“PREVALENCE OF INTERLEUKIN 7 RECEPTOR ALPHA THR244ILE GENETIC POLYMORPHISM AMONG BREAST CANCER PATIENTS”

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COIMBATORE MEDICAL COLLEGE
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CERTIFICATE

This to certify that the dissertation entitled **"PREVALENCE OF INTERLEUKIN 7 RECEPTOR ALPHA THR244ILE GENETIC POLYMORPHISM AMONG BREAST CANCER PATIENTS"** is the bonafide original work done by **DR.RAJESH WILSON,** Post Graduate in **Biochemistry** under overall supervision and guidance in the Department of Biochemistry, Coimbatore Medical College, Coimbatore , in partial fulfilment of the regulations of The Tamil Nadu Dr. M.G.R . Medical University for the award of **M.D. Degree in Biochemistry (Branch XIII).**

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PLAGIARISM CERTIFICATE

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I solemnly declare that this dissertation entitled **"PREVALENCE OF INTERLEUKIN 7 RECEPTOR ALPHA THR244ILE GENETIC POLYMORPHISM AMONG BREAST CANCER PATIENTS"** was written by me in the Department of Biochemistry, Coimbatore Medical College, Coimbatore, under the guidance and supervision of **DR.VEENA JULIETTE.A.,M.D.,** Associate Professor, Department of Biochemistry, Coimbatore Medical College, Coimbatore – 641014.

This dissertation is submitted to **THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,** Chennai, in partial fulfilment of the university regulations for the award of **DEGREE OF M.D BIOCHEMISTRY (BRANCH - XIII)** examinations to be held in **MAY – 2020.**

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“PREVALENCE OF INTERLEUKIN 7 RECEPTOR ALPHA *THR244ILE* GENETIC POLYMORPHISM AMONG BREAST CANCER PATIENTS”

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ABBREVIATIONS

APC	:Antigen Presenting Cells
ASE	: Allele Specific gene Expression
ASM	:Allele Specific DNA Methylation
ASTF	:Allele Specific Transcription Factor binding
c-Myb	: Protooncogene, Transcription Factor
COX -2	: Cyclooxygenase-2
CD	:Cluster of Differentiation
c-Myc	: Master Regulator of Cell Cycle Entry
DNA	: Deoxy ribo Nucleic Acid
DNMT	: DNA methyl transferase
ER positive	: Estrogen Receptor Positive
GWAS	: Genome Wide Association Studies
GATA-1	: An Erythroid Transcription Factor
GM-CSF	: Granulocyte macrophage colony stimulating factor
G-CSF	: Granulocyte colony stimulating factor
HLA	: Human Leukocyte Antigen
HER 2	: Human Epidermal Growth Factor Receptor 2
IgG	: Immunoglobulin G
IL	: Interleukin
JAK	: Janus kinases

Kb	: Kilobase
LFA-1	: Lymphocyte Function Associated Antigen-1
MS	: Methionine synthase
MTHFR	: Methylene Tetra Hydrofolate Reductase Estrogen receptor
MICA	: MHC Class I Polypeptide related Sequence A
qPCR/qrt-PCR	: Quantitative Real Time Polymerase Chain Reaction
KPCR	: kinetic polymerase chain reaction
STAT	:Signal transducers and activators of transcription
SPI	:Salmonella Pathogenecity Island
SNP	:Single Nucleotide Polymorphism
TCR	:T Cell Receptor
TLR	:Toll like receptor
TGF	:Transforming Growth Factor
VEGF	:Vascular Endothelial Growth Factor

INTRODUCTION

INTRODUCTION

Recent increase in the incidence of cancers has turned out to be a major problem of the century impacting physical, mental and social dimensions of health[1]. The most commonly diagnosed cancer in women is Breast Cancer (BC) and the incidence of Breast Cancer continues to rise in both developing and developed countries. It is second only to lung cancer as a cause of cancer death[2].The risk of developing the disease is 100 fold higher in women than men.

Breast cancer is the second most common cancer in the world with overall estimate of 1.7 million cases. Breast cancer remains the most frequent cause of death among women as per GLOBOCON 2012 review[2](Figure 1)

As per 2018 WHO estimate approximately 6,27,000 women died from breast cancer which is 15% of all cancer related death among women[3]. The life time risk of BC is 3.4%. BC is the second most common cause of person years (19.3 years) of life lost to cancer among women[4].

One third of newly diagnosed cancers are Breast cancer and 1 in 8 women are affected by it[4]. The incidence of BC is rare in women younger than age 25 but the incidence increases rapidly after age 30**.**

