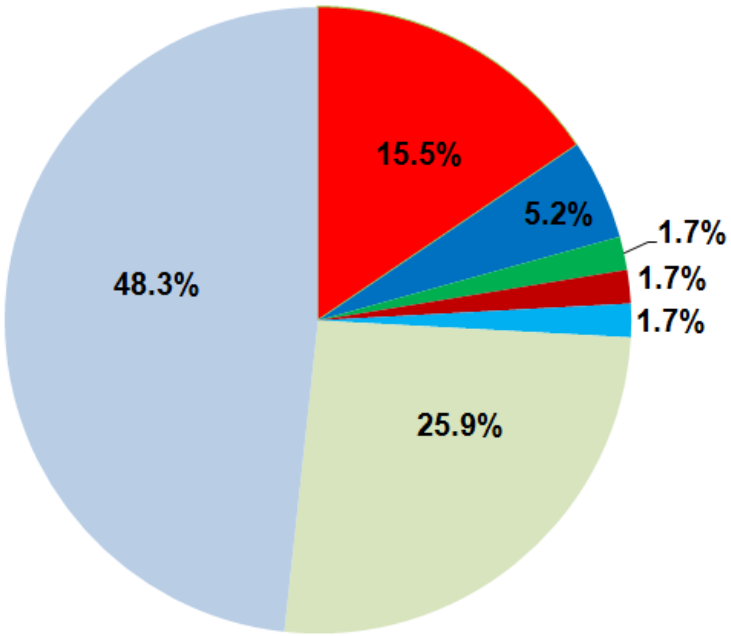
++ p.HGVS – Human Genome Variation Society mutation description on protein translation level

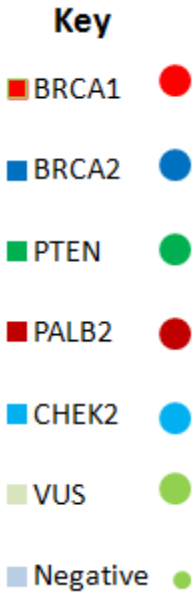
Proband ID	Gene	Reported Ethnicity	Age at Diagnosis	+c.HGVS	++p.HGVS	Class	Breast Cancer Type	Family History (age of diagnosis if known)
*1-1	*BRCA* 1	East Indian	45 Br.Ca	c.4183C>T	p.Gln1395*	Pathogenic by 4 labs	Ductal Carcinoma	• Son – testicular (17) • Daughter – breast (34) • Mother (M) – breast (68)
*1-2	*BRCA* 1	East Indian	34 Br.Ca	c.4183C>T	p.Gln1395*	Pathogenic by 4 labs	Ductal Carcinoma	• Brother – testicular (17) • Mother – breast (57) • Aunt (P) – breast (75) • Grandmother (M) – breast (68)
2	*BRCA* 1	African	23 CRC, 25 Br.Ca	c.4986+6T>C	N/A	Pathogenic by 6 labs	Ductal Carcinoma	None
3	*BRCA* 1	African	40 Br.Ca	c.2766delA	p.Val923Leufs*77	Pathogenic by 4 labs	Ductal Carcinoma	• Sister – breast (40) • Aunt (M) – leukemia (40) • Aunt (P) – breast (42) • Aunt (P) – colon (50) • Aunt (P) – colon (55) • Grandfather (P) – prostate (60)
4	*BRCA* 1	African	29 Br.Ca	c.3756_3759delGTCT	p.Ser1253Argfs*1	Pathogenic by 4 labs	Ductal Carcinoma	• Mother – breast (57) • Grandmother (M) – breast (40)

