

DEVELOPING A FUZZY LOGIC FOR EARLY PREDICTION OF BRCA1/2 NEGATIVE HEREDITARY CANCER ON MATLAB

PEMBE VOLKAN MASTER THESIS

MOLECULAR MEDICINE PROGRAM

THESIS SUPERVISOR Assoc. Prof. MAHMUT ÇERKEZ ERGÖREN

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NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

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ACCEPTANCE/APPROVAL

NEAR EAST UNIVERSITY

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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DEDICATION

This thesis is dedicated to my family who was with me under all circumstances and to my friends who always supported me. I love you all. Thanks for everything.

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ABSTRACT

The importance of early diagnosis in cancer plays an important role in saving time for the patient to recover. The earlier cancer disease is diagnosed, the sooner the treatment can be started. The focus of our study is to achieve cancer diagnosis within a few minutes using the fuzzy logic system. The purpose of our study develop software for early prediction of BRCA1/2 negative hereditary breast cancer using fuzzy logic system with different genetic variation related with breast cancer on MATLAB. Patients' data were collected from both Uludağ University Faculty of Medicine Department of Medical Genetics and Erciyes Üniversitesi Faculty of Medicine Department of Medical Genetics. Overall, 488 different individual data were examined. However, 90 of 488 were applicable for this study as only 90 patients did not have any considerable genetic variations within BRCA1 and BRCA2 genes. The MATLAB program was used to develop fuzzy logic that was harmonized for the risk of breast cancer prediction. We mainly focused on genetic variations associated with 18 hereditary breast cancer genes and used 14 different risk factors related with breast cancer. All data trained into our system and membership functions in the input clusters were offered different degrees of different possibilities for different patients from 0 to 1. Six different validation patients were selected and trained the system. The reliability of the system was measured and the result obtained was accurately. When the data were trained, the results were obtained as two benign variants with 0.25 (%25), two variants with unknown significant variants with 0.5 (%50), and two pathogenic variants with 0.92 (%92). Since, the rate of breast cancer people is very high, early prediction of cancer is crucial. Despite, fuzzy logic system has started to be used in healthcare, there is not much work similar to this current study. This designed artifical inteligence software will be used for early detection of breast cancer in the future.

Key words: MATLAB, Fuzzy Logic, breast cancer, BRCA1, BRCA2

ÖZET

Kanserde erken tanının önemi, hastanın iyileşmesi için zaman kazanmasında önemli bir rol oynar. Kanser hastalığı ne kadar erken teşhis edilirse, tedaviye o kadar erken başlanabilir. Calısmamızın odak noktası, bulanık mantık sistemini kullanarak birkaç dakika içinde kanser teşhisine ulaşmaktır. Çalışmamızın amacı, MATLAB'da meme kanseri ile ilgili farklı genetik varyasyona sahip bulanık mantık sistemini kullanarak BRCA1/ 2 negatif kalıtsal meme kanserinin erken tahmini için yazılım geliştirmektir. Hasta verileri hem Uludağ Üniversitesi Tıp Fakültesi Tıbbi Genetik Bölümünden hem de Erciyes Üniversitesi Tıp Fakültesi Tıbbi Genetik Bölümünden toplandı. Genel olarak, 488 farklı bireysel veri incelenmiştir. Bununla birlikte, sadece 90 hastanın BRCA1 ve BRCA2 genleri içinde önemli genetik varyasyonları bulunmadığından 488 kişiden 90'ı bu çalışma için uygulanabidi. MATLAB programı, meme kanseri tahmini riski icin uyumlu hale getirilerek bulanık mantık gelistirmek icin kullanıldı. Esas olarak 18 kalıtsal meme kanseri geni ile ilişkili genetik varyasyonlara odaklandık ve meme kanseri ile ilgili 14 farklı risk faktörü kullandık. Sistemimizle ilgili eğitilen tüm veriler ve girdi kümelerindeki üyelik fonksiyonları, 0'dan 1'e kadar farklı hastalar için farklı derecelerde farklı olasılıklar sunuldu. Altı farklı validasyon hastası seçildi ve sisteme eğitim verildi. Sistemin güvenilirliği ölçülmüş ve elde edilen sonuç doğru bir şekilde elde edilmistir. Veriler eğitildiğinde, sonuçlar 0.25 (%25) ile iki iyi huylu varyant, 0.5 (% 50) ile bilinmeyen önemli varyantları olan iki varyant ve 0.92 (% 92) ile iki patojenik varyant olarak elde edildi. Günümüzde meme kanseri oranı çok yüksek olduğundan bu konuda araştırmalar yapılmaktadır. Bununla birlikte, bulanık mantık sistemi sağlık hizmetlerinde kullanılmaya baslanmasına rağmen, çalışmamıza benzer fazla çalışma yoktur. Bu çalışma, tüm bu konulara ışık tutacak ve gelecekte tasarlanan yapak zekâ yazılımı meme kanserinin erken teşhisi için kullanılabilecektir.

Anahtar Kelimeler: MATLAB, bulanık sistem, mem kanseri, BRCA1, BRCA2

ABBREVIATIONS

ACMG	American College of Medical Genetics
AI	Artificial Intelligence
APC	Adenomatosis Polyposis Coli
ATM	Ataxia-Telangiesctasia
BART1	BRCA1 Associated RING Domain 1
BLM	Bloom Syndrome Helicase
BPT1	Stands for BRCA1 Interacting Protein C-terminal Helicase 1
BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
CDH1	Cadherin-1
CHECK2	Checkpoint kinase 2
DSB	Double-strand break
FAM175A	Family with sequence similarity 175, member A
MSH6	mutS homolog 6
MSH2	mutS homolog 2
MUTYH	mutY DNA glycosylase
NBN	Nibrin
RAD50	Double-strand break repair protein
RNF	Ring finger proteins
PTEN	Phosphatase and tensin homolog
PALB2	Partner and localizer of BRCA2
SNP	Single Nucleotide Polymorphism

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CHAPTER 1 INTRODUCTION

1.1 Introduction

The detection of cancer at is early stages may lead to the reduction of the rates of death in a longer duration. Cancer may result from genetic changes within the genes that play a role in cellular growth. The variations within the cells continue dividing and multiplying in a manner that is not controlled.

Breast cancer is a cancer that develops within the breast tissue. On a regular basis, cancer develops as lobules or the ducts within the breast. Lobules denote the glands that are engaged in the production of milk, and the ducts denote the pathways that offer milk from the gland up to the nipple. Cancer occurs within the fatty tissue or the fibrous connective type of tissue that is situated in the breast. The disease that is not regulated usually engages in the invasion of the other tissue that is healthy within the breast and is capable of transfer within the nodes below the arms. The lymph nodes are the major pathways that assist the cancer cells in moving to other body parts (DeSantis et. al. 2017).

1.2 Cancer Biology

1.2.1.1 Breast Cancer

The cells responsible for the breast cancer cells often originate from the tumour that may be identified via the x-ray or at times felt via the lump. Breast cancer is more common in in women, however rarely in men. Non-cancerous types of breast tumours are classified as cases of abnormal forms of growth. However, they may continue to cover other areas besides the breast. American Cancer Society (2020) indicated that they might not be hazardous. Still, specific categories of benign breast lumps may be responsible for increasing the risk of a woman getting breast cancer. Any breast type of lump or change requires assessment by a professional in health care in determining whether, it is the malignant or benign type of cancer and if it may have an effect on the future risks of one getting cancer. There is a constant relationship between breast cancer and age, life-style, age and hormonal factors (Newman, 2016).

1.2.1.2 Breast Cancer Aetiology

Breast cancer is very complicated type of disease that is described as being multifactorial. Regardless of certain genetic components being endowed with stronger and clear effect such as genetic variations within the Breast Cancer Associated Gene 1 (BRCA1) and Breast Cancer Associated Gene 2 (BRCA2). Approximately 10% of cancers relating to the breast are regarded as due forms of mutations experienced in high penetrance categories of genes. Breast cancer is probably a group of diseases with various reasonable factors. Until today the exact cause of breast cancer is not clear. Hormonal changes increase the risk of breast cancer, oftenly 'promote' rather than 'initiate' the tumor development (Key et. al. 1999).

Breast cancer essentially starts with cells in the milk-producing channels which is also called invasive ductal carcinoma, moreover may also start in the glandular tissue called invasive lobular carcinoma or tissue in breast or in different cells (Osborne et. al. 2012).

1.2.1.3 Cellular and Molecular Mechanism of Breast Cancer

The evidence present from the postmortem and clinical related studies proposes that between 47-85% of the patients with breast cancer will be identifiable with the bone metastasis (Chen et. al. 2018). Similarly, there have been reports that breast cancer affects the sites and rates of metastases. The low figures associated with the bone metastases included the patients with the estrogen (ER) negative/ human epidermal factor of growth receptor 2 (HER2)-negative tumours which is 55.2%. On the other hand, this has had a significant increment of close to 69.8% 9HER2-positive tumours).

The most renowned sites about bone metastases include the pelvis, skull, ribs, and the proximal femur (Peng et. al. 2018). The actions that lead to destroying these bones, usually result in the excessive skeletal linked complications such as the pain in the bone, features linked with pathology, hazardous life hypercalcemia, and never compression related syndromes. Part of the theme may be fatal and have a significant reduction in life quality.

It is based on cancer cells that secure two special capacities, such as metastasis, invasiveness and increased mobility. Metastasizing cells are basically similar to those in primary tumors (William et. al. 2020). The underlying case of metastasis may be the change of ordinary breast cells to cells with oncogenic transformations. The age of subsequent malignant growth stromal cells can be poorly predicted, since a self-correcting population of stromal cells collects the necessary transformations for tumor formation (Dontu, et. al. 2004).

The bone metastasis is a very complicated process that needs cells that promote breast cancer in detaching from the primary tumour that moves via the blood or the lymphatic system. Eventually, they have their survival in the bone microenvironment and then contributing towards the proliferation of the bone tissue. Up to date, the genomic studies have proposed that every stage with metastasis is linked with the series about the molecular types of events. On the other hand, the interactive network about molecular mechanisms linked with bone metastases ranging from breast cancer that is yet to be understood fully (Zheng et. al. 2018).

1.2.1.4 Breast Cancer Genetics

1.2.1.5.1 Somatic Breast Cancer

Breast cancer has significant effects on post-menopausal women, despite it is approximated at close to 5% of the cases among young adults who are below forty years. Somatic mutations have been widely described in breast cancer (Mertins et. al. 2016). Somatic breast cancer mostly causes by an environmental factors and multiple gene inheritance (polygenic). It stables unknown which of the genetic deviations recognized in sporadic breast cancer tumorigenesis is causative. There are many environmental factors that cause to develop breast cancer, such as lifestyle, exposure to ultraviolet radiation, carcinogenic pollutants and diet. Generally, the rate of exposure to some carcinogens is low and the protection mechanisms of each person are different. The incidence of cancer appears sporadic is formed when combine with the multi-stage nature of cancer initiation (Herold et. al. 2016). There is a model that is currently kept and surrounded by the loss of TP53 or other cell cycle control point genes, as well as early loss of cell cycle control, also the uncontrolled cell cycle of mutagenic signals from the surrounding tumour microenvironment activate to rampant cell cycle. It causes of raise the level of genomic inconstantly and carried on division below the conditions in

conclusion genetic alterations and chromosomal abnormalities formed and breast cancer developed (Donegan, 2002).

Additionally, the ultraviolet radiation is another factor to cause somatic breast cancer (Parsa, 2012). The classic example of uncorrected mutations somatic genes can be shown as $C \rightarrow T$ and $CC \rightarrow TT$ conversion associated with UV radiation (Brash, 1997). Somatic mutations are not found in all cells and occur in a single cell (Minamoto, et. al.1999).