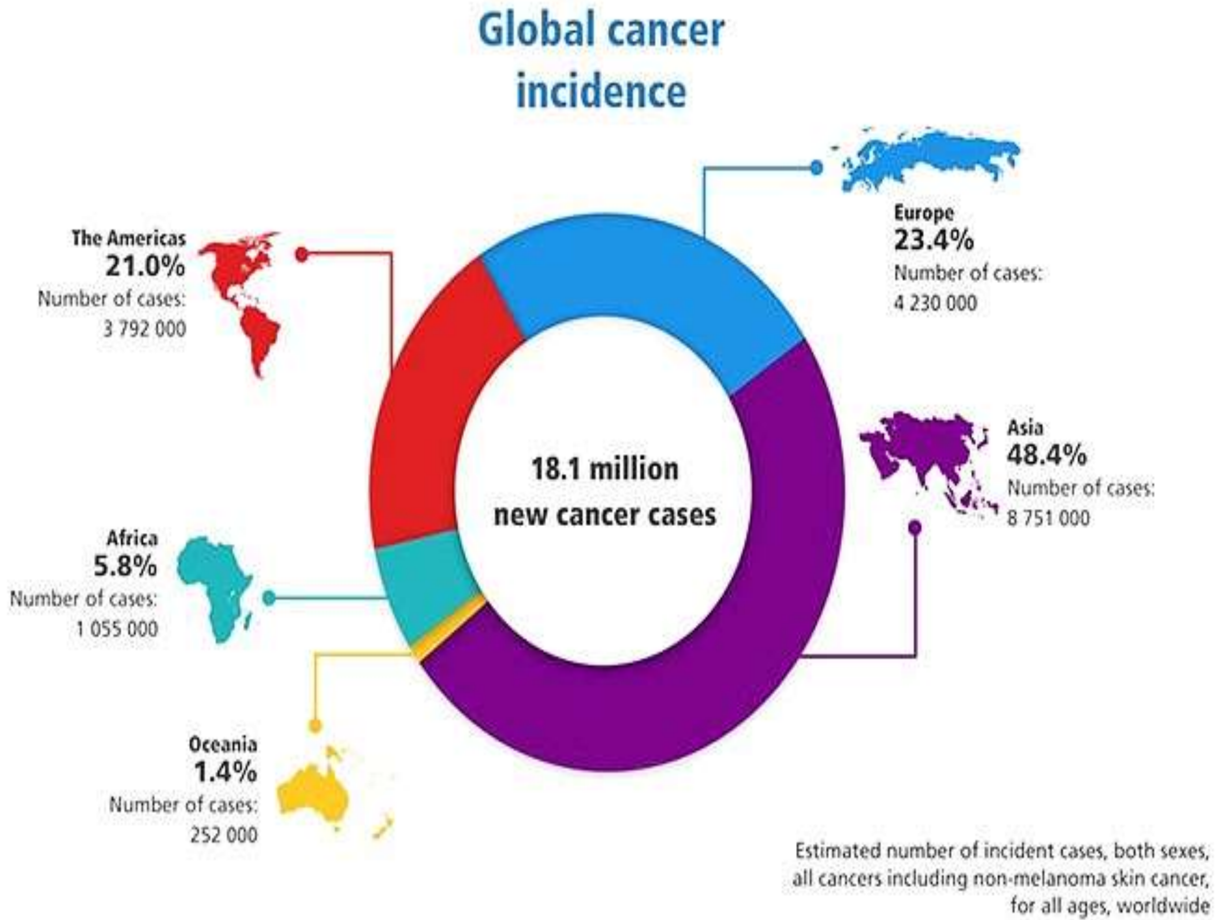


Figure 1: Global Cancer Incidence

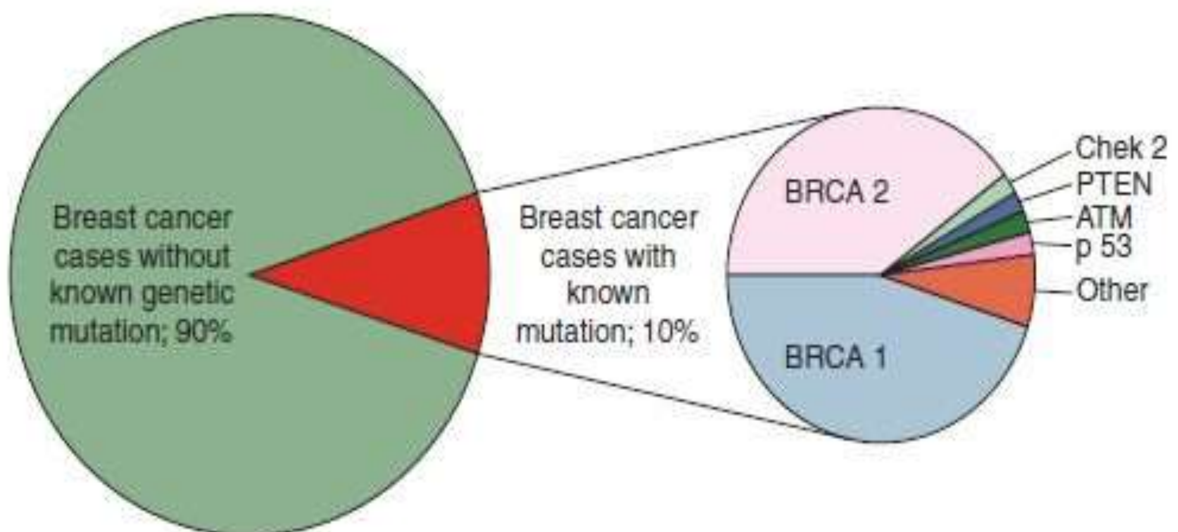


Figure 2: Percentage of Breast cancer cases with a Genetic Mutation

Cancers have a multi-factorial genetic susceptibility. Breast cancers may be hereditary; however environmental factors clearly influence the penetrance of hereditary forms of breast cancer, and both genetic and environmental factors contribute to sporadic forms of Breast cancer.

The identification of BC susceptibility genes (BRCA 1 and BRCA 2) has provided important insights into Breast Cancer. Mutation in tumour suppressor genes BRCA 1 and BRCA 2 are responsible for 80% to 90 % of single gene familial breast cancers and about 3 % of all breast cancers(Figure - 2).

The major risk factors for sporadic Breast Cancers are related to hormone exposure, gender, age at menarche and menopause, reproductive history, breastfeeding, exogenous estrogens, diet(Pala et al.,2009;Huet al .,2012), physical activity (Peters et al.,2009; Elissen et al.,2010),alcohol use (Zhang et al.,2007;Beasley et al.,2010) and smoking (Luo et al., 2011a;2011b). Substantial amount of research have also shown that certain viruses play important role in certain stage of breast cancer pathogenic process[5]. Generally viruses are involved in aetiology and progression of many different cancer types [5].

Almost all breast malignancies are adenocarcinomas and based on the expression of Estrogen receptors and HER 2 they can be divided into

three major biologic subgroups: Estrogen receptor (ER) positive, HER 2-negative (50- 65 % of tumours), HER 2- positive (10-20% of tumours, which may be either ER positive or ER negative) and ER negative, HER 2-negative (10-20% of tumours).

Immune system contributes greatly to cancer risk; innate immunity first provides cell growth stimulation by inflammation (tumour promotion factor) and adaptive immunity detects and eliminates cancer cells based on non surveillance hypothesis[6].

Variations in sequence at defined positions within genomes is the major reason for unique phenotypic characteristics, including proneness of a person towards complex mechanisms involved in oncogenesis[7]. By understanding the variations in human genome and molecular genetics, these sequence variations can be used to understand the molecular mechanisms of drug resistance, increased susceptibility, individual variations in various diseases and cancer progression[7].

Single Nucleotide Polymorphisms (SNP) are defined as single base substitutions involving A,T,C or G. Presently the term "SNP" is changed to single nucleotide variations "SNV"[7]. There is also a public domain archive for Single Nucleotide Polymorphism database (dbSNP) for broad collection of simple genetic polymorphisms[7].

SNPs of breast cancer genes of Gujarati Indians in Houston, USA (GIH) with minor allele frequencies were above 0.05 as per datas obtained from International Hap Map Project data base. The only south Asian population group in the Hap Map Project data base available at the time of study design is GIH.

In this study the prevalence of Interleukin 7 Receptor Alpha Thr244ile genetic polymorphism among breast cancer patients attending Regional Cancer Centre at Coimbatore Medical College & Hospital is evaluated. IL-7 is pleotropic cytokine. Many researchers have established IL-7 as an ideal target to enhance the immune function. It plays an important regulatory role in modulating T and B cell development and T cell homeostasis (Figure-3). IL-7 is also administered to allow the modulation of immune function in patients with immune depletion and autoimmunity. IL-7 also enhances anti-tumour immune responses (Figure-4).Interleukin 7 Receptor Alpha Thr244ile genetic polymorphism is found to be associated with susceptibility and prognostic markers in breast cancer subgroups.

The breast cancer tumour microenvironment is immunosuppressive and is increasingly recognized to play a major significant role in tumorigenesis. A deeper understanding of normal and aberrant molecular level interactions between malignant and immune