Proband ID	*Gene*	Reported Ethnicity	Age at Diagnosis	+c.HGVS	++p.HGVS	Class	Breast Cancer Type	Family History (age of diagnosis if known)
5	*BRCA 1*	African	28 Br.Ca	c.1630C>T	p.Gln544*	Pathogenic by 3 labs	Ductal Carcinoma	• Father – lymphoma (57) • Uncle (M) – Lung • Paternal Aunt – breast (50) • Grandmother (M) – ovarian (70)
6	*BRCA 1*	African	45 Br.Ca	c.1636_1654delATGAATATTACTAATAGTG	p.Met546Valfs*20	Pathogenic by 4 labs	Ductal Carcinoma	• Mother – breast (43)
7	*BRCA 1*	East Indian	35 Br.Ca	c.1961dupA	p.Tyr655Valfs*18	Pathogenic by 8 labs	Ductal Carcinoma	• Aunt (M) – breast (35)
8	*BRCA 1*	African	48 Br.Ca	c.4327C>T	p.Arg1443*	Pathogenic by 4 labs	Ductal Carcinoma	None
9	*BRCA 2*	African, Hispanic	48 Br.Ca	c.1763_1766delATAA	c.1763_1766delATAA	Pathogenic by 3 labs	Ductal Carcinoma	• Brother – lung (50) • Father – breast (50) • Aunt (P) – breast (85)
10	*BRCA 2*	African, East Indian	39 Br.Ca	c.8029G>T	p.Glu2677*	Pathogenic	Ductal Carcinoma	None
11	*BRCA 2*	East Indian	22 Br.Ca	c.8754+1G>A		Pathogenic	Ductal Carcinoma	None
12	*PTEN*	African	50 Thy, 56 Br.Ca, 56 Endo	c.697C>T	p.Arg233*	Pathogenic by 4 labs	Ductal Carcinoma	• Brother – prostate (unknown)
13	*CHEK2*	Not reported	49 Br.Ca	c.817_818delGA	p.Glu273Asnfs*16	Pathogenic	Ductal Carcinoma	• Mother – cervical (72) • Father – unknown
14	*PALB2*	African	56 Br.Ca	c.2120delC	p.Pro707Leufs*2	Pathogenic	Ductal Carcinoma	• Sister - breast (20) • Mother -Breast (50)

Figure 2: Distribution of HBOC genes found in patient sample.

Figure 3: Distribution of HBOC mutations and VUS in the Trinidad and Tobago regions.





The average age of diagnosis for patients with HBOC mutations was 39.8 (range 22-56). For *BRCA*1 it was 36.5 years (range 23-48), and for *BRCA* 2 it was 36.3 years (range 22-48). For HBOC positive patients, the age ranges were: two out of fifteen (0.13) for 18-29 years, three out of fifteen (0.2) for 30-39 years, four out of fifteen (0.27) for 40-49 years, five out of fifteen (0.33) for 50-59 years and one out of fifteen (0.07) for 60 and over. There was a Variant of Uncertain Significance (VUS) rate of 27.6%, and a negative rate of 46.6%

With respect to subtypes of breast cancer, all the HBOC mutation carriers had invasive ductal carcinoma. Most common ethnicity among mutation carriers were those that self-identified as being of African descent (0.35) followed by those of East Indian descent (0.29). In *BRCA* 1 carriers, most common self-identified ethnicity was African descent (0.66). With respect to *BRCA* 2, most common self-identified ethnicity was Mixed (0.66), which to the patients meant African/Hispanic and African/East Indian.

In terms of family history, eight out of twelve (0.66) *BRCA* mutation carriers reported having some form of family history of cancer, six out of twelve (0.50) had at least one first degree relative with breast, ovarian or prostate cancer, five out of twelve (0.42) had extensive family history of cancer (including breast, ovarian or prostate) - of three or more first/second degree relatives. One *BRCA* 2 patient had a first degree male relative with breast cancer. One *BRCA* 1 patient had a family history of ovarian cancer (second degree relative).

The patient with the *PTEN* mutation did have multiple primary tumors suggestive of Cowden syndrome (breast, endometrial, and thyroid), with several of the characteristic benign tumors such as skin hamartomas. She had a family history of a brother with prostate cancer (although she was unsure of his age of diagnosis). Previously she had tested negative on a sole *BRCA*-mutation panel.

The patient with the CHEK2 mutation was diagnosed with breast cancer, and had a family cancer history for both parents (mother: cervical and father: unknown). The patient with the PALB2 mutation had a sister and mother both diagnosed with breast cancer at 20 and 50 respectively.