Germline mutations within BRCA1 and BRCA2 genes may offer support to the carcinogenic actions in close to 20% among the young patients. Other than the germline transformation, tumour movement relies upon the passing of a wild-type allele. Allelic misfortunes in BRCA1 and BRCA2 loci have additionally been identified in a high extent of sporadic breast tumours, recommending the job of these qualities in the improvement of non-acquired breast cancer (Janatova et al. 2005). Mutations in other categories of cancer carrier genes like CHEK2, TP53, and PTEN may justify the additional 4% regarding the early instances of the onsets.

BRCA1 encodes a multifunctional protein that holds a key work within the support of genomic solidness (Huen, et. al. 2009). BRCA1 protein is important for DNA double-strand breaks settle through homologous recombination (HR) a high-constancy settle prepare that utilizes the sister chromatid as a organize for DNA settle (Caestecker et. al. 2013).

Consequently, BRCA1 loss of capacity could incline cells to blunders in DNA replication prompting amassing of substantial changes that would prompt tumour improvement. Also, it has recently been suggested that somatic hyper-methylation of the RAD51C gene promoter results in a mutation sign same to that detected in BRCA1 imperfect tumours. Younger age had been associated with lesser cases of favouring prognosis within the breast cancer, especially because of the early incidences of the on-set linked instances that consist of the lower classes of the relatively better outcome-based luminal. It a sub-type and the higher classes of the more involving triple negative category of the subtype are eminent.

1.2.1.5.2 Hereditary Breast Cancer

The proposed that germline mutations within BRCA1 and BRCA2 genes are associated with most hereditary breast cancers. Mutations within these genes only represent 28% of the family-related risks. However, using high-resolution screening technologies more novel BRCA1/2 variations are detecting every day (Apostolou et. al. 2013). Additionally, Women who are carriers of either BRCA1 or BRCA2 germline variations are also at higher risks for developing ovarian cancer and fallopian tube cancer. On top of this, the BRCA2 type of mutation carriers has also accelerated the risks from other categories of cancers such as male breast cancer, prostate cancer, pancreas cancer, intestines cancers, and melanoma. In a bigger study done by the scholars who investigated the modifiers of BRCA1/2, the average diagnosis age was established to be nearly forty years among BRCA1 and forty years among BRCA1 mutation carriers (Gentilini et. al. 2020).

Despite the germline mutations within BRCA1 and BRCA2 genes posing higher risks linked with the cancer of ovary and breast, the penetrance among these genes indeed is never completed. The risk of developing breast cancer for BRCA1 and BRCA2 carriers before 70 is between 45-87%. In the case of ovarian cancer, the risk was identified as between 45-60% among BRCA1 mutation carriers and 11-35% among the BRCA2 mutation carriers. Regardless of this, the penetrance is dictated by a variety of factors such as the mutation type and the exogenous oriented factors. Similarly, the lifestyle-oriented factors like the physical form of exercises and the absence of obesity among adolescents have been linked with major delays in the onset of breast cancer (Thomassen et. al. 2014). BRCA1 and BRCA2 have a notable complicated genomic constitution, and the coding areas depict the absence of the homology in the past explained genes or the other. The BRCA1 gene comprises of the 24 exons that encode the bigger protein belonging to the 1863 amino acids (Choi, 2003). On the other hand, BRCA2 comprises of 27 exons that engage in the encoding of the bigger protein consisting of 3418 amino acids. Between two genes, the very first exon denotes non-coding while the exon is indeed very big (Eisenhaber, 2011).

BRCA1 and BRCA2 conduct their functions in suppressing the tumour and the crucial, which is the maintenance of the genomic stability via the role they serve in the damage of the DNA signalling and BRCA1 and BRCA2 genes have implications in the mediation while repairing the double strands breaks (DSBs) through the homologous recombination (HR) via interaction with the RAD51 gene. After the DNA damage, BRCA1 associates with RAD51 and also BRCA1 terminates phosphorylation where the damages occur. Meanwhile, BRCA2 carries its duties downwards the BRCA1 through the complicated establishment with RAD51. The major role served by the BRCA2 is the facilitation of HR. Cells that lack BRCA1 or BRCA2 are not able to aid the repair of the DSBs through the error independent HR, and thereby leading to the repair of the error oriented non-homologous end joining (NHEJ) pathway responsible for the production of the chromosomal instability. During S-phase, BRCA1 and BRCA2 expression rise and show the function involved in the maintenance of the genomic stability within the process of the DNA replication Apart from this function, BRCA1 seems to have the added roles in the DNA repair (Jensen et al., 2013).

To date, there are approximately 24.000 genetic variations including single nucleotide polymorphisms (SNPs) and insertion deletions (indels) within the BRCA1 gene variations. These include 3'/5' splice site and 3'/5' UTR mutations, frame shift, synonymous, missense, and nonsense mutations in the coding regions (Tuncel et. al. 2019). Almost 2000 coding region mutation within the BRCA2 gene. Almost 55% of these mutations that were only identified within families. Nearly 1800 SNPs BRCA1 and BRCA2 have been categorized as variant with unknown significance (VUS). The desire to assessing the clinical value of rare personal sequence variants indeed is offering challenges since the present methodologies need higher cases of occurrences a certain type of variant. In 2009, consortium was found with the intent of analysing the clinical values of those SNPs that possible related clinical as well as the history-pathological ideas from the bigger networks (Sergentanis et. al. 2009)

Germline BRCA1/2 mutations only serve as a representative of "the first hit" within the classical Knudson two-hit type hypothesis, where the second non-activating somatic form of mutation normally entails the deletion of the wild category of allele which is identified as the loss of heterozygosity (LOH). LOH has been seen nearly 80% of the tumours that emerge

from the carriers of the mutation (Thommasen et. al. 2014). The other inactivation mechanism such as somatic, epigenetic silencing through the promoter methylation has been noted in BRCA1 with 9-13% of the sporadic breast tumours and a value ups 42% in non-BRCA1/2 hereditary breast tumours. Contrastingly, BRCA1 promoter methylations are not common in tumours for BRCA1/2 mutation carriers. Also, the BRCA2 promoter methylation generally was never observed in hereditary and sporadic breast cancers.

1.2.1.5.3 Familial Breast Cancer

Many rare cases involving gene variants have been explained as conferring the increment in the risk towards breast cancer, where they involve the higher penetrance genes such as FTK11, RAD51D, TP53, RAD51C, PTEN, and CDH1 (Seibert et. al. 2016). Others are the lower or the moderate penetrance forms genes for instance PALB2, ATM, BRIP1, and CHEK2. Generally, the majority of these genes engage in maintaining the genome integrity and the mechanism involved in DNA repair as well as many aspects linked with the diverse cancer syndromes such as the Li-Fraumeni syndrome (TP53), Cowden syndrome (PTEN), and the Peutz Jegheras syndrome (STK11/LBK1). Moreover, genome-wide association studies (GWAS) have been revealed that some genetic changes which were rarely associated with breast cancer such as 2q35, 2q33, 8q24, 5q11, 5p12, 10q26 and 16q12 (Long et al. 2013).

Lower and moderate penetrant loci or genes may only explain the minor sections of the left non-BRCA1/2 families that are capable of showing higher cases of incidences regarding breast cancer. Regardless of the detailed studies by Siu (2016), genetic linkage evaluation, GWAS, and the latest, next-generation sequencing, to are yet to find out about other dominant penetrance breast cancer susceptible types of genes like BRCA1 and BRCA2. None of the single high penetrance genes may account for the more significant fraction of the left familial form of aggregation (Sachs et al., 2018). On the other hand, the left predisposition is expected to constitute a mixture of less common risk form of variants and the polygenic forms of mechanism that entail more familiar and less common penetrance forms of alleles. Similarly, it may involve the moderate form of penetrance genes that act towards conferring the higher risks linked with breast cancer. However, of late, the germline forms of mutations within the RAD5/C have been associated with the higher cancer risk within the smaller cases involving the hereditary breast and ovarian cancer (HBOC) families. In this regard, it supports the hypothesis that lesser cases of risk-related alleles may bring a certain proportion of the left predisposition. At last, Ming et. al. (2019) stated that the exogenous aspects like the oral types of the contraceptives, the therapy involved in the replacement of the hormones, consumption of alcohol, cases of overweight and the instance involving the physical form of inactivity are all classified the risks factors or cancer (Siu, 2016). Fewer fractions involving these particular families with a nearly more reliable history of the family may be attributed to the risk factors within the environment since cancer of the breast is a disease that is common due to the random aggregation of the sporadic cases involving breast cancer.

1.2.1.5 Human Genetic Variations in Breast Cancer Development

1.2.1.5.1.1 BRCA gene family and Breast Cancer

Human genetic variations exist in a lot of models and appears at various frequencies during the whole genome. The various models of genetic variation in the human genome contains single nucleotide polymorphisms (SNPs), null alleles, mutations, small deletions or insertions and repeated DNA. Genetic variation in DNA repair genes causes many diseases such as breast cancer (Crow, 1987).

In the BRCA gene family have two genes namely BRCA1 and BRCA2 which were mentioned earlier. First the BRCA1 gene exists to the RNF group of genes that encrypt protein described a RING-type zinc finger protein. These proteins are so named in light of the fact that the protein atom has locales that overlap around a zinc particle and on the grounds that the subsequent state of such a district looks like a finger. The shapes of RING class zinc finger proteins allow it to bind easily to mostly proteins, nucleic acids and other molecules. After binding to various molecule, they make a few enzymatic actions that improve a cell continue a constant environment. These enterprises contain protein corruption, cell division and growth, as well as tumor suppression.

The second gene, BRCA2, exist to FANC gene family. In this group genes are assembled a compound of proteins this process also called the Fanconi anemia (FA) pathway. First of

pathway aims on repairing and locating DNA damage. Specifically, the proteins target areas of DNA where the contrary strands of the double helix are not appropriately connected. At the point when they discovery such a zone, the FANC proteins tie to the DNA and reconstruct the cross-joins, permitting the DNA to duplicate and capacity ordinarily.

FANC and RNF families assume significant aims safekeeping us healthy. On the off chance that something meddles with the capacity of these qualities, it can prompt various ailments. For instance, interruption of RNF can prompt myotonic dystrophy, which is portrayed by dynamic muscle squandering and misfortune. Disturbance of FANC can bring about, you got it, Fanconi anemia, which can cause bone marrow disappointment, physical irregularities and organ abandons. What's more, obviously, both quality families assume a role in breast cancer (Harres et. al .2020).

The most well-known reason for innate bosom disease is an acquired change in BRCA1 or BRCA2. Transformed renditions of these qualities can prompt unusual cell development, which can prompt cancer growth. In the event that you have acquired a transformed duplicate of either quality from a parent, you have a higher danger of breast cancer. Approximately, women with BRCA1 or BRCA2 variations have up to a 7 out of 10 risk of getting breast cancer by age 80 (Glaser et. al. 2018).

1.2.1.5.1.2 Other genes in Breast Cancer Molecular Pathogenesis

Mutation in some genes may contribute to breast cancer development, specifically genes like TP53, PALB2, ATM, PTEN, CHEK2 or CDH1 (Feng et al., 2018).