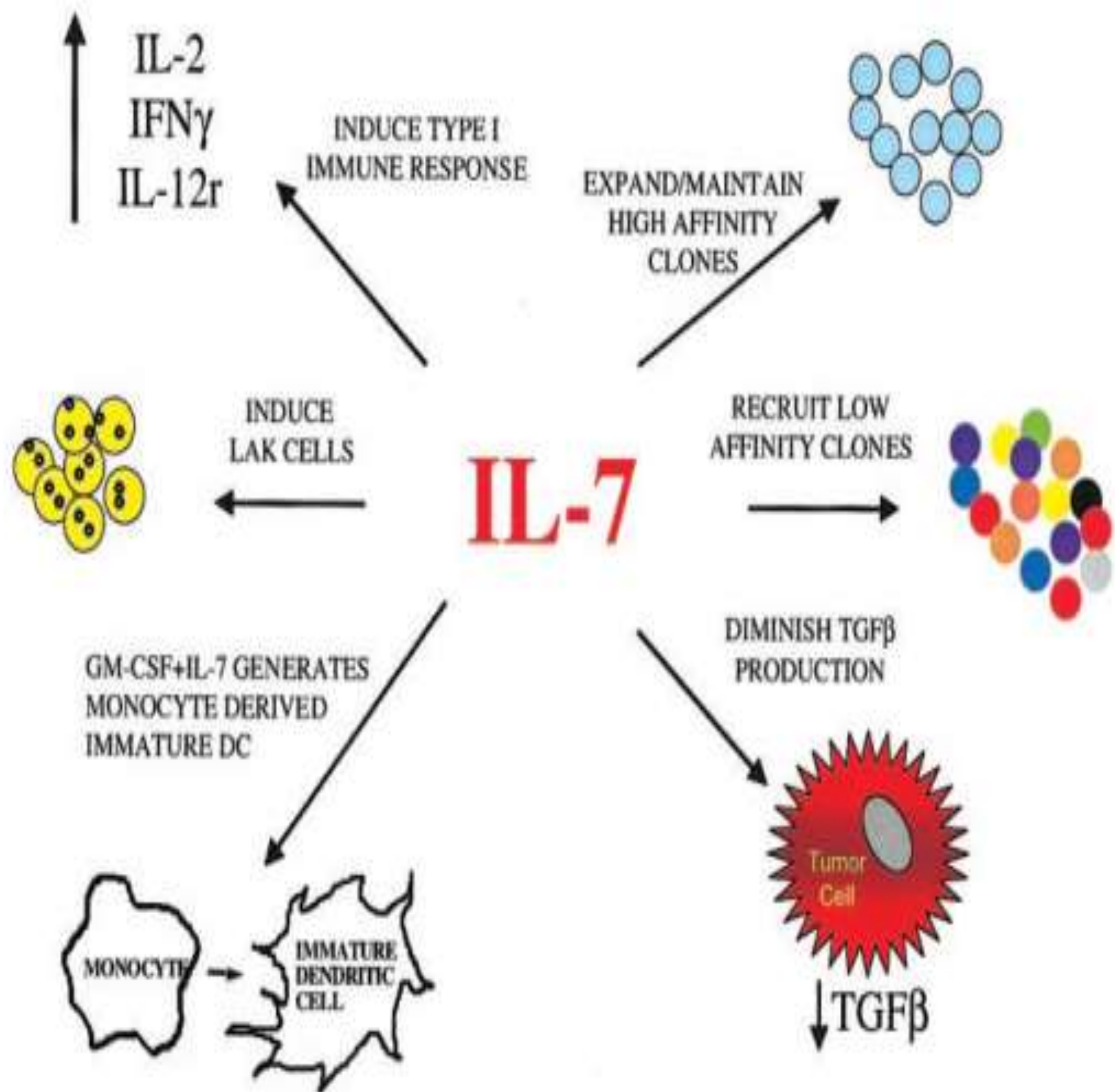


Figure3: **Potential effects of IL-7 on antitumour immune responses** , IL-7 administered systematically or as a local vaccine adjuvant can potentially enhance immune responses against tumour through a variety of mechanisms. In addition to the expansion and maintenance of T cells expressing TCRs with high affinity for tumour antigen, IL-7 may also recruit low affinity T cell clones

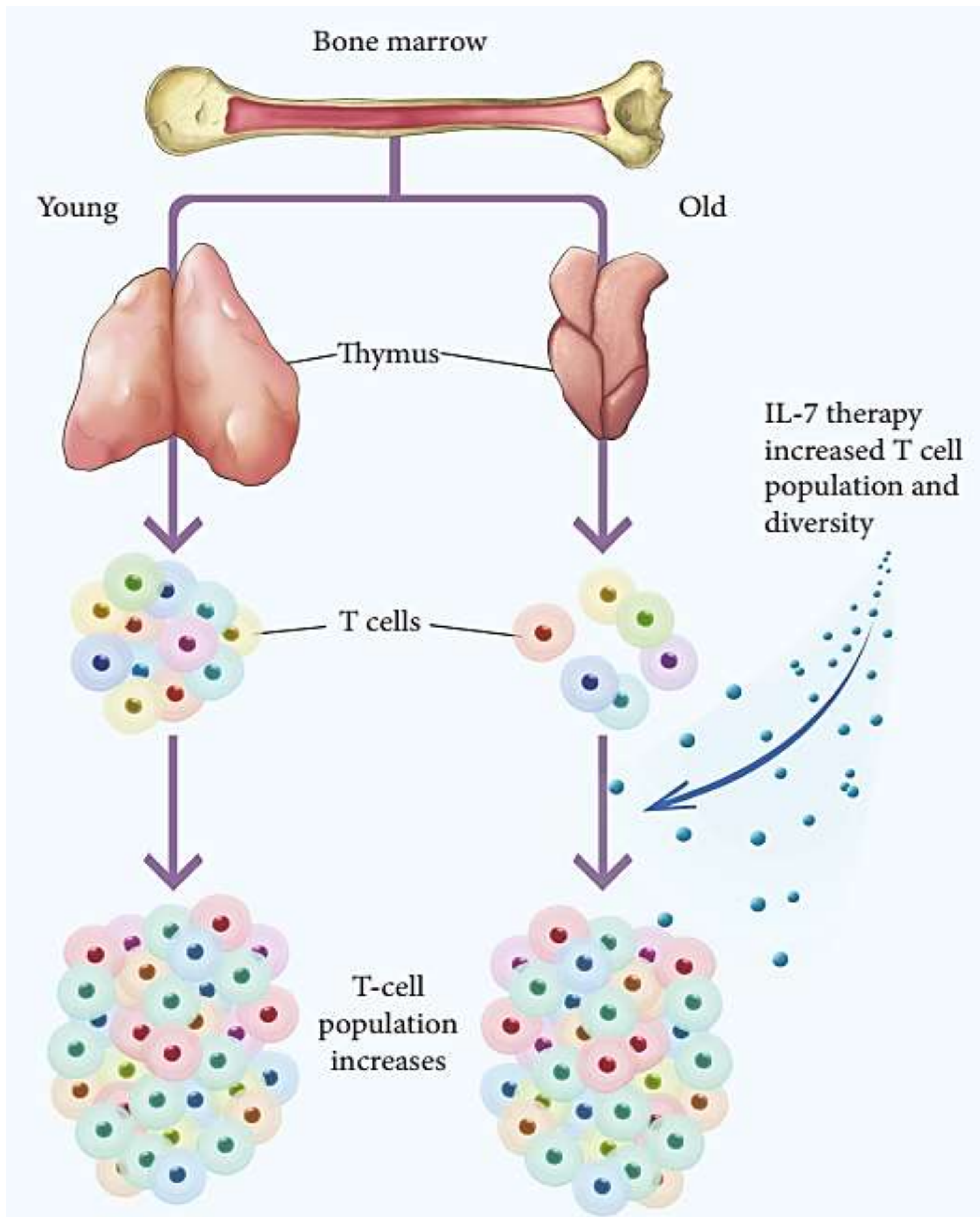


Figure 4: In some studies, **IL-7 Therapy** caused rejuvenation of the T cell population, broadened immune responses in cancer patients and enhanced T cell recovery in HIV-1 infected adults.

cells has allowed researchers and genetic professionals to harness the immune system with novel immunotherapy strategies, many of which have shown huge promise in breast cancer.

The raw genotype data from this study might be relevant to other researchers investigating the association of SNPs involved in the breast cancer related genes in South Asian population.

AIM & OBJECTIVES

AIM AND OBJECTIVES

AIM OF THE STUDY

To observe the prevalence of Interleukin 7 Receptor Alpha Thr244ile genetic polymorphism among breast cancer patients attending Regional Cancer Centre at Coimbatore Medical College & Hospital.

OBJECTIVE:

1. To identify the presence of Single Nucleotide Polymorphism(SNP) among known breast cancer patients attending surgical oncology department at our medical college hospital.
2. To assess whether this polymorphism can be used as a biomarker among breast Cancer patients in our hospital

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The distinguishing feature of class mammalia from other animals is highly evolved and modified skin appendages which are known as mammary glands or breasts. This provides a complete source of nourishment and most importantly the immunological protection for growing offspring[15].

Paired mammary glands in humans rest on the pectoralis muscle on the upper chest wall (Figure-5). The breasts consist of specialized epithelium and stroma. These may give rise to both benign and also malignant lesions due to various causes and risk factors (Figure-6).

CLINICAL PRESENTATIONS

The symptoms most commonly reported by women are pain, a palpable mass, “lumpiness” , or nipple discharge[22].

CLINICAL PROBLEMS FROM BREAST DISEASE

The associated pathological conditions with the breast can be divided into

- Infections and Inflammatory disorders.
- Benign breast disorders.
- Malignant breast disorders / Breast carcinoma