AIM TWO: Determine the proportion of the general population, who met the criteria for having a high risk of carrying the *BRCA* mutation.

Chart reviews were conducted from 1807 records at the Trinidad and Tobago Cancer Society, for women without a history of breast cancer diagnosis. The characteristics of the 520/1807 patients who reported having some family history are shown in Table 4.

Table 4: Characteristics from chart extractions describing clients of the Trinidad and Tobago Cancer Society who reported family history during 2010 - 2013

	N = 520	**Proportion**
Mean Age	55	
Age Range	20 - 59	
Ages		
20-29	41	0.07
30-39	73	0.14
40-49	128	0.25
50-59	149	0.29
≥60	129	0.25
Ethnicity (Self identified)		
Afro-Caribbean	198	0.31
East Indian	161	0.38
Mixed	161	0.31

From that subset which reported family history, the proportion that met NIH/NCI criteria are shown in Table 5 below. The most common criteria reported in charts

was extensive family history (i.e. three or more first or second degree relatives diagnosed with breast cancer or ovarian cancer). This was followed by having at least one relative with a premenopausal breast cancer diagnosis (Table 6).

In short, our results showed that 6.8% (123/1807) of the patients seen at the Trinidad and Tobago Cancer Society during 2010-2013 met the NIH/NCI criteria for referral to a genetic counselor to be further evaluated for genetic testing.

Table 5: Number of Family history factors met by each client, as suggested by the NIH/NCI that are associated with an increased likelihood of having a *BRCA* mutation

Number of NIH/NCI Family History Factors Met by Each Patient	**N= 520**	**Proportion**
1	105	0.20
≥2	18	0.03
No factors	257	0.49

Table 6: Family history criteria suggested by the NIH/NCI that are associated with an increased likelihood of having a *BRCA* mutation.

Family history factors suggested by the NIH/NCI	**N**
Three or more first or second degree relatives diagnosed with breast cancer or ovarian cancer	102
Two first degree relatives diagnosed with breast cancer, with one below age 50	83
An immediate family member with bilateral breast cancer	41
Two or more first- or second-degree relatives diagnosed with ovarian cancer (regardless of age at diagnosis)	35
Breast cancer diagnosed in a male relative	1
Three or more first or second degree relatives diagnosed with breast cancer or ovarian cancer	1
Family history reported, but did not meet family history criteria	257

DISCUSSION

Currently, the infrastructure for genetic counseling and testing in Trinidad and Tobago is limited in terms of accessibility, as it is only available in the private sector of local healthcare system for $1500-2000 USD. In addition, there are no trained genetic counselors within the country. However, Trinidad and Tobago has an average age of breast cancer diagnosis at about 45-50 years, which is significantly younger than the average age in the United States (61 years). The significant prevalence rate of HBOC mutations found in this study of 25.8% in breast cancer patients meeting NCCN criteria strongly suggests a major role of HBOC syndrome in young breast cancer patients in T&T. More importantly, this high mutation rate also serves to emphasize the need for genetic counseling and testing services to be made obtainable in T&T.

Our results are consistent with a previous study in T&T, which demonstrated a significantly high rate of *BRCA* mutations in the country. This study tested 268 unselected local patients for *BRCA* and *PALB2* mutations. They found an estimated *BRCA* mutation rate of approximately 9.5%, and estimated the *PALB2* mutation rate at approximately 1.1% (16). Our study, which was the country's first multi-gene panel investigation, further confirmed that indeed there are significantly higher rates of HBOC mutations in T&T, but that they go beyond *BRCA* and *PALB2* within this population.

To extend the importance of our findings to a region-wide scale, it should be noted that there have not been, until this moment, published studies investigating

the prevalence of the other HBOC mutations beyond *BRCA* in the Caribbean region. Therefore, our study marks the first of this kind to investigate HBOC mutations further than the sole *BRCA*-panel genetic testing.

The significance and clinical relevance of extending beyond *BRCA*-focused testing in novel populations was demonstrated in our sample. For example, one of our patients who tested positive for *PTEN* reported having negative results with previous genetic testing, but this was done solely with a *BRCA* mutation panel. This caused her local physicians to rule out an underlying genetic influence to her cancer diagnoses, which in turn influenced her oncology care and prevention options for future cancers. Therefore, by widening the panel to incorporate more HBOC mutations (particularly for families who are not familiar with their genetic status), we can provide a more thorough screening to this population.