The TP53 gene, also called tumour protein p53, stops the growth of the tumour by making its protein. When the TP53 gene mutated causes of Li-Fraumeni syndrome which is a rare syndrome has more than normal rate of breast cancer in the people (Duha et. al. 2017). The PALB2 gene is also known as localizer of the BRCA2 gene. It gives guidelines to make a protein that works with the BRCA2 protein to fix DNA damage and stop tumour development. PALB2 mutations have 14% rate of increasing breast cancer in women over 50 years old additionally, in age 70 that risk increases to 35% (Feng et. al. 2018). The ATM

gene is also the known ataxia-telangiectasia causing gene. The function of this gene to repair DNA damage (Carbajal-Mamani et al., 2020). Inheritance of an abnormal Ataxia-Telangiectasia mutated gene is associated to raise risk of breast cancer because of the abnormal gene as will not be able to repair DNA damage. People who inherit a mutated copy of ATM from one parent are at increased risk of female breast cancer (up to 52% lifetime risk). Many investigations proposed that in age 80 have a risk of breast cancer development between 33% and 38% when they carry ATM mutation (Sizilio et. al. 2012). The PTEN gene name is known phosphatase and tensin homolog. It helps coordinate cell development. PTEN mutations cause breast tumour formation and tumour progression. About 25% to 50% PTEN mutations occur in women's life cycles, and breast cancer develops (Carbognin et. al. 2019). The Checkpoint Kinase 2 (CHECK2) gene functions to form a protein that inhibits the tumour growth. Mutations within CHECK2 gene could be double the average the rate of breast cancer development because it cannot do function when mutated. The E-cadherin or the epithelial cadherin (CDH1) gene creates a protein that encourages cells associate together to make tissue. Women with a mutated CDH1 gene have a 39% to 52% lifetime rate of breast cancer (Corso et. al. 2018).

1.3 Artificial Intelligence (AI)

1.3.1.1 Machine Learning

Machine learning offers the optional approach to the standard model of estimating the modelling that may tackle the present setbacks and also leading to the improvement in the accuracy. The techniques of modern language were formulated from past studies about the computational statistical-based mode of learning and the recognition pattern. Fewer observations depending on the computational based algorithms as well as the models in identifying the complicated relationships among the diverse heterogeneous risk-oriented factors have been stated (Ming et. al. 2019). In this regard, it is realized by the iterative minimization of the certain objective roles of the estimated and observed findings or outcomes. Machine learning has been applied in models linked to cancer forms of prognosis, survival, and eventually have resulted in the production of good accuracy and reliability approximations. Up to the moment, lesser studies have used the machine language methodologies in personalizing the prediction of the risk linked with the prediction of cancer

or giving the comparison of the reliability and the accuracy with the models utilized within the community practice.

1.3.1.2 Deep Learning

The drastic invention in the machine learning with specific reference to deep learning, It is enhancing the medical form of imaging of the interest from the community in using the techniques towards realizing the improvements in the accuracy regarding the accurate screening of cancer. Breast cancer comes second as the cause of deaths linked with cancer in women from the US (Shen et. al. 2019). However noticeably, mammography screening has been reduced the mortality cases.

Regardless of the benefits, screening is linked with the higher risks concerning the false cases of the positivity plus other false forms of negatives. The average sensitivity about the digital form of screening mammography within the US stands at 86.9%, and the average specificity is at 88.9% (Dai et. al. 2017). To assist the radiologists in realizing the improvements in the predictive model of accuracy concerning the screening mammography, computer-aided detection (CAD), and diagnosis software have been formulated and also in the clinical form of applications. Unfortunately, the findings proposed that new cases about the systems of CAD had not brought significant improvement in the performance. Hence, there was stagnation in progress for at least a decade from the time of its introduction. With the notable success about deep learning on visual object identification and even detection plus various domains, there exist multiple interests in the development of tools for deep for learning to help the radiologists and the improvement in the accuracy regarding the screening mammography. The latest studies by Shen et. al. (2019) have confirmed that deep learning regarding CAD system, including the radiologist in the independent mode, including the performance from the radiologist in supporting the way.

1.3.1.3 Fuzzy Logic and Probability Theory

The fuzzy logic model handles the reasoning that is estimated instead of being fixed and exact. Unlike the ancient Boolean logic, in which the objects are categorized as being true or false, that within the fuzzy logic, they may be classified as having values that range between zero and one (Kempowsky-Hamon et. al. 2015). Fuzzy logic has been more applicable in the controlling of systems because of its less complexity and effectiveness, especially when handling the non-linear and higher systems of dimensions because of the concept that supports the handling and the manipulation and the imprecise and the noisy type of data. On top of this, it offers an intuitive way of understanding the interpretation of the outcomes. Regardless individual efforts towards the usage of the fuzzy logic in performing selection being suggested by Sizilio et al. (2012) pointed out that these methodologies work effectively when handling the imprecise and the noisy results though at last become more sophisticated. In either way, they rely on the certain method; the Fuzzy C means that is meant for the clustering or use in within the based arbitral decision in determining the linguistic terminologies about the 'fuzzified' elements that often are never easy and not highly accurate when more significant features have to be handled. On top of this, with the desire to reduce the cost linked with computation, the fuzzy mechanism of selection with the genetic algorithms or also was responsible for the introduction of the fuzzy concept about entropy in selecting the features that are relevant (Steele, 2015).

According to fuzzy classifiers have of late exhibit their respective effectiveness in classifying tasks because they foster the handling of the noisy and the imprecise ideas that normally is available in various appliances. Regardless of the decline, their respective performances about the dimensional or the heterogeneous issues. Similarly, irrespective of the drawbacks, in the application of the fuzzy forms of classifiers to prognosis regarding the cancer of the breast that uses the gene expression information had been witnessed. Contrastingly, the past studies in which the fuzzy logic was utilized in assessing the classification of the patients, were also developed to factor the classification and the selection of the algorithms, both founded on the fuzzy logic concept about the degree of membership (Kempowsky-Hamon et. al. 2015).

1.3.1.5 Generic Algorithm Synthesis and Applications

A combination of the cancer of the breast mRNA profiling research findings has gone ahead with the stratification and the definition of the sets of the genes that correlate with the outcome. The other studies have developed into the strategic plans for the possible use of the nucleic acid-oriented sets in selecting patients that need no more therapy after their major resection (Newman, 2016). Regardless, various genes applicable in predicting the outcome among the patients or defining the tumour subtypes through the expression of the RNA studies are still showing variation, non-overlaps, and in many. Situations need specialised types of technologies. Lesser markers may conduct Immuno-histochemical types of studies though they end in suffering from the inherent cases of the flaw about the subjective evaluation and variable form of reproduction. Hence, it would be realistic in situations where the familiarity and streamlined nature regarding the immune-histochemistry may be integrated with the specific quantitative and more specific roles linked with the nucleic acidbased evaluation in predicting the outcome from the patient. The AQUA methodology could integrate the desirable features associated with these forms of essays. Likewise, it is more of the same to the immune-histochemistry based on its structure and the preparation of slides (Tidhar et. al. 2009).

Tissue microarrays is the methodology for the higher throughput protein way of expression evaluation of more significant cohorts about the cancer patients via a single slide that leads to standardization to various variables and the capacity for the embedded form controls. Often, the technology has been prolonged for use in the discovery since the evaluation of the arrays has ever remained more subjective. Hence, this contributes to the invalidation of the algorithms applied in discovering the nucleic acid array form of experiments. The latest developed and the automated quantitative evaluation type of technology enable the immune-fluorescent quantification and eventually giving the reflection of the continuum of the protein expression (Shen et. al. 2019).

Laboratories have confirmed that the scores linked with AQUA have more correlation with the protein content (Dolled-Filhart et. al. 2006). Hence, the combination of the tissue forms of tissue microarrays and AQUA offers the follow-up and the extension regarding technology for the cDNA microarray-oriented discovery by enabling for in situ proteins assay of markers

of the interest on more significant cohorts about tumours with the factoring the spatial subcellular way of localizing information and then multiplexed.

1.3.1.5 Artificial Intelligence in Medicine

AI assists the radiologist in improving the accuracy in the detection of the cancer of the breast with minimal recalls. AI only has exhibited 88.8% sensitivity in the detection of the cancer of the brain, where the radiologists only exhibited 75.3% after aiding the radiologist with the AI, there was a notable of increment in the AI by 9.5% up to 84.8% (Sizilio et. al. 2012).

A new study done by Koh (2020) showed that the benefits linked with AI aided detection of the cancer of the breast from the mammography related images. The main finding confirmed that AI when compared to the radiologist, showed good sensitivity in the detection of cancer with mass (90% against 78%) and the also the distortion or asymmetry (90% against 50%). Hence, these outcomes affirm that AI is more desirable in detecting the TI cancers that Koh (2020) classified as the early-stage invasive categories of cancer. AI managed to detect 91% of the TI related cancers and 80% of the node-negative types of cancers, while the radiologists only managed to detect 74% of both of them.

The diagnosis of the mammograms is the density of the breast and the dense tissues forming the breast. Often, this phenomenon originates from the population among the Asian community, and it is never easy in interpreting the dense tissue since it is more likely to be eminent in the mask cancers within the mammograms. The diagnostic performance linked with AI was below the density of the breast affected while the radiologist's performance was more susceptible to density and consequently exhibiting more sensitivity about the fatty breasts being at 79.2% in comparison to the dense breast at 73.8% (Chen et. al. 2018).

1.3.1.6 Artificial Intelligence using Fuzzy Logic: The Next Generation

Computational Intelligence (CI) assist via the intelligent based techniques besides drawing their respective inspirations from nature, aid in the development of the systems of intelligence that can imitate the elements linked with the behaviour of the human beings. The behaviours are learning perception, evolution, adaptation, and reasoning. Evolutionary computation through the inspiration by the biological form of evolution and inference related processes and the Fuzzy logic under the inspiration of the language form of processing (Kopchak et. al. 2018).

The theory of the fuzzy systems is a formal form of approach that is after addressing the modelling, reasoning, representation and the procedure regarding the information that is not accurate as of the strategy of troubleshooting (Hadjileontiadou et. al. 2015). The theory of the fuzzy set is the tool that aids in the modelling of the imprecision and the cases of the ambiguity that develops within the complicated systems. Also, the theory was formed with the objective of combining the ideas related to classical logic and the groupings that define the levels of relevance (McBratney et. al. 1997).

A fuzzy set is far from the classic set regarding the way of assigning every element a value within the unit interval. On a specific note, a fuzzy set is a function A of the combination x (Briganti et. al. 2020). The function A is the membership function, and the value defined as A(x) is the level of relevance or the compatibility regarding the element x in which the concept has its representation in each fuzzy set. Hence, the fuzzy set offers the model of mathematics for processing non-accurate or the vague data as well as the concepts, to ensure that computes make people as the inferences (Sivanandam et. al. 2012).

1.3.1.7 Fuzzy Logic Approaches for Breast Cancer Diagnosis: Decision Making

The decision to choose the most appropriate follow-up treatment for a suspected breast cancer case largely depends on the correct assessment and diagnosis of the breast cancer hazard. Despite the latest technological advances, the criteria and strategies used to distinguish the stage of healing of breast cancer and thus measure the characteristics of the recognized injury to reach the most likely hazard prediction have yet been poorly and subjectively defined for some clinicians (Mohammed, 2016).

Normally, the process about fuzzy comprises of the Rules Base offered by the technocrats or the got from the numerical data set, Fuzzification stage that aids in the activation of the rules from the entries, Inference stage that aides in the determination of the way of enabling the rules and the Defuzzification stage that offers the precise output, generation of the fuzzy output set as depicted in Figure 1.1 below.



Figure 1.1 Decision Making in Fuzzy logic (adapted from Sizilio et. al. 2012).

1.4 Work in This Thesis

In this thesis, we developed fuzzy logic system for early prediction of BRCA1/2 negative hereditary cancer patients on MATLAB. In chapter 2, the methodology of fuzzy system and training process of the data were explained. The used methods in this study were described step by step. Chapter 3 as a results chapter, the results of this study were presented. Briefly, 14 Breast cancer associated risk factors were merged with 18 different genes with different genetic variations that are thought to related to breast cancer molecular pathogenesis in 90 patients were studied. The patient data came from Bursa Uludağ University Faculty of Medicine, Department of Medical Genetics and Erciyes University Faculty of Medicine, Department of Medical Genetics. Each patient ha 14 input clusters (risk factors) in fuzzy logic system for this study. In the last chapter, we compared literature with our study results and emphases the significance and contribution of this study to the literature as well as future remarks were indicated.