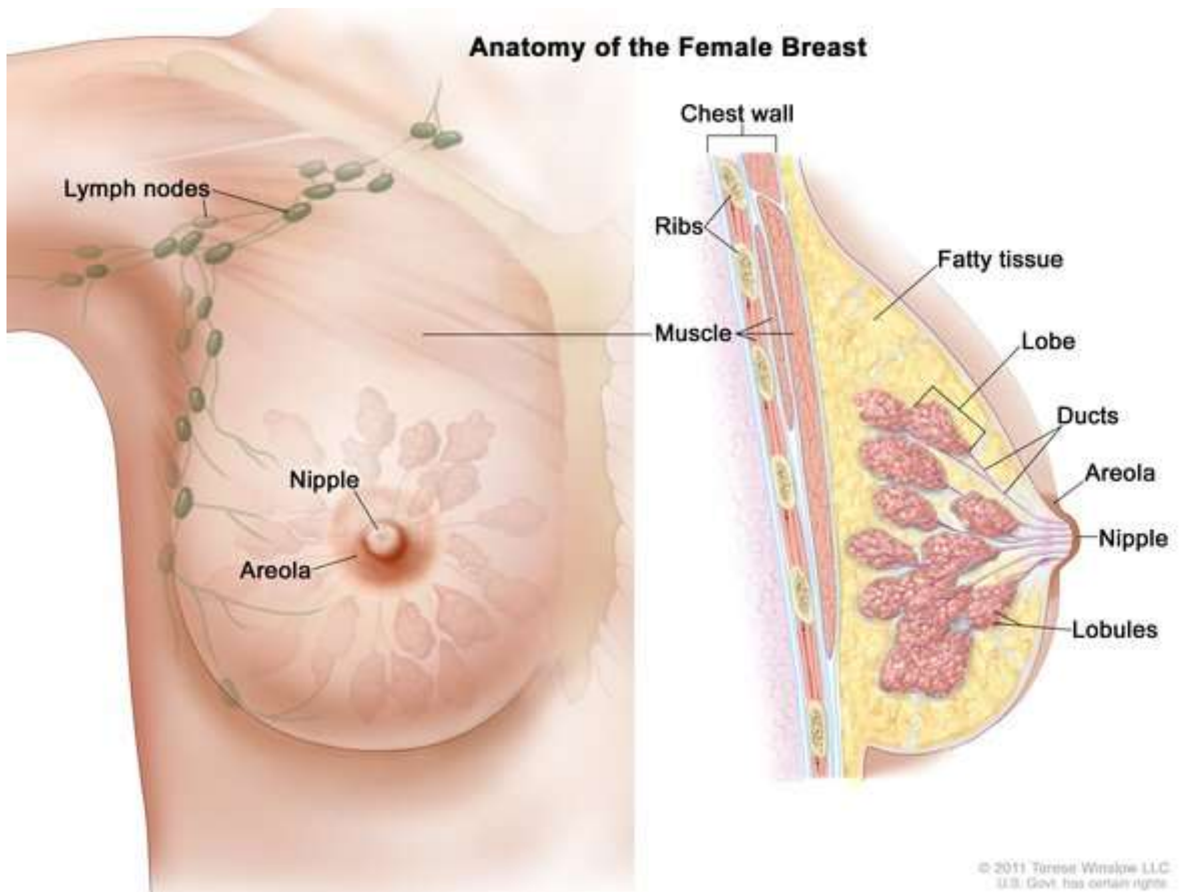


Figure 5: Anatomy of Female Breast

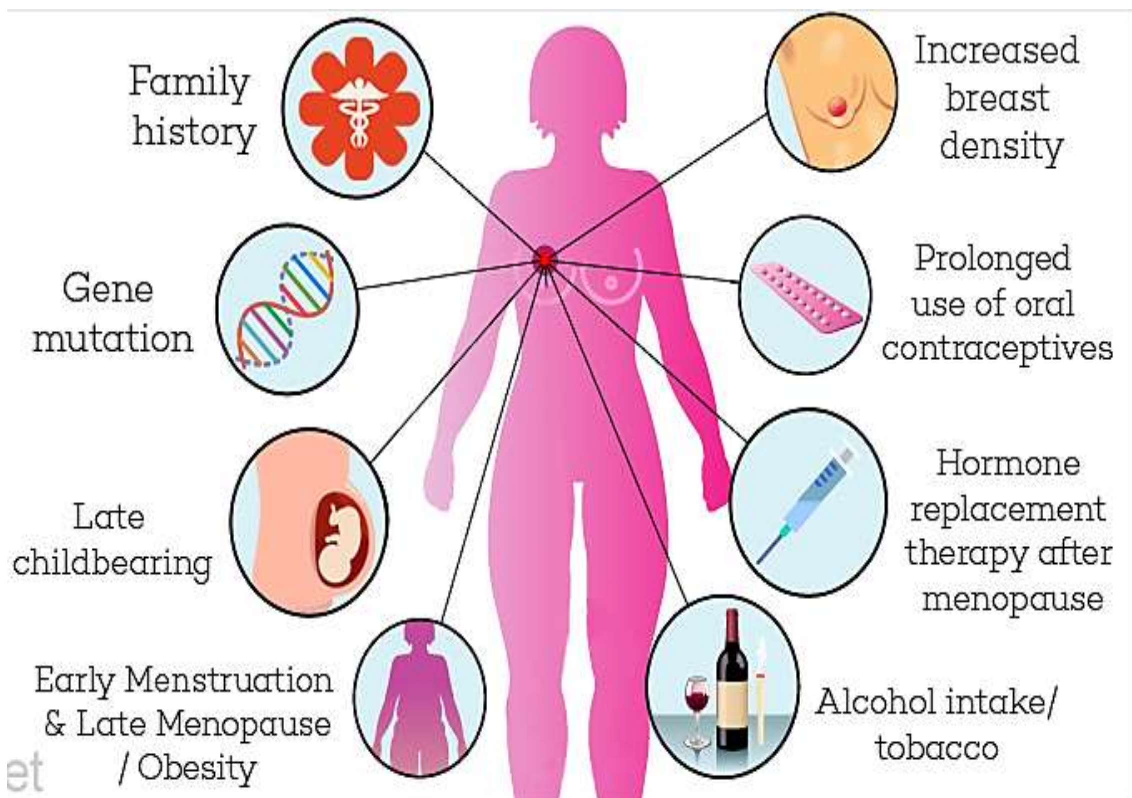


Figure 6: Causes and Risk Factors of Breast Cancer.

INFECTIONS AND INFLAMMATORY DISORDERS OF THE BREAST

Infections of the breast are rare except during the postpartum period. They are classified as

Intrinsic – occur as secondary to abnormalities in the breast.

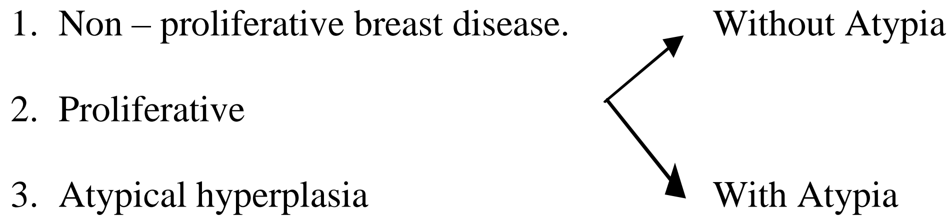
Extrinsic – occur as secondary to infections in the adjacent structures. E.g. Skin, Thoracic cavity.

Inflammatory conditions such as [15]

- Acute pyogenic infections
- Hidradenitis suppurativa
- Mondor's disease
- Mammary duct ectasia
- Fat necrosis

BENIGN BREAST DISEASE

Benign breast diseases and disorders encompass a wide range of clinico pathologicalentities[15].These lesions have been classified into three groups, according to the risk of developing breast cancer[22]:



Clinical significance of the benign breast disorder*:*

Non-proliferative changes actually do not increase the risk of cancer. Proliferative disease is usually associated with a mild increase in risk. The proliferative diseases with atypia confer a moderate increase in risk. Both breasts are equally at risk, although the breast once affected is at increased risk of developing recurrent cancers in the future[22, 24].

Multiple epidemiologic studies in the past have classified benign histological changes of the breast and have determined their association with later development of the invasivecancer[22, 25-27].

THE RISK OF DEVELOPING INVASIVE CARCINOMA IN EPITHELIAL BREAST LESIONS

Pathologic Lesion	Relative Risk
Non-Proliferative Breast Changes	1.0
Proliferative Disease Without Atypia	1.5 – 2.0
Proliferative Disease With Atypia	4.0 – 5.0
Carcinoma in situ	8.0 – 10.0

CARCINOMA OF THE BREAST:

The most common non-skin malignancy in women is Carcinoma of the breast. A woman in her lifetime has 1 in 8 chance of developing breast cancer. In spite of ready accessibility to self- examination and clinical diagnosis, the incidence rates and mortality rates are very high.

EPIDEMIOLOGY

Breast cancer accounts for about 31% of all cancers in women[23]. It is the leading cause of death due to cancer among women aged 20-59 years[15, 28].