It should be noted that in Trinidad and Tobago, there is an extensive ethnic variety, and our sample captured this appropriately. The local population of Trinidad and Tobago is mostly composed of African and East Indian descendants owing respectively to the slave-trade, and the arrival of indentured laborers from southern Asia seeking employment after the abolishment of slavery (17).

Trinidad and Tobago also has a rich European colonizing history. The nation was first discovered by the Spanish explorer, Christopher Columbus. This was followed by the colonization of the Dutch, French and ultimately the British before the nation gained independence in the mid 20th century. In addition to these

many cultures present on the island, there is the native population which consists mostly of Arawaks and Carib ancestries (17). Our sample seemed to capture similar proportions to the population makeup. In addition, 39% of our participants identified as ‘mixed’, and did not wish to identify as being exclusively from one ethnicity.

Interestingly, the genetic sequences found in the patients who tested positive reflected this ethnic diversity. In the 15 positive mutations, with the exception of the single mother/daughter pair, no two mutations were the same. Also, the genetic mutations seen in our sample were previously reported in studies regarding distinct populations from a wide variety of countries. For example, one patient, a 29 year old *BRCA* 1 carrier had a mutation (c.3756_3759delGTCT), and a 48 year old *BRCA* 1 carrier had a mutation (c.4327C>T). While both of these patients, self-identified as being of African descent, their mutations have been well described as founder mutations in the French Canadian population in Quebec. This mutation was thought to have originated from France, and brought to Quebec during the 17^{th}-18^{th} century settlement period. Given Trinidad and Tobago’s past French colonization history, it can be assumed that similar settlements came to T&T from common origins in France (18) (19).

Another mutation (c.1961dupA) is generally assumed to be a founder mutation in the Pakistani population (20), was found in a 35 year old *BRCA* 1 carrier, who self-identified as being of East Indian descent. One of our BRCA 2 carriers (48 years at diagnosis) who identified as both Hispanic and African had the genetic

mutation (c.1763_1766delATAA) which has previously been found in unrelated Muslim families from Israel (21).

Therefore, the rich cultural variation in Trinidad and Tobago can explain the broad diversity of genetic mutations seen in this sample. However, for an island population, which typically lends itself to geographic and reproductive isolation, it was initially hypothesized that T&T's high mutation rate was attributed to some founder mutation effect within the country, such as that seen in Bahamas (11). But, this phenomenon would have resulted in little diversity in our genetic results. Thus, the extensive genetic variability in these mutations, does not lend itself to the founder effect hypothesis, but rather reflects the vast ethnic diversity that is present within T&T due to its past migration patterns.

In terms of distribution of the sample, most of the patients found to have mutations were from the northwestern and central parts of Trinidad (Figure 3), which were the areas closest to the National Radiotherapy Center, located in the northwest part of the island. As the main cancer institute for the public health-sector, it provides complete oncology services for citizens, which includes surgeries, radiotherapy, chemotherapy and post-treatment surveillance. Therefore, it attracts patients from all over the country. This allowed us to gather a sample that was as close to an accurate representation of the national population as possible from a single location. Despite this, we still were limited in terms of our reach to the southern and eastern portions of Trinidad, as well as Tobago.

Another interesting aspect of the results was the family history reported by those enrolled in the study. In terms of family history, 67% *BRCA* mutation carriers reported having some form of family history of cancer, and 50% had at least one first degree relative with either breast, ovarian or prostate cancer, 42% had extensive family history of cancer of three or more first/second degree relatives with breast, ovarian or prostate cancer. However, 33% of the *BRCA* mutation carriers, reported having no family history, and met the criteria for the study solely based on age. It should be noted that many of the study participants were uncertain of their complete family history, as cancer remains a fairly taboo topic in the nation.