CHAPTER: 2

MATERIALS AND METHODS

2.1.1 Materials

2.1.1.1 Human Data Collection

In this Retrospective study, patients' data were collected from both Uludağ University Faculty of Medicine Department of Medical Genetics and Erciyes University Faculty of Medicine Department of Medical Genetics. Overall, 488 different individual data were examined. However, 90 of 488 were applicable for this study as only 90 patients did not have any considerable genetic variations within *BRCA1* and *BRCA2* genes. In this study, we focused on 18 hereditary risk factor genes apart from *BRCA1* and *BRCA2* which were indicated Table 2.1. Moreover, 14 cancer associated risk factors also determined for those 90 patients as shown Table 2.2 The risk factors were utilized as input clusters for fuzzy logic system. Each input clusters were inserted with membership function. Therefore, every membership function reflected various membership degrees within the cluster.

TP53 (Tumor protein p53)	FAM175A (Family with sequence similarity 175, member A)	RAD50 (Double Strand Break Repair Protein)	
NBN (encodes nibrin)	MSH6 (DNA mismatch repair protein Msh6)	APC (Adenomatosis polyposis coli)	
MSH2 (DNA mismatch	ATM (Ataxia-	CDH1 (Hereditary Diffuse	
repair protein Msh2)	Telangiesctasia mutated)	Gastric Cancer syndrome)	
MUTYH (MutY DNA	PALB2 (Partner and	BLM (Bloom syndrome	

glycosylase)	localizer of BRCA2)	helicase)
MRE11A (Homolog A, double strand break repair nuclease)	PMS2 (Mismatch repair system component)	CHEK2 (Checkpoint kinase)
PTEN (Phosphatase and tensin homolog)	BART1 (Protein coding)	BRIP (Stands for BRCA1 Interacting Protein C- terminal Helicase 1)

Table 0.1	Handitow	mials footon	~~~ 41. ~ 4		and in a dim	Alada atrada
Table 2.1	Hereditary	risk factor	genes that	were ex	ammed m	this study

Age	Sex	Consanguinity	Family History
Membership Degree	Tumour Size	Lymph Node	Malignity
Location of the Tumour	Oestrogen Receptor Positiveness	Progesterone Hormone Positiveness	Gene Variation
Diagnosis	Gene Classtification		

Table 2.2 Determinant cancer associated risk factors in this study

2.1.1.2 Computer

Windows 10 Pro was used as computer operating system to design an artificial intelligence software on MATLAB. Intel $\[mathbb{R}$ Core $\[mathbb{TM}$ is CPU and 2.53 GHz was the system processor. Computer memory was 444 GB and the access memory was 3.00 GB. System type was X64-based processor and 32-bit operating system. Fuzzy logic system on MATLAB was used to create the software which constructed the database via the computer.

2.1.1.3 Matrix-Laboratory (MATLAB) Software

A multi-paradigm numerical computing software matrix laboratory (MATLAB) is a certified programming language developed by MathWorks. It allows the user to carry out function, data drawing, algorithm implementation, matrix processing, and interfacing with programs written in other languages such as C, Java Fortran and C ++ (Potter et al., 2019). Currently, MATLAB is highly being utilized in multiple fields of Image Processing. Signal Processing

and Artificial Intelligence. Mathematical calculation operations, algorithm development and code writing for example programming, numeral integration and optimization can be examples of usual applications of MATLAB. MATLAB r2018a version was utilized in this study.

2.1.1.4 Creating Fuzzy Logic System on MATLAB

Artificial intelligence systems involves in multiple software and hardware systems that have many abilities, for example, sound perception, speech and motion, performing digital logic, and exhibiting behaviours like human beings. Fuzzy logic is the basis of creating an artificial intelligence application. The convention of fuzzy logic is to arrive directly to results.

On the contrary to classical clusters, fuzzy logic shows difference between items in "fuzzy" sets by giving members between 0 and 1 interval. Fast variables generally are classified as light-dark colours in sharp clusters, such as, cold or hot, fast or slow. On the other hand, fuzzy logic are softened by flexing the qualifiers for instance slightly hot, hot, slightly cold or cold or slightly dark, dark, slightly light, or light.

In our study, MATLAB program was used to improve fuzzy logic which was harmonized for breast cancer prediction/ scoring risk. We mainly focused on genetic variations associated with 18 hereditary breast cancer genes.

2.2.1. Methods

2.2.2.1 Setting up fuzzy logic with patients' data

On the system 90 patients' data were selected after creating input clusters. Input clusters were 14 cancer associated risk factors (Table 2.2) for breast cancer. Each cluster contained a various number of membership functions and functions within the cluster represent different degrees of membership.

The membership functions and inputs were established into the system, and then the patients' data were introduced into the system. Thus, finally 90 various patients' data were utilized for the system. However, the system always work better with increased data number. In order to get the correct result, it is crucial that the data is accurate and proportional, if not the results will not be correct. Moreover, incorrectly entered data into the system will give incorrect results.

2.2.1.2. Creating Fuzzy Logic System on MATLAB

Fuzzy logic applications were utilized in many various areas and can be easily applied with the fuzzy logic toolbox on MATLAB program.

Operations can be simplified by numerous available functions which will be written by the programmer. MATLAB has five different windows; workspace, current directory, array editor, command history and command window. Investigators can build their own programs or perform simple mathematical operations by coding in the window.

Type fuzzy in the command window and press enter is enough to run the toolbox as shown in Figure 2.1. The image that appeared at the end of this command is shown in Figure 2.2. Fuzzy Logic Designer features are accessed in the toolbox and utilized for system creation and fuzzy modelling.

Command Window
New to MATLAB? See resources for Getting Started.
fx >>fuzzy

Figure 2.1 Accession of the Fuzzy Logic system

承 Fuzzy Logic Designer:	Untitled			- 🗆 ×
File Edit View				
Untitled (mamdani) input1				
FIS Name: Un	titled		FIS Type:	mamdani
And method	min	~	Current Variable	
Or method	max	~	Name	input1
Implication	min	~	Туре	input
Aggregation	max	~	Range	[0 1]
Defuzzification	centroid	~	Help	Close
System "Untitled": 1 input, 1 output, and 0 rules				

Figure 2.2 Fuzzy Logic Designer Window

In this window, by clicking the Edit, variables can be added or removed as illustrating in Figure 2.3



Figure 2.3 Adding and removing the variable on the fuzzy logic system

Other important stage is the Membership Function Editor. Here, function parameters, the width of data exchange in variables, member functions, name of input and output variables can be established. Membership Function Editor aids with eleven membership functions within the menu as indicated in Table 2.3.

d sigmoidal membership function (dsigmf)	Generalized bell membership function (gbellmf)
Gaussian 2 membership function (gauss2mf)	sigmoidal membership function (sigmf)
---	---------------------------------------
Gaussian membership function (gaussmf)	Pi membership function (pimf)
P sigmoidal membership function (psigmf)	S membership function (smf)
trapezoid membership function (trapmf)	triangle membership function (trimf
Z membership function (zmf)	

Table 2.3 Membership Function Editor supports eleven membership functions.

Using this menu, investigators can choose the membership function which suitable for their own purposes. As in Figure 2.4, the MFE is eventually opens after three triangles membership functions with an input and an output variable of each variable are now accessible. Additionally, researchers can use the Add Custom MF, Add MF and Remove All MFs whenever they want to remove or add a new membership function, it also shown Figure 2.5.



Figure 2.4 the illustration of the Membership Function Editor



Figure 2.5 shows remove or add a new membership function on the system

System rules were written according to the data entered, and the rules were written between AND/OR connectors and IF/THEN conditions. Changes can be made using add or change button in all used rules window. Introduced results from all different values were presented in the window.

2.2.1.3 Fuzzy Logic Assumption Methods

There are variety of methods to develop fuzzy logic, however some of the only built for fuzzy logic. Each method basically achieves the same aim but some aspects, for example complexity and efficiency, can affect the methods. Preferred method is the one who can be built up with parameters. The most widely recognized two methods for fuzzy logic systems are the Takagi-Sugeno Fuzzy Inference and the Mamdani Fuzzy Inference Methods. In this study, Mamdani Fuzzy Inference Method (MFIM) were used. (Mayorga et. al. 2019).

The development of the load sensor was done by utilizing the MFIM model which involves two inputs displacement and load sensor. On the other hand, the Tkagi-Sugeno Fuzzy Inference system has the same initial stage as the MFIM (Kamboj et. al, 2013).

A fuzzy logic method that entails expert information and can be implemented to any problem is called Mamdani Fuzzy method. This type of fuzzy model is very simple to build for human being.

Fuzzification (0 to 1) \rightarrow Utilizing fuzzy logic operations \rightarrow Implementation of fuzzy cluster (and/or) \rightarrow Collection results \rightarrow Defuzzification

These arrangement above shows the five main steps of MFIM model. An initial fuzzification step was designed to decide membership degrees of input variables between 0 and 1. Utilizing fuzzy logic operations were depend on the values. Third step named as implementation of fuzzy cluster was designed to modify "or","and". The penultimate step assemblage the final data (Pourjavad et al. 2019). Additionally, Takagi-Sugeno Fuzzy Inference Model do not have output membership function but MFIM model has.

CHAPTER 3

RESULTS

3.1.1 Introduction

Cancer is uncontrolled cellular division whereas cancer has various features of different types and additionally, it can form any tissue of the body. Extensively separated into malignant or benign tumours; moreover, cancers are also classified and identified by tissues, organs of origin or cell types (Herrington, 1997).

Globally, breast cancer is the most common cancer in women. Mortality and incidence rates have been raising in developing countries such as Turkey (Ferlay et al., 2018). The breast cancer incidence was 24/100.000 in 1993 and then it raised to 50/100.000 in 2013. In 2018, the number of patients were approximately 22.345 in Turkey (Fidaner et al., 2018). Many factors including environmental and genetic may affect the development of breast cancer in

humans. *BRCA1* and *BRCA2* genes that assemble tumour suppressor proteins, show increase number of suspepility to develop breast cancer (Ozmen et al., 2019).

Additionally, with advanced high-throughput DNA sequencing technologies using genomic capture and massive parallel sequencing revealed other genes that are associated hereditary breast cancer (Walsh et al., 2010). Early detection of pathogenic gene variations may be crucial for patients' survival. Nevertheless, treatment methods vary according to cancer types. Often, multiple treatments are used to achieve a definitive result (An et al., 2020).

New developments in genomic technologies have led multiple genes to be tested in parallel sequencing. Personalized new generation sequencing panels offer simultaneous analysis of breast cancer associated genes (Apostolou et al.,2013). Therefore, early prediction or using the suitable treatment for the patients may be possible when the accumulated variation data from high-throughtput DNA sequencing analysis and the data of patients' clinical and family history will be merged (Venkitaraman, 2019).

Most known breast cancer susceptibility gene *BRCA1/2* sequencing is one of the gold standart nowadays. Despite, many gene variations effect within *BRCA1* and *BRCA2* genes are still not known, negative test results (no variation found) understanding often more difficult to conclude rather than positive test results. Here with negative test results for *BRCA1* and *BRCA2* may be rely on other factors such as individual's family cancer history as well as other related genes (Long et al., 2013)

Today's medical and current biotechnology approaches well determined the breast cancer risk factors. In this thesis, we utilized fuzzy system to develop software early prediction of *BRCA1/2* negative hereditary cancer on MATLAB. The main aim was to create a software program for cancer prediction as applying all risk parameters to fuzzy logic system.