Carcinoma of the breast is predominantly a disease of females (female to male ratio of approximately 200 to 1)[29]. One million new breast cancer cases are diagnosed every year world-wide[2]. This was the cause of cancer deaths in women in the developed countries like United States until 1986, until it was supplanted by carcinoma of the lung[29].

Among the Indian women population, carcinoma of the breast and cervix account for mostly 60% of total cases. Out of these cases, breast carcinoma accounts for 10.4% in total[47]. WHO conducted a study in Chennai which revealed that the highest incidence among all leading centers located in India is about 26/1, 00, 000 women[50]. The mean age of occurrence of breast cancer is 42 years. The mean age is found to be decreasing in the recent years[49]. The burden of Breast cancer varies by geography, life style, and racial or ethnic background.

In general, the incidence and mortality of breast cancer are comparatively lower among the Asian and African populations, which are relatively underdeveloped nations and they have not adopted the westernized dietary and reproductive patterns. In contrast, women from heavily industrialized or westernized countries like Europe and North America have a significantly higher breast cancer burden[15](Figure-7).

This clearly indicates that there are more important genetic, cultural, environmental and epigenetic factors involved in the development of breast cancer [28, 32, 33].

An estimated 1,78,480 women were found to be diagnosed with invasive breast cancer and 62,030 with carcinoma in situ, and it has been found that over40,000 women died of breast cancer in 2007[21,34]. The incidence of breast cancer is at constant rise for many years. This is mainly due to the detection of increased number of breast cancer cases by means of introduction of screening mammographic techniques in the early 1980s[21].

The foremost benefit of screening is the identification of predominantly small ER positive invasive carcinomas and also the carcinomas in situ. DCIS is detected mostly by mammography, thus providing a major reason for sharp increase in the diagnosis of DCIS since early 1980s.

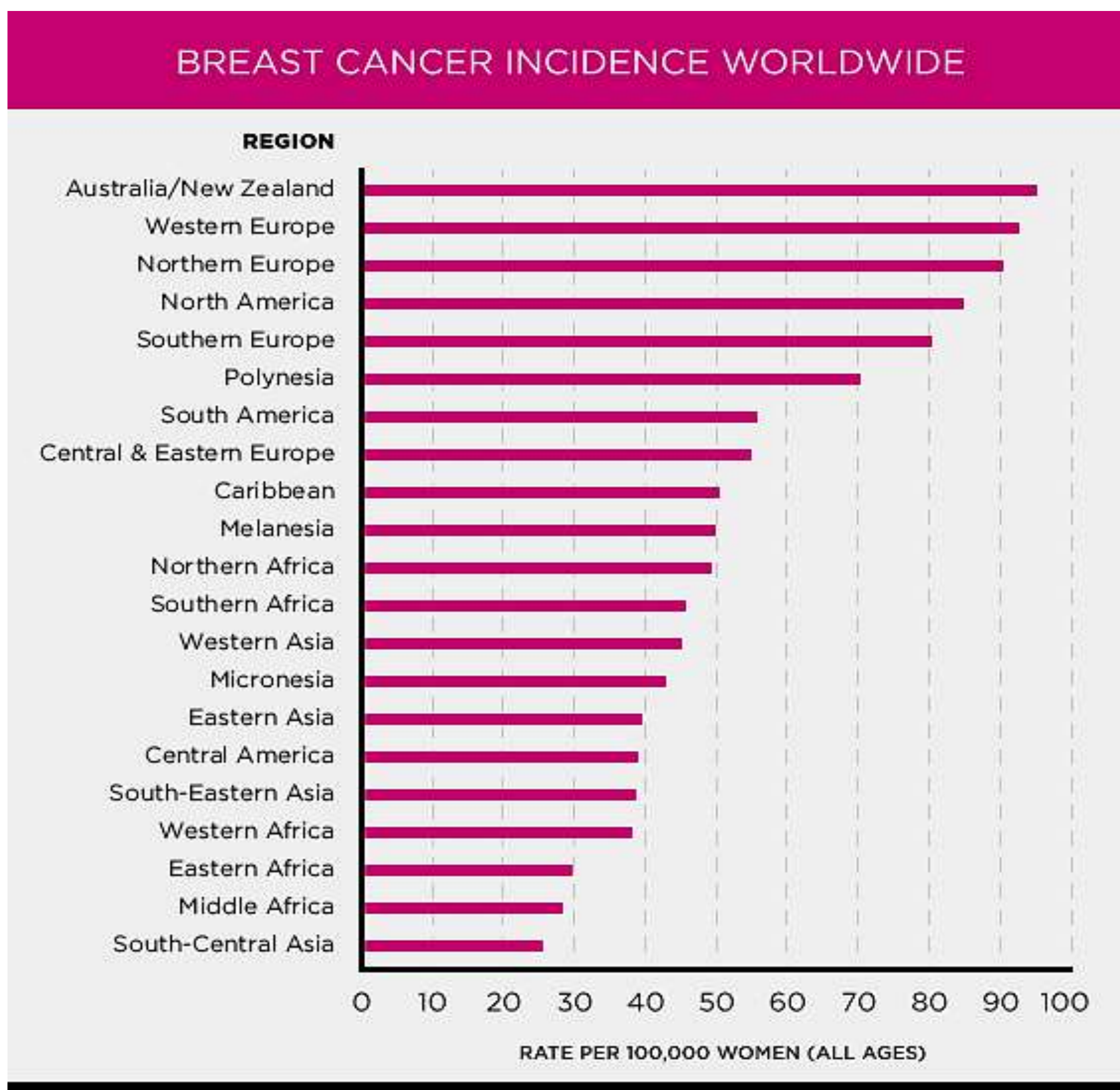


Figure 7: Incidence of Breast Cancer World Wide.

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There is a decreasing trend in the incidence of ER positive cases of invasive cancer from 2001 to 2004. The reasons for this trend could be probably multifactorial. This could be due to that in 2002 many women stopped using hormone replacement therapy among postmenopausal after the results of Women's Health Initiative trial, which clearly shows that this treatment had limited benefits[21, 35].

ETIOLOGY AND RISK FACTORS

Genomic alterations leading to the initiation, transformation and progression of normal cells to abnormal malignant neoplastic cells are the crucial causes of all cancers. These genetic alterations which are mostly unknown, may be inherited or acquired[28].

The risk factors identification is most important because they provide insight into the root causes of breast cancer and paves way to reduce its incidence[28]. The various genetic risk factors are shown in Figures - 8 &9. Factors known to be associated have a significantly elevated risk and can also be classed as hormonal and non-hormonal, which are listed in table[14, 28] as shown below

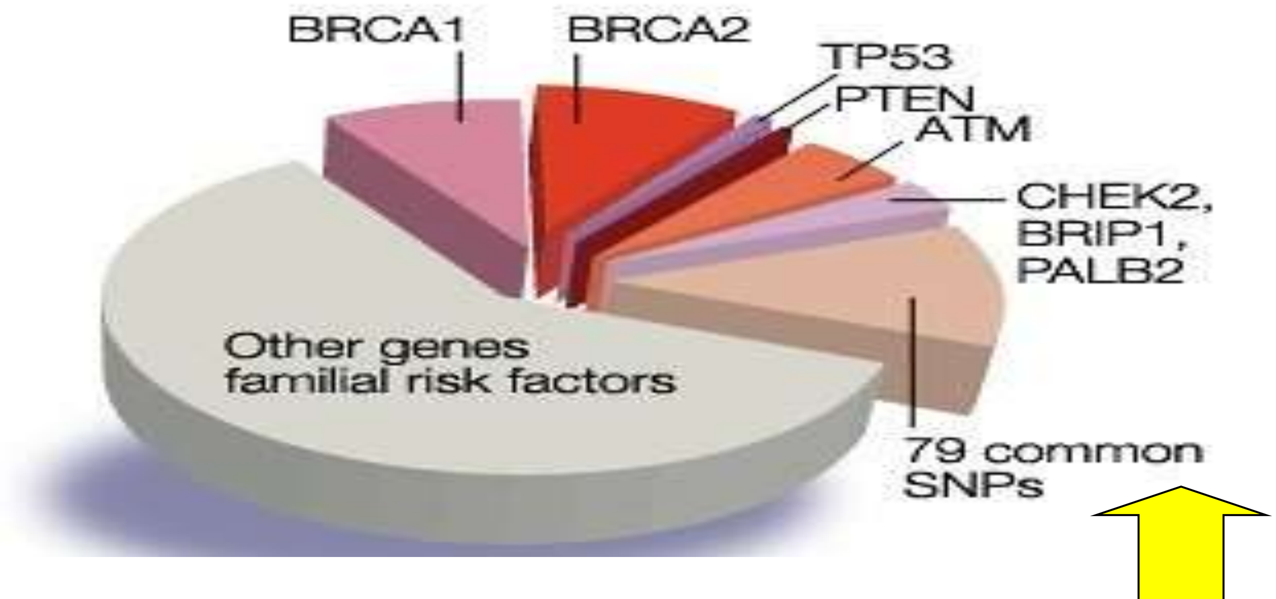


Figure 8: Contribution of known genes to familial aggregation of Breast cancer.