Therefore, this showed that when evaluating risk to ascertain who needs genetic testing, perhaps T&T requires different selection criteria, perhaps one that relies less on family history. When considering cancer patients, the criteria should rely mostly on age of diagnosis parameters. Therefore, more research is needed to determine which criteria should be used for the local population of T&T to accurately evaluate who needs these services.

The high prevalence of HBOC mutations in our breast cancer patient sample strongly indicates the need for genetic testing infrastructure in the country. This need was further demonstrated by our second aim which found that 6.8% of the women (with no previous cancer history) who visited the Trinidad and Tobago Cancer Society during 2010-2013, had family history criteria that recommended that they visit a genetic counselor to evaluate their risk of developing breast and/or ovarian cancer in the future (3) (22). Of the total number of patient

records analyzed, 5.8% (105/1807) of the patients reported having one criteria, and 1% (18/1807) of the patients reported having two or more criteria. The second most common criteria reported in charts was having a relative with a premenopausal breast cancer diagnosis (usually before 50 years), which aligns with the high rates of young breast cancer diagnoses in Trinidad and Tobago.

However, it should be noted that despite family history being the most common factor, about 61% of the women had another factor that made them at high risk for having a genetic mutation. Therefore, this contributes to the previous deduction that a criteria specific for T&T needs to go beyond the presence of family history when detecting high risk women in Trinidad and Tobago. The two least reported factors were: having male relatives affected with breast cancer, as well as, having multiple relatives with breast or ovarian cancer. It should be noted that this portion of the study did not inquire about prostate or pancreatic cancer in male relatives. Therefore, future investigations should contain family history questions that inquire beyond breast and ovarian cancers to attain a complete risk assessment.

The Trinidad and Tobago Cancer Society was chosen as our single location to investigate the second aim because it is one of the major local non-profit organizations, and it attracts citizens from all over the country, similar to the National Radiotherapy Center. Therefore, it allowed us to gather a sample from a single location that was closest to an accurate representation of the national population.

In summary, these two aims show that genetic counseling and testing should be accessible in Trinidad and Tobago for both cancer patients and unaffected citizens, as HBOC mutations are prevalent in the cancer patient population, and a number of women without cancer diagnoses meet the criteria for genetic counseling.

Access can offer women preventative measures such as prophylactic surgeries (mastectomy and salpingo-oophorectomy), chemoprevention options and increased screenings, which have all been proven to lower mortality rates regardless of a previous cancer diagnosis (14) (13). Therefore, it is imperative that a cancer screening and prevention program be established in this country. This can, ultimately, help lead to the reduction in local breast cancer incidence and mortality.

LIMITATIONS

Our data collection for patients was either done from chart records or from the patients themselves. Therefore, more fundamental clinical details of patients' diagnoses could not be obtained in a timely manner. For example, tumor receptors were not automatically attained at the time of diagnosis in Trinidad and Tobago. In the public sector this can take weeks to months for the results to return. Therefore, many patients we recruited at the time did not have their tumor receptors on file. We hope to gather this information to complete our interpretations of the results, particularly with regard to the role of triple negative breast cancer and genetic mutation carriers within this population.

Topics surrounding cancer are still very taboo in T&T's culture. Even though there have been more awareness and educational campaigns, unfortunately, many family members do not share their diagnosis with those close to them. This impacted the study because some of our pedigrees for patients were incomplete due to uncertainty of family history. Some patients did share instances of unknown causes of death for family members, which were preceded by weight loss, abdominal pain and rapid deterioration. However, they were ultimately uncertain whether these were attributed to an underlying cancer diagnosis. Considering this aspect of the local culture, during genetic counseling sessions, patients were offered options to confidentially notify family members that they thought may benefit from testing, without having to reveal their personal diagnosis.

In addition, we did have some delay in the return of results, as initially there seemed to be a cultural barrier with telemedicine which limited the effectiveness of the post-test genetic counseling. For example, our first patient who tested positive for a genetic mutation did not appear to benefit fully from the telemedicine session, and seemed to have some difficulty understanding her results. This was perhaps due to most medicine in T&T being performed in-person. Therefore, in order to ensure effective and safe return of the results, we needed to accommodate genetic counselors traveling to T&T to deliver some in-person counseling. This delayed this portion of the study by approximately four months. However, the in-person return of the results were more beneficial, particularly to the patients who tested positive, as they had the most clinical impact from the results.