3.1.2 Data Collection and Study Method

Initially, data from all identified *BRCA1/2* negative breast cancer patients were collected from Bursa Uludağ University and Erciyes University. A data was consisting of 488 breast cancer patients, but since we were only interested in *BRCA1* and *BRCA2* negative hereditary breast cancerpatients, only 90 were suitable for this study. Thus, 90 patients have been used to teach the fuzzy logic system to create the software. An ethical approval for the study was

obtained from the Near East University Scientific Research Ethics Committee (YDU/2019/72-893). Informed consent was obtained from each participant.

The collected data were divided into fifteen various input clusters known as risk factors. These input clusters were age, sex, consanguinity, family history, relativeness degree, tumour size, lymph node, malignancy, location, oestrogen receptor positiveness, progesterone positiveness, gene, gene variation, diagnosis and classification. We used 18 different genes which have been associated with breast cancer development before.

In figure 3.1 shows the distribution of tumour locations in 90 female patients. 38% (34 patients) had tumour only on the left breast, 29% (26 patients) were with only right breast tumour whereas 7% (6 patients) had bilateral tumours. However, 26% (24 patients) of the patient's tumour locations was not indicated.

Genetic variant classification was done according to The American College of Medical Genetics and Genomics ACMG Guideline as benign, likely benign, variant with unknown significance (VUS), likely pathogenic and pathogenic (Richards et al., 2015).

A total number of 23% (21 patients) had benign variants within the genes that have been associated with hereditary breast cancer, whereas 29% (26 patients) were likely benign, 22% (20 patients) were VUS, 20% (18 patients) were lightly pathogenic and 6% (5 patient) were classified as pathogenic (Figure 3.2).





Figure 3.2 Distributions of the gene variants to their ACMG classifications (B: benign, LB: likely benign, VUS: variant with unknown significance, LP: likely pathogenic, P: pathogenic).

3.1.3 Collection of BRCA1 and BRCA2 Negative Patients

The main point of this study was to examine *BRCA1/2* negative hereditary breast cancer patients focusing on different gene variations. In our study, we obtained the results using data from 90 *BRCA1/2* negative hereditary breast cancer patients who had genetic variation in 18 different genes. Furthermore, we aimed that different gene polymorphisms might indicate the effect of hereditary breast cancer development and might play role of molecular pathogenesis of breast cancer. All genetic variation with 18 different genes were shows as Table 3.1.

Tumor Protein p53 (TP53)	Family with Sequence	Double Strand Break
	Similarity 175, member A	Repair Protein (RAD50)
	(FAM175A)	
Encodes Nibrin (NBN)	DNA Mismatch Repair	Adenomatosis Polyposis
	Protein msh6 (MSH6)	Coli (APC)
DNA Mismatch Repair	Ataxia-Telangiesctasia	Hereditary Diffuse Gastric
Protein msh2 (MSH2)	mutated (ATM)	Cancer Syndrome (CDH1)
MutY DNA Glycosylase	Partner and Localizer of	Bloom Syndrome Helicase
(MUTYH)	BRCA2 (PALB2)	(BLM)
Homolog A, Double Strand	Mismatch Repair System	Checkpoint-Kinase
Break Repair Nuclease	Component (PMS2)	(CHECK2)
(MRE11A)		
Phosphatase and Tensin	Protein Coding (BART1)	Stands for BRCA1
Homolog (PTEN)		Interacting Protein C-
		terminal Helicase 1 (BRIP)

3.1.4 Identifying Risk Factors of Breast Cancer

Despite, having any identified cancer symptoms does not mean that you have a cancer but identifying cancer symptoms is crucial for early diagnosis. There are many different recognized risk factor classifications. Gender, race and age are biological risk factors. Gender is sometimes related to some specific types of cancer such as prostate cancer is seen only in men as they have prostate glands, however breast cancer can be observed in women and men because both have breast tissue but mainly in females. Age is another important risk factor. People over 50 are at higher risk of cancer development. More importantly, genetic factors are another risk factor for cancer development as gene alleles are inherited from parents so family history sometimes are significant for tracing the cancer prognosis and treatment.

In this study we focused on 14 various determined risk factors; age, sex, consanguinity, family history, relative degree, tumour size, lymph node, malignancy, location, oestrogen receptor, progesterone, gene/ gene variation, diagnosis and variant classification. These risk factors were consisting of input and output data of this study. Membership functions in artificial intelligence occur when each risk factor was divided into groups. These functions granted us to rate risk factors. The input part of the system has been developed utilizing the membership functions and risk factors illustrated in Table 3.2

Risk Factors	Membership Functions

	<15
	16-29
Age	30-39
	40-59
	>=60
Sex	Female
	Male
Consanguinity	Yes
	No
Family History	Yes
	No
	0
Membership Degree	1 & 2
	>=3
	0-19cm
Tumor Size	20-39cm
	>=40cm
Lymph Node	Yes
	No
	Grade 1
Malignancy	Grade 2
	Grade 3
Location	Right Breast

	Left Breast
	Both Breast
	Other
Oestrogen Receptor	Positive
	Negative
Progesterone	Positive
	Negative
	TP53
	FAM175
	RAD50
	NBN
	MSH6
	APC
	MSH2
Gene/Gene Variation	АТМ
Gene/ Gene Variation	CDH1
	MUTY
	PALB2
	BLM
	MRE11A
	PMS2
	CHEK2
	РТЕМ

	BART1
	BRIP
Diagnosis	Yes
	No
	Benign (B)
	Likely Benign (LB)
Classification input and output	VUS
	Likely Pathogenic (LP)
	Pathogenic(P)

Table 3.2 Identified risk factors and membership functions.

3.1.5 Combining the Massive Data

A total of 90 patients' data was utilized in our study. All 14 significant risk factors and membership functions are shown in Table 3.2. 55 different membership functions of 14 risk factors have been determined and trained into the system. This large stack of consolidation data was utilized to train the fuzzy logic system for early prediction of negative hereditary breast cancer. The main and final list of 90 carefully selected and pre-determined breast cancer patients and these data were registered in our advanced fuzzy logic system. Risk factors and membership functions are created as input parameters for each patient. Tables 3.3a, b and c illustrate information of randomly selected breast cancer patients through the established database.

Input Parameters	Sample Patient Datas
Age	51

Sex	Female
Consanguinity	No
Family History	Yes
Membership Degree	unknown
Tumor Size	10cm
Lymph Node	No
Malignancy	2
Location	Both Breast
Oestrogen Receptor	Positive
Progesterone	Positive
Gene Variation	APC
Diagnosis	Positive
Classification	Likely Benign

Table 3.3a the first example of patient data.

Table 3.3a was presented the first example of the patient data which has been utilized towe train the system. A 51-year-old woman without any consanguineous marriage was selected Tumor locations were bilateral and there was a cancer history in her family. She was progesterone hormone and oestrogen receptor hormone positive. Additionally, as we were only interested in BRCA1/2 negative patients she had a genetic variation within the *APC* gene and classified as likely benign variant.

Table 3.3b illustrates a 50 year-old female patient without any consanguineous She had a 16 cm tumour on her left breast. Progesterone and oestrogen receptor hormones were positive.

The patient had a family history with cancer and her malignancy degree was grade 1. She had a gene variation within the *ATM* gene which was classified as a VUS variant.

Input Parameters	Sample Patient Datas
Age	50
Sex	Female
Consanguinity	No
Family History	Yes
Membership Degree	1
Tumor Size	16cm
Lymph Node	No
Malignancy	Unknown
Location	Left Breast
Oestrogen Receptor	Positive
Progesterone	Positive
Gene Variation	АТМ
Diagnosis	Positive
Classification	VUS

Table 3.3b The second example of patient data.

Table 3.3c represents the third patient example that trained the system. A 60 year-old woman with a family history of cancer had a 20 cm size tumour on her right breast. A pathogenic gene variant has been detected within RAD50 which is a double-strand repair protein gene.

Input Parameters	Sample Patient Datas
Age	62
Sex	Female
Consanguinity	No
Family History	Yes
Membership Degree	1
Tumor Size	20cm
Lymph Node	Unknown
Malignancy	Unknown
Location	Right Breast
Oestrogen Receptor	Negative
Progesterone	Negative
Gene Variation	RAD50
Diagnosis	Positive
Classification	Pathogenic

Table 3.3c The third example of patient data.

In line with all this information, they were added to the system for the train and contributed to the early estimation of breast cancer.

3.1.6 Fuzzy Logic System was created on MATLAB r2018a Edition

The MATLAB r2018a version was used in this thesis to develop the fuzzy logic system for early prediction of *BRCA1/2* negative hereditary breast cancer (Themes, 2020). Many of the

functional processes were carried out on MATLAB, such as the fuzzy logic interface should

be opened via MATLAB.

Command Window
New to MATLAB? See resources for <u>Getting Started</u>.

Figure 3.3 Accession of the Fuzzy Logic system in Command Window

In standard fuzzy logic interface has three different parts; input, rules and output. Membership functions and fuzzy clusters were created in the input section. Rules were created in the rule section using the created sets and membership functions.

In addition, breast cancer patient data were utilized in the rule section to train the system. A summary of the decision-making process was included in the output section. Standard fuzzy logic interface on MATLAB shown in Figure 3.4.

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Figure 3.4 Fuzzy Logic Designer on MATLAB

Input and output parameters were added to the system unlimitedly. Moreover, as a rule an unlimited number of data was utilized. As shown in Figure 3.5, after clicking the edit section on the main page of the interface, any number of inputs or outputs could be added from the add variables section.



Figure 3.5 Adding input and output on fuzzy logic system

Figure 3.6 shows 14 different input parameters and one output parameter that was created in this study.



Figure 3.6 Developed input and output parameters on the system

The fuzzy set of various membership functions were created and these functions were identified members of different degrees in each set. In fuzzy logic, each element of the membership function gives a value between 0 and 1. Therefore, the member function could be mapped while this value is described as the degree of membership. Triangular functions were utilized as membership functions in the input section and trapezoidal functions were utilized as membership functions in this study. Membership functions approved to represent a fuzzy group graphically. The X axis symbolized the discourse universe and the y axis symbolized membership degrees in the range between 0 and 1.

A fuzzy logic system was created by following the four main steps in the Mamdani's method (Dadios, 2012). First of all, the proposal for each rule were evaluated in the step described input fuzzification and secondary, the result of each rule was obtained by this evaluation. First of all, the proposal for each rule were evaluated in the step described input fuzzification and secondary, with this evaluation, the result of each rule was obtained. In the third step, we combined all the outputs for each rule in the second step into one fuzzy set utilizing a fuzzy operator. In the last step, we converted the fuzzy set we obtained in the third step into a single numerical value and this step is called defuzzification.

3.1.7 Creating Fuzzy Logic System with Collected Patients' Data

It was very important to train patient data properly to the system. Incorrect information of patients' data was avoided. Accordingly, it was crucial to transfer appropriate and correct patients' data to the system. We used data from a total of 90 different breast cancer patients.

Membership functions in the input clusters were offered different degrees of different possibilities for different patients from 0 to 1. For system training, values and membership functions of rick factors were determined by us. 55 different membership functions of 14 different input sets were created in the fuzzy logic system as shown Table 3.3.