	BRCA1	BRCA2
Breast Cancer, in unaffected women (up to age 80)	60-90%	45-85%
Lifetime risk of a new cancer in the other breast in woman who have already had a breast cancer in one breast	50% 5 year risk of new breast cancer 10%	50% 5 year risk of new breast cancer 5-10%

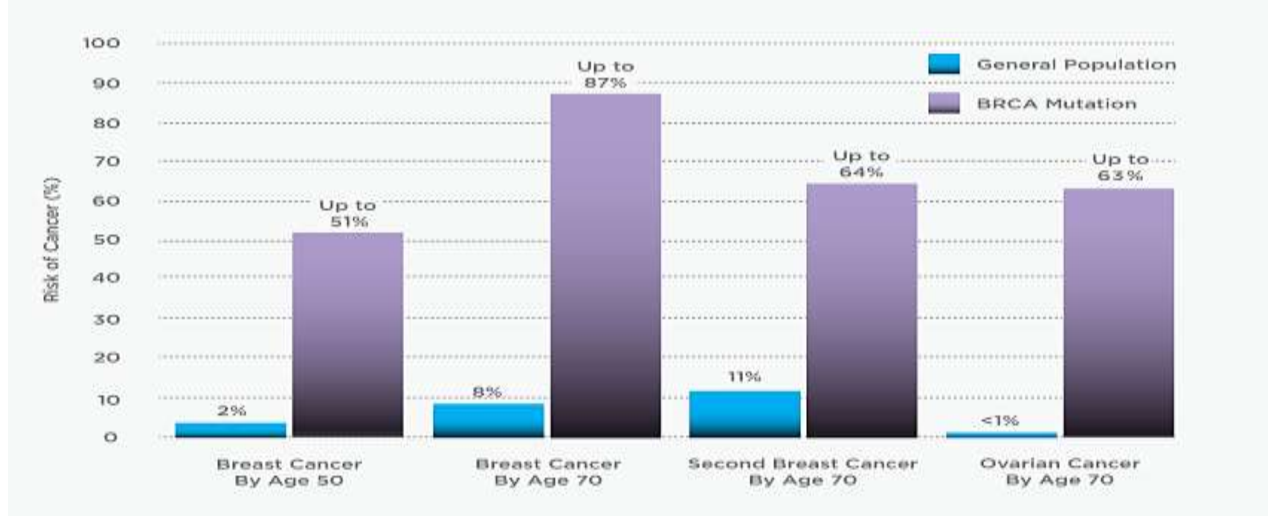


Figure 9: Risk of cancer percentage associated with BRCA mutation.

RISK FACTORS FOR DEVELOPING BREAST CANCER

FACTOR	RELATIVE RISK
HORMONAL FACTORS:	
Excess estrogen exposure	
Early menarche (before 14 years of age)	2.0
Late childbearing(nulliparous or after 30years of age)	4.0 – 6.0
Late menopause (after 55 years of age)	1.3
Postmenopausal estrogen therapy	1.9
Oral contraceptive use	1.5
Benign Breast Disease	
Hyperplasia	1.5 – 2.0
Atypical hyperplasia	4.0 – 5.0
NON – HORMONAL FACTORS	
Family history	
Mother affected before 60 years of age	2.0
Two first-degree relatives affected	4.0 – 6.0
RADIATION EXPOSURE	
Atomic bomb	3.0
Repeated fluoroscopy	1.5 – 2.0
Alcohol abuse	1.4 – 2.0
Obesity	1.2

FEMALE SEX AND AGE

1% of all breast cancers occur in men, so female gender is an important risk factor[22].The incidence of breast cancer varies significantly withage[28].

Similar to all carcinomas, increasing age is another important risk factor in breast cancer. Till 50 years of age, the rate of incidence is sharp; then it slows down, though the incidence continues to increase with increasing age[22].

FAMILY HISTORY AND GENETIC FACTORS

4 – 6% of breast cancers are approximately associated with a very strong family history[22].Women who had mothers with breast cancer before 60 years of age have double the risk of developing the disease than those women whose mothers did not have breast cancer. The relative risk increases up to 4 to 6 times if two of the first degree relatives (such as mother and sister) have breastcancer[28].

However family history is a heterogeneous high risk factor with different implications, it usually depends on the number of relatives with breast cancer, relationship, age at diagnosis and number of unaffected relatives[28].

EXCESS ESTROGEN EXPOSURE

Exposure to high levels of estrogen is associated with an increased probability for developing the breast cancer, while reducing exposure is found to be protective[14].

Early menarche and late menopause

Women with early menarche, or late menopause, are more prone for developing breast cancer[15, 22]. The factors which increase the number of cycles, such as early menarche, nulliparity, and late menopause are mostly associated with increased risk[14]. Therefore women with more than 40 years of active menstruation have double the breast cancer risk compared to counterparts with fewer than 30 years of menstrualactivity[22].

Age at first full-term pregnancy

The risk of developing breast cancer in women who have their first child birth after the age of 30 is two times higher than that of women who have had their first child birth before the age of 20. Therefore, young age at the time of first delivery protects against breast cancer[15]. Nulliparous women usually have an increased risk of developing breast cancer[22].

Breast feeding

The longer duration of breast feeding in women, the greater the risk reduction. Lactation suppresses ovulation and protects the women

from hormonal action and may trigger terminal differentiation of luminal cells[21].

The lower incidence of breast cancer in developing countries can be largely explained by the evidence of more frequent and longer duration of nursing in infants[21].

Oral contraceptives / Hormone Replacement Therapy (HRT)

There is a considerably increased risk for the current and recent users of oral contraceptive pills but there is no long term increase associated with the above. Combined estrogen and progesterone hormone replacement therapy greatly increases the relative risk of developing breast cancer for users by approximately twofold rise and is greater, the longer the treatment duration. The risk decreases with cessation of oral contraceptives[8].

ATYPICAL HYPERPLASIA:

A history of prior breast biopsies with atypical hyperplasia increases the risk percentage of developing invasive carcinoma. There is a slighter increase in risk associated with proliferative breast changes without atypia[35].

OBESITY:

For women above average body weight and those below 50 years of age, there is small or no increased risk of developing breast cancer. Women with age 60 or above and whose weight is increased, have a

high risk of cancer[22]. This is mainly due to the major source of estrogen in postmenopausal women is by the conversion of androstenedione to estrone by the adipose tissue, so obesity is usually associated with a long term increase in the risk of high estrogen exposure[14].

DIET:

Obviously diet is a determinant of weight. So high fat diet may be a high risk factor, but the scientific evidence is not clear.

RADIATION EXPOSURE:

Radiation exposure to the chest wall due to cancer therapy, exposure to atomic bomb or nuclear accident spill, results in a higher risk rate of developing breast cancer. The risk is greatest when exposure is at young age and with high doses of radiation[14].

ALCOHOL ABUSE:

The risk of developing the breast cancer increases with the increased amount of alcohol a woman consumes. Alcohol consumption is therefore known to increase the serum levels of estradiol[14].

CLASSIFICATION

Breast cancers originate from the epithelial cells which line the terminal duct lobular unit[15.] More than 95 % of breast malignancies are adenocarcinomas[21].

Breast cancers are divided into two broad categories depending on whether they invade through the basement membrane or not [14, 36, 37] as shown in Figure – 10.