As mentioned in the Discussion, Figure 3 shows that our sample mainly came from areas closest to the National Radiotherapy Center. This could possibly be because we required that participants travel to northwestern Trinidad, which could have been a hindrance for potential participants. All Tobago participants needed to commute to Trinidad which required a flight or water ferry transportation. Given that Tobago has about a quarter of the country's population, our sample may not have captured the prevalence adequately of this particular island, as only 3.4% (2/58) of the patients were from Tobago. Therefore, future studies need to expand to other oncology treatment sites across the islands to incorporate a wider distribution of patients.

Finally, our study intended to sample about 350 women at the National Radiotherapy Center. However, funding could only be obtained for 60 samples. Therefore, we hope to receive additional funding to continue pursuing research within this population.

CONCLUSIONS AND FUTURE DIRECTIONS

Trinidad and Tobago has one of the highest breast cancer rates in the Caribbean region. This disease represents 30% of all new female cancer cases and 24% of all female cancer related deaths. In addition, a large proportion of breast cancer cases in T&T appear to occur at a young age. HBOC can be associated with a younger age of breast cancer diagnosis. Therefore, we investigated the prevalence and spectrum of HBOC mutations in the local population of high risk breast cancer patients (by NCCN guidelines), and also investigated the proportion of women who met NIH/NCI criteria for further genetic counseling and testing.

Through our sample, we found that for the women who met the NCCN guidelines, approximately 25.8% had an HBOC mutations: 21% *BRCA*, 1.7% *PTEN*, 1.7% *CHEK2* and 1.7% *PALB2*. In addition, approximately 6.8% of adult women (with no cancer diagnosis) who visited the T&T Cancer Society during 2010-2013 met the criteria for further genetic counseling and evaluation according to U.S. guidelines.

These two conclusions strongly suggest that genetic testing infrastructure needs to be established in this country in an accessible manner. Therefore, it is recommended that the government put policies and resources in place to facilitate this new addition to local oncology in the public system.

Future works in Trinidad and Tobago include the establishment of a high-risk clinic that promotes continued genetic testing and counseling to families of affected patients, and other high risk patients who are referred by local physicians. By using the framework provided by this research project, we are able to continue our partnership with Color Genomics and the local oncologists.

It is our goal to continue assembling funding to help subsidize the cost of the test for patients, which will enhance accessibility. Ultimately, we hope that genetic counseling and testing would be implemented in the public healthcare sector, which can thereby remove the cost from patients entirely. With genetic testing as part of Trinidad and Tobago's oncology regimen, there can be a reduction of breast cancer incidence and mortality rates throughout the nation, and this can ultimately be used as a model for other Caribbean islands.

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APPENDIX

APPENDIX 1: LABORATORY METHODS FOR COLOR GENOMICS INC – GENETIC SEQUENCING OF SAMPLES

“The Color Test is designed to assess clinically relevant mutations in 30 genes associated with hereditary cancer risk. Genomic DNA is extracted from a saliva sample using standard methods. Next Generation Sequencing libraries compatible with the Illumina platform are generated and enriched for the 30 genes via a custom designed Agilent SureSelect bait library. DNA fragments enriched from these genes are retrieved and analyzed using 2x150 paired end sequencing with an Illumina NextSeq 500 instrument. After alignment to reference genome GRCh37.p12 (hg19), low quality and duplicate reads are removed and variants are detected with GATK Haplotypecaller. This test detects single nucleotide substitutions, small insertions and deletions, and copy number variations located in the DNA coding sequences, nearby flanking regions (+/20bp) and known splice regions in the genes targeted by the Color panel. Any exceptions to this are noted in the Limitations section.

Variants are classified according to the standards and guidelines for sequence variant interpretation of the American College of Medical Genetics and Genomics (ACMG). Variant classification categories include pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. All variants are evaluated by a board certified medical geneticist or pathologist. Identified likely benign and benign variants are not reported. The presence of a VUS is always

reported, and the details are available upon request. All VUS and likely pathogenic variants are reviewed bi-annually for updates in the scientific literature. As part of the Color service, we will attempt to recontact the client if any reported variant's classification changes.