Risk Factors	Membership Functions	Values	
	<15	0	
	16-29	0.25	
Age	30-39	0.5	
	40-59	0.75	
	>=60	1	
Sev	Female	1	
	Male	0	
Consanguinity	Yes	1	
	No	0	
Family History	Yes	1	
	No	0	
	0	0	
Membership Degree	1 & 2	0.5	
	>=3	1	
Tumor Size	0-19cm	0	

	20-39cm	0.5	
	>=40cm	1	
Lymph Node	Yes	1	
	No	0	
	Grade 1	0	
Malignancy	Grade 2	0.5	
	Grade 3	1	
	Other	0.25	
Location	Right Breast	0.5	
	Left Breast	0.75	
	Both Breast	1	
Oestrogen Receptor	Positive	1	
	Negative	0	
Progesterone	Positive	1	
	Negative	0	
	TP53	0.1	
	FAM175	0.15	
	RAD50	0.2	
Gene Variation	NBN	0.25	
	MSH6	0.3	
		0.35	
	MSH2	0.4	
	ATM	0.45	

	CDH1	0.5
	MUTY	0.55
	PALB2	0.6
	BLM	0.65
	MRE11A	0.7
	PMS2	0.75
	CHEK2	0.8
	РТЕМ	085
	BART1	0.9
	BRIP	1
Diagnosis	Yes	1
	No	0
	Benign (B)	0
	Likely Benign (LB)	0.25
Classification input and output	VUS	0.5
	Likely Pathogenic (LP)	0.75
	Pathogenic(P)	1

Table 3.4 Values of membership functions for each risk factor in each input clusters are shown.

Figure 3.7a, b, c, d, e, f, g, h, I, j, k shows examples of membership function for each risk factor cluster; age, sex, family history, tumour size, malignancy, tumour location, oestrogen receptor, progesterone, gene/gene variation, diagnosis, variant classification, respectively.



Figure 3.7 Membership functions of age clusters.



Figure 3.7b Membership functions of sex clusters.



Figure 3.7c Membership functions of family history clusters.



Figure 3.7d Membership functions of tumour size clusters



Figure 3.7e Membership functions of malignancy clusters.



Figure 3.7f Membership functions of location clusters.



Figure 3.7g Membership functions of oestrogen receptor clusters.



Figure 3.7h Membership functions of progesterone clusters.



Figure 3.7i Membership functions of gene variation clusters.



Figure 3.7j Membership functions of diagnosis clusters.



Figure 3.7k Membership functions of variant classification clusters.

In the rule section, with the data of these patients, the system was aimed to appraise it with 90 various perspectives and possibilities to achieve a more accurate result. In Figure 3.9 shows the data of 90 patients trained in the system with risk factors in the rule section. All trained data and selected risk factors appear together in this section.



Figure 3.8 Rules section in the Fuzzy Logic System

In the last part of the output, five membership functions were described and a value was given to each membership function. We appraised the results and accessed all while the test phase as state in the classification values and we generate in the output set. In table 3.5 shows membership functions of output and values of these functions.

Membership Functions of Output	Values of Membership Functions
Classtification	
Benign	0
Likely Benigh	0.25
VUS (Variant with Unknown Significance)	0.5
Likely Pathogenic	0.75
Pathogenic	1

Table 3.5 Output cluster and Values of Membership.

3.1.8 Verification of Fuzzy System

After training patient data and creating the system, the verification tests were carried out. We determined the test group and performed the test process with six different patients which were not introduced to system. These patients' gene variant classification were likely benign, two VUS and two pathogenic.

The test was carried out to verify that the system was correctly analysing the genetic variants and could be a possible early detection of breast cancer tool. The system provided values between 0 and 1 for each individual at the end of the test. Thus, these values were converted to percentages and the system evaluated the percentages of individuals' probability of cancer risk. The classification interval was established to determine the cancer status of the individual shown in Figure 3.10. Variants from 0 to 1 were evaluated in the classification and all variants were correlated with each other's. Based on the values collected from the system which was established by us.



Figure 3.9 View of the output set in the fuzzy logic interface on Matlab.

Three different tests with six different patient data were performed for the system's validation. All our patients were female. When these results were analysed and compared by one by with previously trained data, the system was working accurately. The system gave us results between 0 and 1. As all of our results have almost the same percentage, we can say that we trained the system correctly. Our results were shown below.

Table 3.6 represents the data test group for the validation study. For two breast cancer patients who had a pathogenic variants in *RAD50* and *NBN* genes, respectively the system

gave us 0.92 pathogenic of these two tests. As a result, since these individuals have the same percentage in the system, we showed once again that the system we installed was reliable.

In Tests 3 we re-affirmed the accuracy of the system using data from different patients. As seen in Table 3.6, breast cancer patient who had a likely benign variant in the *RAD50* gene and the system gave us 0.25 likely benign of this test. According to the results we examined, our youngest patient was test 3 and it was confirmed to be likely benign.

The last fourth, fifth and sixth patients were VUS variants and the result for both tests was 0.5. All these patients had a VUS variants within *MSH6*, *APC*, *MSH2*. There were VUS variants in these three individuals we tested so patients 4,5,6 have same classifications. In accordance with our classification table, we detected that the system made a possible VUS correct prediction.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age	42	34	52	47	49	42
Sex	Female	Female	Female	Female	Female	Female
Consanguinity	No	Unknown	Unknown	Yes	Unknown	Unknown
Family History	Positive	Positive	Positive	Negative	Positive	Positive
Tumour Size	1.9x1.8	3x2.5	2x2	1.2x0.5	3x2x2	Unknown
Membership Degree	Grade 3	Grade 2	Grade 2	Grade 2	Grade 3	Grade 2
Location	Right Breast	Right Breast	Left Breast	Right Breast	Left Breast	Right Breast
Oestrogen receptor	Positive	Positive	Positive	Positive	Negative	Positive
Progesterone	Negative	Positive	Positive	Positive	Negative	Positive

Gene/Gene	Double	Double	DNA	Adenomatosis	DNA	Encodes Nibrin
Variation	Strand	Strand	Mismatch	Polyposis	Mismatch	NRN
	Break	Break	Repair	Coli	Repair	
	Repair	Repair	Protein	APC	Protein	
	Protein	Protein	MSH6	AI C	MSH2	
	RAD50	RAD50				
Variant	c.2014C>T	c.980G>A	c.663A>C	c.296G>A	c.435T>G	c.1154_1155del
Diagnose	YES	YES	YES	YES	YES	YES
Classification	Р	LB	LB/CIP	VUS	VUS	Р
Output	Р	LB	LB/CIP	VUS	VUS	Р
Percentage	0.92	0.25	0.5	0.5	0.5	0.92
Of	%92	%25	%50	%50	%50	%92
Results						
ICSUID						

Table 3.6 Results from the system validation (P: pathogenic, LB: likely bening, VUS: variant with unknown significance)

Overall, designed fuzzy logic system accurately predicted all individuals in the test group. If these values that were obtained from the system were converted to percentage, the system gave us the percentage of an individual's early risk score prediction for breast cancer as shown in Table 3.7.

Tests of Individuals	Collected Values	Converted Percentage
		Values
Patient 1	0.92	92%
Patient 2	0.92	92%
Patient 3	0.25	25%
Patient 4	0.50	50%
Patient 5	0.50	50%
Patient 6	0.50	50%

Table 3.7 Results of the test group with risk scores as percentage

CHAPTER 4

DISCUSSION

4.1 Discussion

Integration of artificial intelligence (AI) approaches such as machine learning and fuzzy logic transforms big data into clinically actionable knowledge and becoming the foundation of precision medicine. Since uncertainty is the inseparable nature of medicine, fuzzy logic methods have been used as one of the best methods to decrease this ambiguity. Early diagnosis as the initial step of medical practice. This has given rise to evidence based computerized diagnostic tools, intended to aid the physician in making primary medical decisions and hence an early diagnosis which helps reduce the treatment time or may save lives. The complicated clinical decision making which is usually accompanied with the degree of ambiguity and uncertainty. Since uncertainty is the inseparable nature of medicine, fuzzy logic methods have been used as one of the best methods to decrease this ambiguity. Some of the most important areas in medicine research are related to cancer and cardiovascular diseases. Specifically, heterogenous disease breast cancer, is the cause of the most common cancer death in women globally, early detection of the breast cancer is an effective method to reduce mortality. The majority (~70%) of breast cancer cases are sporadic. Familial breast cancers (~30%), often seen in families and have been associated with a number of susceptibility genes. Family-linkage studies have identified high penetrance genes (BRCA1/2, PTEN and P53) that are responsible for inherited syndromes. Recently, the fuzzy logic system was used to predict breast cancer mortality while creating a scoring system to estimate breast cancer using breast cancer class, family history, TNM stage and breast cancer history as risk factors (Tawfeeq, 2018). In other study conducted by Valarmathi et al. (2012) in the Western Region of Tamil Nadu in India, breast cancer risk assessment was performed based on staging lymph node, tumour, and metastasis using the ID3 algorithm and unity rule. The intensity range of breast cancer was estimated using the Fuzzy logic toolbox. The parameters were age, gender, location, diet, year, marital status, heredity, disease period, treatment, diagnostic stages like TNM (Valarmathi et al., 2012).

However, in this current study 14 different risk factors have been used: age, sex, consanguinity, family history, membership degree, tumour size, lymph node, malignancy, location, oestrogen receptor, progesterone receptor, gene/ gene variation, diagnosis and genetic classification according to ACMG guidelines (Richards et al., 2015). More importantly, 18 different genes (*TP53*, *FAM175*, *RAD50*, *NBN*, *MSH6*, *APC*, *MSH2*, *ATM*, *CDH1*, *MUTYH*, *PALB2*, *BLM*, *MRE11A*, *PMS2*, *CHEK2*, *PTEN*, *BART1*, *BRIP1*) and their genetic variations which thought to be associated with breast cancer molecular pathogenesis have been included in this study.

Previously, a combination of family-based and population-based approaches revealed that genes involved in DNA repair associates with breast cancer risk. BRCA1 and BRCA2 genes are involved in maintaining genome integrity, at least in part, by engaging in DNA repair, cell cycle checkpoint control and even the regulation of key mitotic or cell division steps. Thus, the complete loss of function of either protein leads to a dramatic increase in genomic instability. Thousands of different disease-associated variations of BRCA1/2 genes have been detected. As the role and effect of BRCA1/2 genes are well studied, we focused on other hereditary cancer related genes in this study for early prediction for breast cancer using fuzzy logic. Mainly, this study focused on the complex behaviour of genetic variations selecting only BRCA1/2 negative patients. 90 patients were selected from 488 patients' data. They had variations in other various 18 different hereditary cancer genes but not neither BRCA1 nor BRCA2. The data of 90 patients have been thought to developed the fuzzy logic system we. To the best our knowledge, this study is unique in terms of merging a wide number off cancer risk factors as well as genetic variants. In artificial intelligence or fuzzy logic are very significant to include and train the system with more risk factors to obtain more accurate and consequently more consistent results.

In our study, MATLAB program was used to improve fuzzy logic which was harmonized for breast cancer prediction/ scoring risk. We mainly focused on genetic variations associated with 18 (*TP53*, *FAM175*, *RAD50*, *NBN*, *MSH6*, *APC*, *MSH2*, *ATM*, *CDH1*, *MUTYH*, *PALB2*, *BLM*, *MRE11A*, *PMS2*, *CHEK2*, *PTEN*, *BART1*, *BRIP1*) hereditary breast cancer related genes. We used variant classification guidelines of ACMG to classify our outputs as benign, likely benign, VUS, likely pathogenic and pathogenic. According to another study, output clusters were determined as very serious, very serious moderate and not serious in order to give breast cancer risk prognosis (Richards et al., 2015). As these output clusters were not certain, we can conclude that our study is more reliable than the other study as giving more precise results. The important point is when the new patient's written into the trained system in the future, the system will give accurately the risk score of breast cancer percentage for *BRCA1/2* negative patients.

Another study was focused on *BRCA1* and *BRCA2* positive breast cancer patients to develop early prediction and precise medicine approach using the fuzzy logic system (a personal communication with Assoc. Prof. Mahmut Çerkez Ergoren from Near East University Faculty of Medicine, Department of Medical Biology). As well as with this study, they used 16 different risk factors and focus on only 268 *BRCA1* and *BRCA2* positive patients. However, we obtained the results using data from 90 *BRCA1/2* negative hereditary breast cancer patients who had genetic variation within 18 different genes. Different genetic variants in different genes were obtained in this current study. However, both these studies had same five membership functions in the output section such as Benign, Likely Benign, VUS, Likely Pathogenic and Pathogenic.