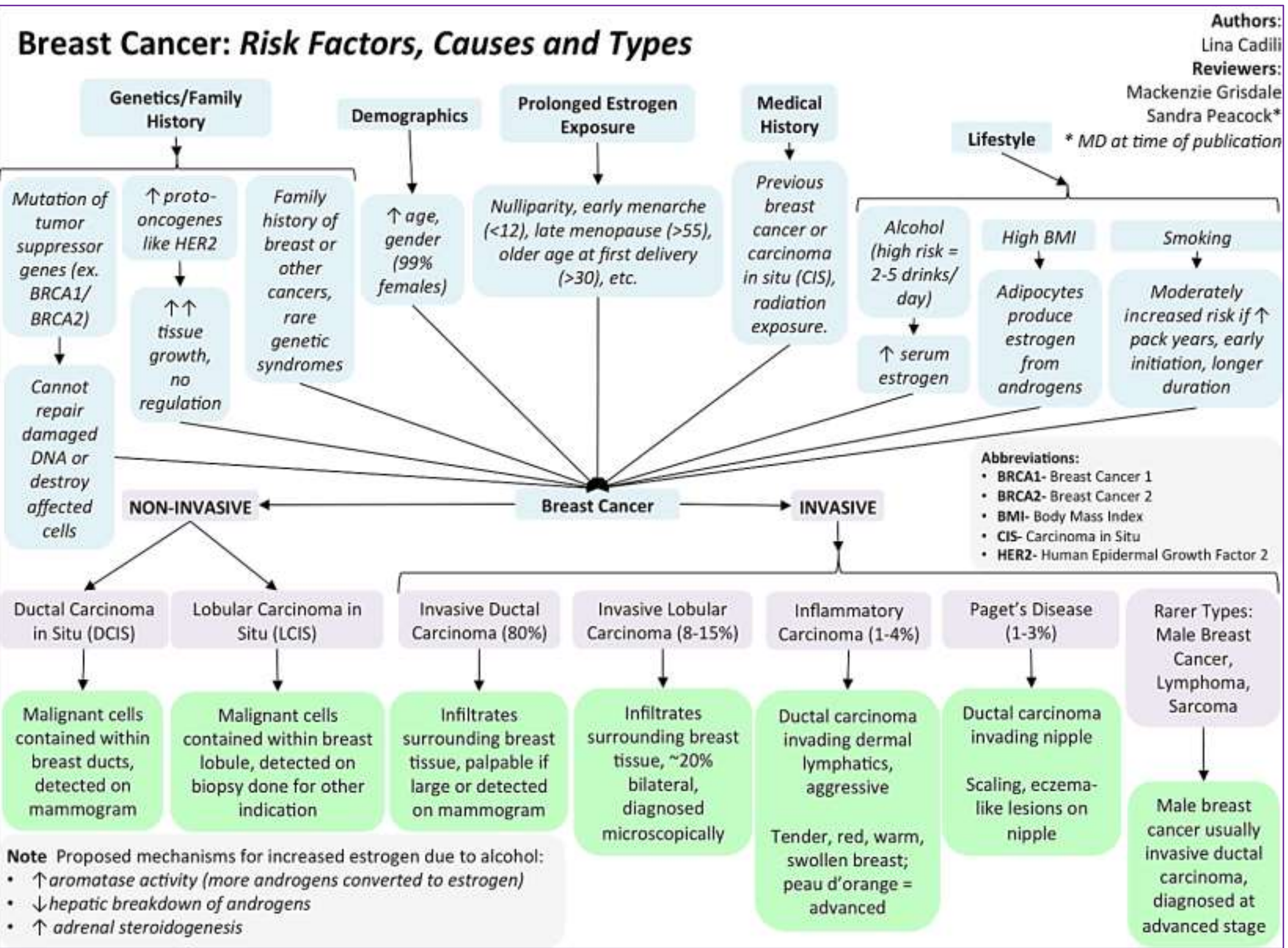


Figure 10: Breast Cancer Risk, Causes and Types

(1) Non-invasive carcinoma or carcinoma insitu

(2) Invasive carcinoma

Gallagher and Martin in the late 1960s published their study of whole breast sections and elaborately described a stepwise progression of breast cancer from benign breast tissue to carcinoma in situ and subsequently to invasive cancer. They coined the term “minimal breast cancer” (LCIS, DCIS and invasive cancers smaller than 0.5 cm in size) and laid the clinical importance of earlydetection[38].

NON-INVASIVE CARCINOMA OR CARCINOMA IN SITU

Carcinoma in situ refers to neoplastic proliferation which is limited to the ducts and lobules by the basement membrane[21]. Carcinoma in situ was originally classified based on the resemblance of the involved spaces to normal ducts or lobules as

a) Ductal carcinoma insitu

b) Lobular carcinoma insitu

It is recognized by the diverse patterns of the growth in situ which are usually not related to the site or cell of origin but reflects the differences in cell tumour biology, such as whether the tumour cells express the molecules like cell adhesion protein E- cadherin or not.

Currently "Lobular" is used to carcinoma of specific type, and "ductal" refers to more generally for adenocarcinomas that have no other designation[21].

Ductal Carcinoma in situ / Intraductal Carcinoma

DCIS is predominant carcinoma seen in the female breast, it accounts for upto 5% of male breast cancers[14]. With the advent of screening techniques like mammography, the diagnosis of DCIS rapidly increases from less than 5% of all carcinomas upto 15 to 30 % of carcinomas in the screened populations, most of them are detected as a result of calcifications[21, 39]. DCIS represents approximately more than 30% of breast carcinomas that are diagnosed by screening mammography[40].

The term “Intraductal carcinoma” frequently applies to DCIS, which carries an increased risk for progression to an invasive cancer[28]. DCIS has been divided into five architectural histological subtypes[35]:

- Comedo carcinoma
- Solid carcinoma
- Cribriform noncomedo carcinoma
- Papillary carcinoma
- Micro papillary carcinoma

DCIS is frequently based depending on nuclear grade and the presence of necrosis[14]. Though there is no universal agreement on

classification of DCIS, most system endorse the use of cytological grade and presence or absence of necrosis[41].

The risk for invasive breast cancer is increased nearly five times in women with DCIS[28]. The invasive cancers are seen in ipsilateral breast, usually in the same quadrants where the DCIS was originally detected. This suggests that DCIS is an anatomic precursor of invasive ductal carcinoma[14].

LOBULAR CARCINOMA IN SITU

LCIS originates from terminal duct lobular units and originates only in the female breast[14].

This is a non-palpable microscopic lesion which is always encountered as an incidental finding in the breast of the premenopausal women[28, 43]. It is usually not associated with calcifications or stromal reactions which produces mammographic dense lesion as a result of its incidence, (1 to6% of all carcinomas) has not been affected by the introduction of mammographic screening[14].

LCIS is more commonly seen in young women, with 80 to 90% of the cases occurring before menopause[14]. The total frequency of LCIS in the general population cannot be determined reliably because it usually presents as an incidental finding[14].

70% of LCIS cases are multicentric and bilateral breast involvement is present almost in about 30 to 40%[44]. Invasive breast cancer develops in about 25 to 30 % of women with LCIS.

Invasive cancer may develop in either of the breasts, regardless of which breast harbored the initial focus of LCIS, and is detected along with LCIS in 5% of cases. In women with a past history of LCIS, upto 65% of following invasive cancers are mostly ductal and not lobular in origin. For all the above reasons, LCIS is regarded as marker of greater risk for invasive breast cancer than its anatomic precursor[14].

INVASIVE OR INFILTRATING CARCINOMA

Invasive carcinoma – the tumour cells get infiltrated through ductal or acinar basement membranes to reach connective tissue.

In the absence of screening techniques like mammography, invasive carcinoma most always presents as palpable mass. Palpable tumours are mainly associated with axillary lymph node metastases in over 50% of patients. Cancers which are detected by mammography average half the size of palpable cancers. Less than 20% will have node metastasis[21].

Invasive breast cancers are described mainly as lobular or ductal in origin[14, 38, 45, 46]. Earlier classifications have used the term "lobular" and have described invasive cancers which were associated with LCIS, although all other invasive cancers were referred to be as "ductal".