This test was developed and its performance characteristics determined by Color Genomics, a clinical laboratory accredited by the College of American Pathologists (CAP) and certified under the Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity testing (CAP #8975161 - CLIA #05D2081492). This test has received the European Conformity (CE) mark approval in compliance with the EU legislation. This test has not been cleared or approved by the United States Food and Drug Administration (FDA). The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research.

Genes *APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4*, CDKN2A (p14ARF), CDKN2A (p16INK4a), CHEK2, EPCAM*, GREM1*, MITF*, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2*, POLD1*, POLE*, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP5 3*

* These genes are only analyzed at specific locations (see Limitations).

Limitations: This test aims to detect all clinically relevant variants within the genes analyzed (defined above). The majority of these genes are assessed for variants within all coding exons (+/- 20bp in the nearby flanking regions). Exons 12-15 of PMS2 and homopolymer regions outside of the coding regions cannot

be reliably assessed with standard target enrichment protocols. For the CDK4, MITF, POLD1 and POLE genes, the elevated risk of cancer is associated with distinct functional genomics regions. The complete coding sequences of these genes are not reported, but instead only the following regions: CDK4 - chr12:g.58145429-58145431 (codon 24), MITF chr3:g.70014091 (including c.952G>A), POLD1 - chr19:g.50909713 (including c.1433G>A) and POLE - chr12:g.133250250 (including c.1270C>G). In EPCAM, only large deletions and duplications including the 3' end of the gene are reported. These are the only variants known to silence the MSH2 gene and therefore increase risk of associated cancer. GREM1 is only analyzed for duplications in the upstream regulatory region.

The test might have reduced sensitivity to detect insertions and deletions between approximately 40-250bp. While this test does detect large deletions and duplications, it is not designed to detect chromosomal aneuploidy or complex gene rearrangements such as translocations, large insertions, and inversions. It also does not reliably detect mosaicism.

Color only reports findings within the genes that are on the panel. It is important to understand that there may be variants in those genes that current technology is not able to detect. Additionally, there may be genes associated with hereditary cancer risk whose clinical association has not yet been definitively established. The test may therefore not detect all variants associated with hereditary cancer risk. Additionally, in the unlikely event a variant is detected that is associated with a disorder or disease other than cancer, this information will be included in the

report. Genetic counseling and/or physician consultation may be warranted to ensure complete understanding of your test results.

Environmental and other factors are thought to cause the majority of cancers. Consequently, tests that do not detect a pathogenic or likely pathogenic mutation do not eliminate an individual's hereditary cancer risk and do not guarantee present or future health. In addition, the causes of cancer are multifactorial and can be influenced by both inherited and acquired genetic mutations, age, environment and various lifestyle choices. An individual's risk of cancer is dependent upon each of these factors as well as family disease history. In very rare cases, such as circulating hematolymphoid neoplasm, allogeneic bone marrow transplant, or recent blood transfusion (within 7 days of testing), the results of germline DNA analysis may be complicated by somatic and/or donor mutations.

Disclaimers: Color implements several safeguards to avoid technical errors, such as 2-dimensional barcoding and barcode scanning at several steps throughout the sequencing process. Color Genomics is not responsible for errors in specimen collection, transportation, and activation or other errors made prior to receipt at our laboratory. Due to the complexity of genetic testing, diagnostic errors, although rare, may occur due to sample mix-up, DNA contamination, or other laboratory operational errors. In addition, poor sample DNA quality and certain characteristics inherent to specific regions of an individual's genomic DNA may limit the accuracy of results in those regions.

In the absence of an identified pathogenic or likely pathogenic mutation, several standard risk models may be employed to determine potential risk of breast cancer and guidelines displayed on this report. All risk estimation is approximate, sometimes cannot be specifically calculated, and is based on previously analyzed cohorts. Additionally, risk estimation may be incorrect if inaccurate personal or family history information is provided. An elevated risk of cancer is not a diagnosis and does not guarantee that a person will develop the disease.**”** (23)