Six individual test groups were conducted to test the developed fuzzy logic system. These patients were not included to the system before. An accurate and a consistent result were obtained using six new individual test groups. Therefore, we can conclude that the obtained results using artificial intelligence methods can be utilized in early prediction of BRCA1/2 negative hereditary breast cancer patients.

4.1.1 Future Remarks

Nowadays, artificial intelligence in health industry is also developing day by day. Our work is important as future precise medicine approach. As cancer rates have been increasing globally, new treatment strategies should be invented. Using advanced technology and artificial intelligence will reduce the risk of mistakes of people.

The purpose of our study was to create a software for personal medicine and preventive medicine while from diagnose to treatment might take some times for clinician and it can be frustrating for the patient as well as considering the early diagnosis is life-saving for cancer patients. We believe that our study has been conducted to find solutions for these problems.

For the first time, a program has been developed for the pre-diagnosis of breast cancer using the fuzzy logic system for 14 different factors including 18 different genes and genetic variations. Thus, our work has a very significant contribution to the literature very important. In the future, our study can be used for early prediction of many patients at risk of breast cancer and will be used for pre-diagnosis in the clinic, hospital or by doctors. Our model will be a useful tool for preventive medicine and will be a unique example of personalized medicine.

Overall, our aim here was to develop a software to give risk score for early diagnosis of BRCA1/2 negative hereditary breast cancer patients to save time from diagnosis to treatment. And our results approved this thesis hypothesis as giving a rise of importance of using artificial intelligence systems in medicine.

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PATIENT NO	DATE	INDICATION	СІТҮ	SEX	AGE	DIAGNOSTIC AGE	FAMILY HISTORY	SAMPLE TYPE
1896	14/06/2019	BREAST	BURSA	F	45	30	Pozitif	Peripheral Blood
387885	15.02.2019 - 01.03.2019	OVER	VAN	F	51	43		Peripheral Blood
480510	24/05/2019	BREAST	BURSA	F	42	35	Pozitif	Peripheral Blood
503745	28/09/2018	BREAST	BURSA	F	32	31	Pozitif	Peripheral Blood
543417	04/02/2019	BREAST	BALIKESİR	F	54	40	Negatif	Peripheral Blood
665477	08/03/2019	BREAST	BURSA	F	34	33	Pozitif	Peripheral Blood
752351	28/12/2018	BREAST	KONYA	F	34	33	Pozitif	Peripheral Blood
1012151	07/12/2018	BREAST		F	52	44	Pozitif	Peripheral Blood
1723683	05/04/2019	BREAST	VAN	F	47	45	Negatif	Peripheral Blood
	29/03/2019	BREAST	BULGARİSTAN	F	59		Negatif	Peripheral Blood
1799155	15/03/2019	BREAST	КÜТАНҮА	F	42			Peripheral Blood
1806752	24/12/2018	BREAST	КÜТАНҮА	F	28			Peripheral Blood
1867503	24/05/2019	BREAST	ΗΑΤΑΥ	F	42		Pozitif	Peripheral Blood
4963	17/05/2019	BREAST	BURSA	F	49	43	Pozitif	Peripheral Blood
19626	19/01/2018	BREAST	NEVŞEHİR	F	44	25	Pozitif	Peripheral Blood
51597	11/03/2019	BREAST	MUŞ	F	54	32	Pozitif	Peripheral Blood
72028	01/11/2018	BREAST	BURSA	F	60	58	Pozitif	Peripheral Blood
96739	19/01/2019	BREAST	BURSA	F	51	45	Pozitif	Peripheral Blood
118040	18/01/2019	BREAST	BURSA	F	47	44	Negatif	Peripheral Blood
358853	08/04/2019	BREAST	BALIKESİR	F	45	36	Negatif	Peripheral Blood
367109	11/03/2019	BREAST	KARAMAN	F	51	39	Pozitif	Peripheral Blood
447825	23/11/2018	BREAST	BURSA	F	67	55	Pozitif	Peripheral Blood
467999	10/05/2019	BREAST + OVER	BULGARİSTAN	F	53	41	Pozitif	Peripheral Blood
479063	24/05/2019	OVER	ORDU	F	59	57	Pozitif	Peripheral Blood

APPENDIX I

487518	20/07/2018	BREAST	BURSA	F	55	53	Pozitif	Peripheral Blood
503580	03/08/2018	BREAST	DUDSA		42	42	Nogatif	Peripheral
512765	08/07/2019	BREAST	DALWEGID	г г	45	38	Desitif	Peripheral
581346	16/11/2018	BREAST	BALIKESIK	г _	48	62	POZILII	Peripheral
581547	05/02/2018	BREAST	ERZURUM	F	64	45	Pozitif	Blood Peripheral
561547	03/02/2018	BREAST	KAYSERİ	F	49	45	Pozitif	Blood Peripheral
588195	13/02/2018	BREAST	BALIKESİR	F	57	47	Pozitif	Blood
616422	11/06/2019	BREAST	BURSA	F	52	48	Pozitif	Blood
666460	18/01/2019	OVER	BURSA	F	54	51	Pozitif	Peripheral Blood
747474	18/12/2018	BREAST	BALIKESİR	F	38	35	Pozitif	Peripheral Blood
VARYANT 3								
749300	11/12/2018	BREAST	TRABZON	F	70	62		Peripheral Blood
782923	22/03/2019	BREAST + COLON	BALIKESİR	F	74		Negatif	Peripheral Blood
911189	17/08/2018	BREAST	BURSA	F	50	49	Pozitif	Peripheral
991841	18/04/2018	BREAST	BURSA	- '	30		Pozitif	Peripheral
994388	05/04/2019	BREAST	BURSA	г 	44	41	POZICII	Peripheral
1002036	08/03/2019	OVER	KUTAHYA	F	49		Pozitif	Blood Peripheral
1002030	00/03/2019	OVER	ANKARA	F	46		Pozitif	Blood Peripheral
1012588	05/04/2019	BREAST	TRABZON	F	47	46	Pozitif	Blood
1024968	05/04/2019	BREAST	ERZURUM	F	39	27/2 3)/42		Blood
1165863	05/04/2019	BREAST	KÜTAHYA	F	44	37 (Sag) / 43 (Sol)	Pozitif	Blood
VARYANT 3								
1208372	18/03/2019	BREAST	BURSA	F	41	41	Pozitif	Peripheral Blood
1243965	05/07/2019	BREAST	TEKİRDAĞ	F	42	36		Peripheral Blood
1246715	28/06/2018	OVER	MUS	F	38		Pozitif	Peripheral Blood
1288720	22/02/2019	BREAST	DALIKESID	E	20		Dozitif	Peripheral
1331609	20/07/2018	BREAST	BALIKESIK	г _	58		POZILII	Peripheral
1339112	12/10/2018	BREAST	BURSA	F	35	32	Pozitif	Blood Peripheral
1202271	14/02/2010	DDEACT	BURSA	F	34	46	Pozitif	Blood Peripheral
1383371	14/03/2019	BREAST	NİĞDE	F	48	40	Negatif	Blood Peripheral
1396963	24/05/2019	BREAST	KARS	F	55	50	Pozitif	Blood
1398126	27/07/2018	BREAST	AĞRI	F	27		Pozitif	Blood
1403852	12/04/2019	BREAST	SİVAS	F	41	36?	Negatif	Blood
1412788	10/05/2019	BREAST	BALIKESİR	F	43	39	Pozitif	Peripheral Blood
1461494	17/05/2019	BREAST	TRABZON	F	45	41	Pozitif	Peripheral Blood
1487647	11/07/2019	BREAST	DİYARBAKIR	F	49	49	Pozitif	Peripheral Blood
1501231	03/05/2019	BREAST	BALIKESIR	F	65	61	Pozitif	Peripheral Blood
1511223	24/01/2019	BREAST	SİNOP	F	65		Pozitif	Peripheral

								Blood
1629572	12/12/2019							Peripheral
1038573	12/12/2018	OVER	DİYARBAKIR	F	23		Negatif	Blood
1645648	26/04/2019	BREAST	DALIKECID	-	24	32	Desitif	Peripheral
VARVANT			BALIKESIK	F	34		POZIŢI	BIOOD
3								
1657174	19/10/2018	BREAST				35		Peripheral
103/1/1	13/10/2010	BREACT	BURSA	F	38	33	Pozitif	Blood
1662804	05/11/2018	BREAST		F	36	33	Pozitif	Peripheral
	14.02.2018 -				50		1 021011	Peripheral
1666781	03.08.2018	BREAST	ADANA	F	37	34	Pozitif	Blood
1686052	05/02/2019	BREAST						Peripheral
	,,		KARS	F	24			Blood
1704805	17/07/2018	BREAST	GIRESUN	F	46			Peripheral
			GINESON		40			Peripheral
1718575	03/08/2018	BREAST	BURSA	F	36			Blood
1773051	25/04/2019	BREAST						Peripheral
1775051	23/01/2013	BILLIOT	ANKARA	F	36		Negatif	Blood
1773109	17/05/2019	BREAST	DALIKESID	E	44	44	Pozitif	Peripheral
			BALIKESIK	1	44		FUZILII	Peripheral
1773479	10/12/2018	BREAST	ANKARA	F	65		Pozitif	Blood
1789199	05/04/2019	BREAST				23		Peripheral
1785155	03/04/2013	DIREAST	BİNGÖL	F	34	23	Negatif	Blood
1791546	10/09/2019	BREAST	SIVAS		52		Dozitif	Peripheral
			SIVAS	Г	52		POZILII	Peripheral
1793523	20/10/2018	BREAST	AĞRI	F	38	37	Pozitif	Blood
VARYANT								
3								
1862496	22/02/2019	BREAST	ΔΝΙΚΑΡΑ		E 4	46	2	Peripheral
			ANKARA	F	54		1	Peripheral
1868617	25/01/2019	BREAST	DIYARBAKIR	F	39		Pozitif	Blood
1870779	26/03/2019	OVER						Peripheral
10/0//5	20/03/2013	OVEN	BURSA	F	64			Blood
1875575	11/02/2019	BREAST	EDZUDUM		60			Peripheral
			ENZONOM	1	00			Perinheral
1894428	11/07/2019	BREAST	BALIKESİR	F	48		Pozitif	Blood
1906874	16/05/2019	BREAST						Peripheral
15000,4	10,00,2015		KADİRLİ	F	34		Pozitif	Blood
VARYANT 3								
1000109	21/06/2010	DDEACT			1			Peripheral
1909108	21/00/2019	DREAST	YALOVA	F	53			Blood

METHODLOG Y	PATHOLOGICAL DIAGNOSIS	GRADE	TUMOR SIZE	AKRABA EVLİLİĞİ (ANNE- BABA)	LOCATIO N	ÖSTROJEN RESEPTÖR Ü
NGS	Patoloji raporu yok	-	-	Anne Baba akraba değil		
NGS	Patoloji raporu yok					
NGS	Invaziv duktal karsinom	3	1.9X1.8	Anne Baba akraba değil	Sağ Meme	30
NGS	Patoloji raporu yok			Anne Baba akraba değil		
NGS	Invaziv duktal karsinom			Anne Baba akraba	Sol Meme	
NGS	Invaziv duktal karsinom				Sol Meme	95
NGS	Invaziv duktal	2	3x2.5		Sağ Meme	10

	karsinom					
NGS	Invaziv duktal karsinom	2	2x2		Sol Meme	POZİTİF
NGS	Invaziv duktal karsinom	2	2 1x1 7 ve 1 2x0 5	Anne Baba akraba	Sağ Meme	80
NCS	Invaziv duktal		2.1.1.1.1 VC 1.2.40.5	Anne Baba	Sug Mente	NECATIE
NGS	Invaziv duktal			akraba degii	Sag Meme	NEGATIF
NGS	karsinom Malign iğsi hücreli	2	0.3x0.2		Sağ Meme	90
NGS	tümor Invaziy duktal				Sağ Meme	
NGS	karsionoma	2			Sağ Meme	95
NGS	karsinom	3	3X2X2		Sol Meme	NEGATİF
NGS	Infiltratif duktal karsinom				Sol Meme	
NGS	Patoloji raporu yok					
NGS	In situ duktal karsinom		1x0.8		Sağ Meme	70
NGS	Invaziv duktal karsinom	2	1x1		Bilateral	70
NGG	Invaziv duktal	2	2 51/2		G 114	70
NGS	karsinom	2	3.5X3		Sol Meme	70
NGS	Patoloji bilgisi yok			Anne Baba	Sag Meme	POZITIF
NGS	Dış merkezli hasta			akraba değil		
NGS	karsinom			akraba değil		
NGS	seröz papiller karsinom (over)					
NGS	Patoloji raporu vok			Anne Baba akraba		
NGS	Invaziv duktal	2			Sol momo	NECATÍE
NUS	Invaziv duktal	2			Sol meme	NEGATIF
NGS	karsinom	2	2.8x2		Sol meme	95
NGS	bilateral meme ca				Bilateral	
NGS	Intraduktal papilom			Anne Baba	Sol Meme	95
NGS	Patoloji raporu yok			akraba değil		
NGS	Patoloji bilgisi yok			akraba değil		
NGS	Dış merkezli hasta					
NGS	Patoloji bilgisi yok					
NGS	karsinom	5	4.5x3		Sağ Meme	NEGATİF
NGS	Intratubuler karsinom	1	0.5x0.3		Sağ Meme	90
NGS	Invaziv duktal karsinom	2	2.2X1.9		Sağ Meme	POZİTİF
NGS	Invaziv duktal karsinom		2x0.8	Anne Baba akraba değil	Sol Meme	95
NCS	Invaziv lobüler	2				80
	Invaziv duktal	۷		Anne Baba		00
NGS	karsinom Yüksek dereceli seröz	3	1x1	akraba değil Anne Baba	Sol Meme	NEGATİF
NGS	karsinoma			akraba değil		POZİTİF
NGS	karsinoma	2	3.1x2.3	akraba	Sol Meme	95
NGS	Invaziv duktal karsinom	2	13x10x3			80
NGS	Invaziv duktal karsinom	3	1,2x0,6	Anne Baba akraba değil	Bilateral	NEGATİF
·	•	•	•	. 5	-	•

	Invaziv duktal			Anne Baba		
NGS	karsinom	2		akraba değil	Sağ Meme	90
	In situ duktal					
NGS	karsinom	3	2.8x2.5		Sağ Meme	90
				Anne Baba		
NGS	Patoloji raporu yok			akraba değil		
	Invaziv duktal					
NGS	karsinom	2	1,5X0.1X0.1		Sağ Meme	95
NGS	Patoloji raporu yok				Sağ Meme	
	Invaziv duktal					
NGS	karsinom				Sol Meme	
	Invaziv duktal					
NGS	karsinom	2			Sağ Meme	90
				Anne Baba		
NGS	Patoloji raporu yok			akraba değil		
NGS	Patoloji bilgisi yok				Sağ Meme	
	Invaziv tübüler					
NGS	karsinom	2	0.6		Sağ Meme	90
	Invaziv duktal					
NGS	karsinom	3	2,4x1.7x1.5		Sağ Meme	NEGATİF
				Anne Baba		
NGS	Patoloji raporu yok			akraba değil		
	Invaziv duktal			Anne Baba		
NGS	karsinom	3	2	akraba değil	Sol Meme	NEGATÍF
NGS	Metastatik Meme					
	Invaziv lobüler					
NGS	karsinom	2	1.4 ve 0.4		Sol Meme	80
	Müsinöz Borderline					
NGS	karsinom				Sağ Over	NEGATİF
				Anne Baba		
NGS	Patoloji raporu yok			akraba değil		

NGS	Patoloji raporu yok				Sol Meme	
NGS	Patoloji raporu yok					
NGS	Invaziv duktal karsinom					60
NGS	Invaziv duktal karsinom	2	6.5x4.5		Sağ Meme	90
NGS	Invaziv duktal karsinom		1.6x0.1x0.1 ve 1.5x0.1x0.1		Sağ Meme	60
NGS	Invaziv duktal karsinom					90
NGS	Invaziv duktal karsinom	2	1.5x1.3	Anne Baba akraba	Sağ Meme	95
NGS	Invaziv duktal karsinom	2			Sol Meme	95
NGS	Invaziv duktal karsinom	1	1.5x1.3		Sol Meme	80
NGS	Invaziv duktal karsinom	Büyük Tümor:2,Küçük T:3	Büyük T: 4x2.2 Küçük T: 3x1.5		Sol Meme	Büyük: 70, Küçük:95
NGS	Invaziv duktal karsinom	Büyük Tümor:2,Küçük T:1	Büyük T: .8x1.5 Küçük T: 1x0.9		Sol Meme	Büyük: 95, Küçük:90
NGS	Invaziv duktal karsinom	3	1.9x1.7		Sağ Meme	95
NGS	Invaziv duktal karsionoma	2			Sol Meme	95
NGS	Patoloji raporu yok					
NGS	Yüksek dereceli seröz karsinoma		10.5x5x0.9		Bilateral	POZİTİF
NGS	Patoloji raporu yok				Sol Meme	
NGS	Invaziv duktal karsionoma	1			Sağ Meme	95
NGS	Patoloji raporu yok			Anne Baba akraba değil		

	Invaziv duktal			
NGS	karsionoma		Sol Meme	70

PROGESTERON	CERB B2	LENF NODU MET	Gene	Transcript (NM)	Variant
			TP53	NM_000546.5	c.40C>G
			FAM175A (ABRAXAS1)	NM_139076	c.853C>T
NEGATİF	POZİTİF	NEGATİF	RAD50	NM_005732	c.2014C>T
			RAD50	NM_005732	c.980G>A
80	FOKAL POZİTİF	NEGATİF	NBN	NM_002485	c.880A>T
1	POZİTİF	POZİTİF	RAD50	NM_005732	c.980G>A
POZİTİF	NEGATİF		MSH6	NM_000179	c.663A>C
80	NEGATİF	POZİTİF	APC	NM_000038	c.296G>A
NEGATİF	POZİTİF		RAD50	NM_005732	c.1277A>G
10	NEGATİF	NEGATİF		T	1
			MSH6	NM_000179	c.2161A>G
95	NEGATİF	NEGATİF	NBN	NM_002485	c.1154_1155del
NEGATİF	NEGATİF	POZİTİF	MSH2	NM_000251	c.435T>G
			MSH2	NM_000251	c.1787A>G
			MSH6	NM_000179	c.1069G>A
70	NEGATİF			1	,
70	ZAYIF POZİTİF	NEGATİF	APC	NM_000038	c.3949G>C
70	NEGATİF	POZİTİF	F	47	44
POZİTİF	POZİTİF		ATM	NM_000051	c.7424T>G
			CDH1	NM_004360	c.1766A>G
				NM_005732	
			RAD50		c.326_329delCAGA
NEGATİF	NEGATİF	NEGATİF	ATM	NM 000051	c.4329C>A
95	NEGATÍF	NEGATIF		100200001	0110250711
,,,	NEGHTI				
95	NEGATİF		MUTYH	NM_001128425	c.884C>T
			NBN	NM_002485	c.283G>A
					,
			ATM	NM_000051	c.7500C>G
NEGATİF	POZÍTÍF		ATM	NM_000051	c.6860G>A
			PALB2	NM_024675	c.315G>C
60	NEGATİF	NEGATİF		1	,
POZİTİF		NEGATİF	RAD50	NM_005732	c.379G>A
95	NEGATİF	NEGATİF	ATM	NM_000051	c.2494C>T

80	NEGATİF		BLM	NM_000057	c.2407-06AG
NEGATİF	NEGATİF	NEGATİF			
POZİTİF			PALB2	NM_024675	c.3306C>G
60	NEGATİF	NEGATİF	ATM	NM_000051	c.3676G>C
NEGATİF	NEGATÍF	POZİTİF	FAM175A (ABRAXAS1)	NM 139076	c 853C>T
NEGATİF	NEGATÍF	TOLIN	APC	NM 000038	c.1287A>G
			MRE11A	NM 005591	c.2046A>G
90	POZİTİF	POZİTİF	PMS2	NM 000535	c.1211C>G
75	NEGATİF	NEGATİF	CHEK2	NM_007194	c.592+3A>T
			ATM	NM_000051	c.5882A>G
95	POZİTİF	NEGATİF		·	·
			ATM	NM_000051	c.4329C>A
90	POZİTİF	NEGATİF			
90	NEGATİF	NEGATİF	CHEK2	NM_007194	c.1427C>T
NEGATİF	NEGATİF	POZİTİF			
				1	
NEGATİF	NEGATİF	POZİTİF	RAD50	NM_005732	c.379G>A
			ATM	NM_000051	c.2932T>C
40	NEGATİF		MUTYH	NM_001128425	c.1431G>C
NEGATİF			ATM	NM_000051	c.1054A>G
			TP53	NM_000546	c.47_48delAGinsT
		1	ATM	NM_000051	c.4424A>G
NEGATİF	NEGATİF		CDH1	NM_004360	c.1610G>T
30	NEGATİF				
NEGATİF	NEGATİF		PALB2	NM_024675	c.711A>G
100	POZİTİF		PTEN	NM_000314	c.955_958delACTT
90	NEGATİF	NEGATİF	ATM	NM_000051	c.5111A>G
95	NEGATİF	POZİTİF			
20 Büyük: 50 Küçük:	NEGATİF				
95 95	NEGATİF (büyük				
	NEGATİF (büyük tümör)				
Büyük: 95, Küçük:100	NEGATİF (büyük tümör) POZİTİFx2	POZİTİF			
Büyük: 95, Küçük:100 95	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF	POZİTİF	APC	NM_000038	c.3220A>G
Büyük: 95, Küçük:100 95	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF	POZİTİF	APC PALB2	NM_000038 NM_024675	c.3220A>G c.3306C>G
Büyük: 95, Küçük:100 95 90	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF NEGATİF	POZİTİF	APC PALB2 CDH1	NM_000038 NM_024675 NM_004360	c.3220A>G c.3306C>G c.892G>A
Büyük: 95, Küçük:100 95 90	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF NEGATİF	POZİTİF NEGATİF	APC PALB2 CDH1	NM_000038 NM_024675 NM_004360	c.3220A>G c.3306C>G c.892G>A
Büyük: 95, Küçük:100 95 90 POZİTİF	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF NEGATİF	POZİTİF NEGATİF POZİTİF	APC PALB2 CDH1	NM_000038 NM_024675 NM_004360	c.3220A>G c.3306C>G c.892G>A
Büyük: 95, Küçük:100 95 90 POZİTİF	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF NEGATİF	POZİTİF NEGATİF POZİTİF	APC PALB2 CDH1 BART1	NM_000038 NM_024675 NM_004360 NM_000465	c.3220A>G c.3306C>G c.892G>A c.103G>A
Büyük: 95, Küçük:100 95 90 POZİTİF 95	NEGATİF (büyük tümör) POZİTİFx2 NEGATİF NEGATİF NEGATİF	POZİTİF NEGATİF POZİTİF NEGATİF	APC PALB2 CDH1 BART1 PMS2	NM_000038 NM_024675 NM_004360 NM_000465 NM_000535	c.3220A>G c.3306C>G c.892G>A c.103G>A c.620G>A

		BRIP	NM_032043	c.2741T>C
40	NEGATİF	ATM	NM_000051	c.8147T>C