Currently histological classification recognizes special types of breast cancers (10% of total cases), which are precisely defined by specific histologic features and types. For classifying as special type of

cancer, a minimum 90% of the cancer must contain the defining histologic feature. 80 % of the invasive breast cancers are described as invasive ductal carcinomas of no special types (NST). These cancer generally have a worse prognosis than that of special type cancers[14].

Foot and Stewart originally proposed the following classifications for invasive breast cancers[37].

- Paget's disease of the nipple
- Invasive ductal carcinoma
- Adenocarcinoma with productive fibrosis (schirrhous, simplex, NST)
- Medullary carcinoma,4%
- Mucinous or colloid carcinoma,2%
- Papillary carcinoma,2%
- Tubular carcinoma,2%
- Invasive lobular carcinoma,10%
- Rare cancers (Adenoid cystic, Squamous cell, Apocrine)

DIAGNOSTIC MODALITIES OF BREAST CANCER

Mostly in 33% of breast cancer case, the women usually discover a lump in her breast [14]. Several methods are used to investigate various breast lesions. They include the following

1) Imaging techniques
2) Fine needle aspiration cytology
3) Biopsy

IMAGING TECHNIQUES

a) Mammography

Mammography is the radiography of the breast tissue which is used mainly to help in the diagnosis of palpable and non - palpable lesion of the breast[22].

There is no increased risk of breast cancer associated with the radiation dose that is delivered with screening mammography technique.

Screening mammography is used to detect unsuspected breast cancers in asymptomatic women. Diagnostic mammography technique is used to evaluate women with abnormal finding such as a breast mass or nipple discharge.

Mammographic techniques are also used to guide interventional procedures which include needle localization and needle biopsies. Specific mammographic features which suggests a diagnosis of breast cancers includes;

i) A solid mass with or without stellate feature

ii) Asymmetric thickening of breast tissue and

iii) Clustered micro calcification.

Mammography is more accurate than that of clinical examination for the detection of early breast cancer lesions thus providing a true positive rate of ninety percent[14] (Figure – 11).

b) Ductography

The primary indication for the ductographic method is nipple discharge particularly when the fluid contains blood. Radio opaque contrast media is injected into the major ducts and the routine mammography is performed.

c) Ultrasonography

Ultrasonography is used second only to mammography for breast imaging. Ultrasonography is a significant method of resolving equivocal mammographic findings, defining the cystic masses and demonstrating the echogenic qualities of these specific solid abnormalities[14]. Ultrasound imaging is a major imaging technique among younger patients for defining the lesion edges e.g. Cysts[22]. Ultrasonography is used as a guide for fine needle aspiration biopsies, core needle biopsies and needle localization of breast lesion. The findings are highly reproducible and has a high rate of patient acceptance, but ultrasound does not reliably detect lesions that are $\leq$ 1 cm in diameter.

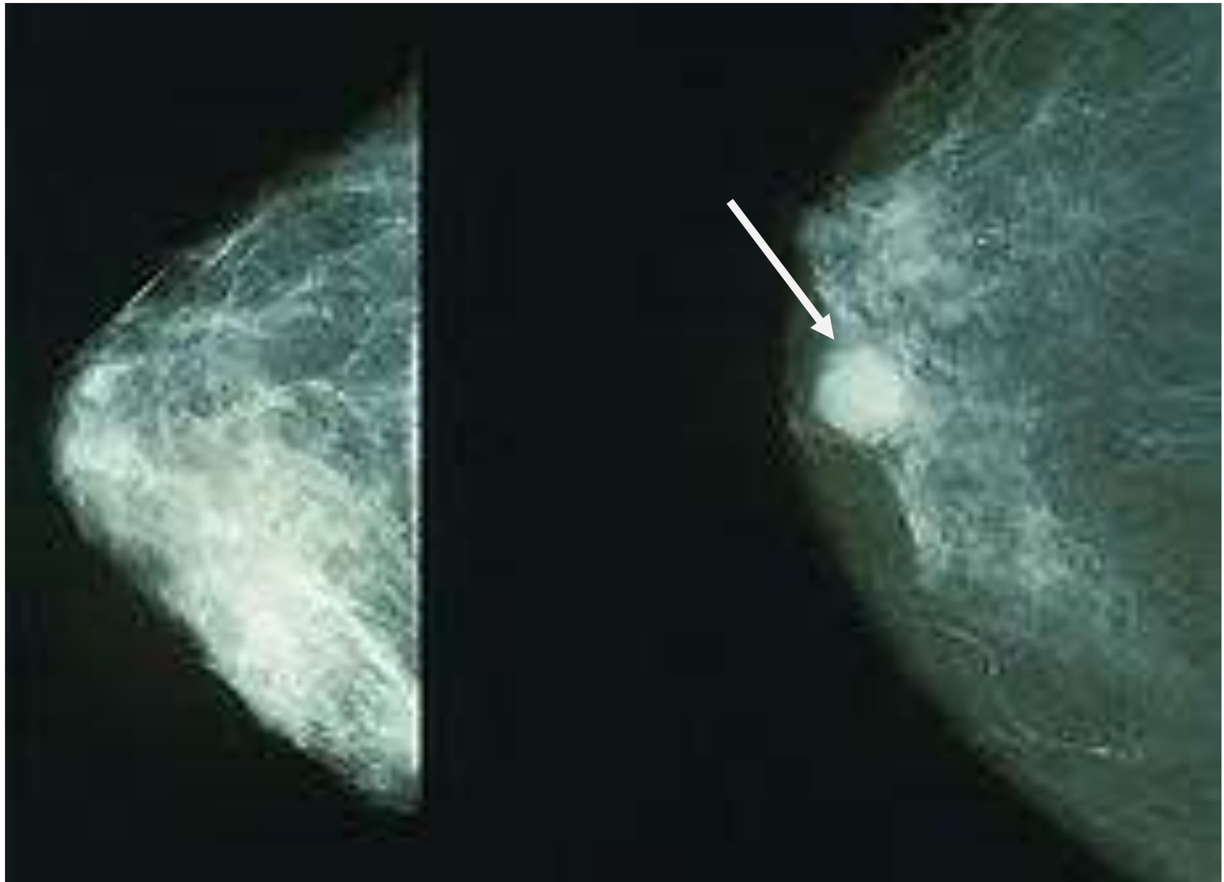


Figure 11: Mammograms which shows a Normal breast (Left) and a Cancerous breast (Right).

d) Magnetic Resonance Imaging (MRI)

There is a current trend in the use of MRI in screening for

- Women with High risk
- Newly diagnosed breast cancer.

In women who have a positive family history of breast cancer or those who carry genetic mutations screening at an early age, but evaluation by mammography is limited because of dense breast tissue in younger women. In newly diagnosed breast cancer, MRI study of the contralateral breast with a known breast cancer has mostly shown a contralateral breast cancer in 5.7 percent of these women[14].

MRI much more effective than routine mammographic screening of women with less than 50 years are at very high risk of breast cancer either because that they carry a BRCA1 or BRCA2 mutation or probably because of their family history[34].

(ii) Fine needle aspiration cytology

Fine needle aspiration cytology can differentiate between solid and cystic lesions of the breast[2]. The combination of the diagnostic mammography, ultrasound technique or stereotactic localization and fine needle aspiration biopsy achieves just about 100% accuracy in the diagnosis of breastcancer[1].

Image guided breast biopsies are frequently required to diagnose non- palpable lesions in clinical settings.

FNAC of a palpable breast masses can easily proceed in an outpatient settings[14].

(iii) Breast biopsy

- Core biopsy
- Open biopsy

Core biopsy

Several cores of tissues are removed from a mass or an area of micro- calcification with help of a cutting needle. Core biopsies can be performed using palpation to guide biopsy but is the most successful when combined with image guidance [15].

Core-needle biopsy is preferred over than open biopsy for non-palpable breast cancer lesions because a surgical procedure can be planned based on the outcome of core biopsy.

The advantages of core-needle biopsies include a very low complication rate, less scarring, and a lower cost[14].

Open biopsy

An open biopsy can be performed in patients who have been suitably investigated by imaging, FNAC techniques, and/or core biopsy.

Breast biopsy is a morbid procedure and one-fifth of patients who have a biopsy performed develop a further lump under the scar, or pain specifically related to the operated site[15].

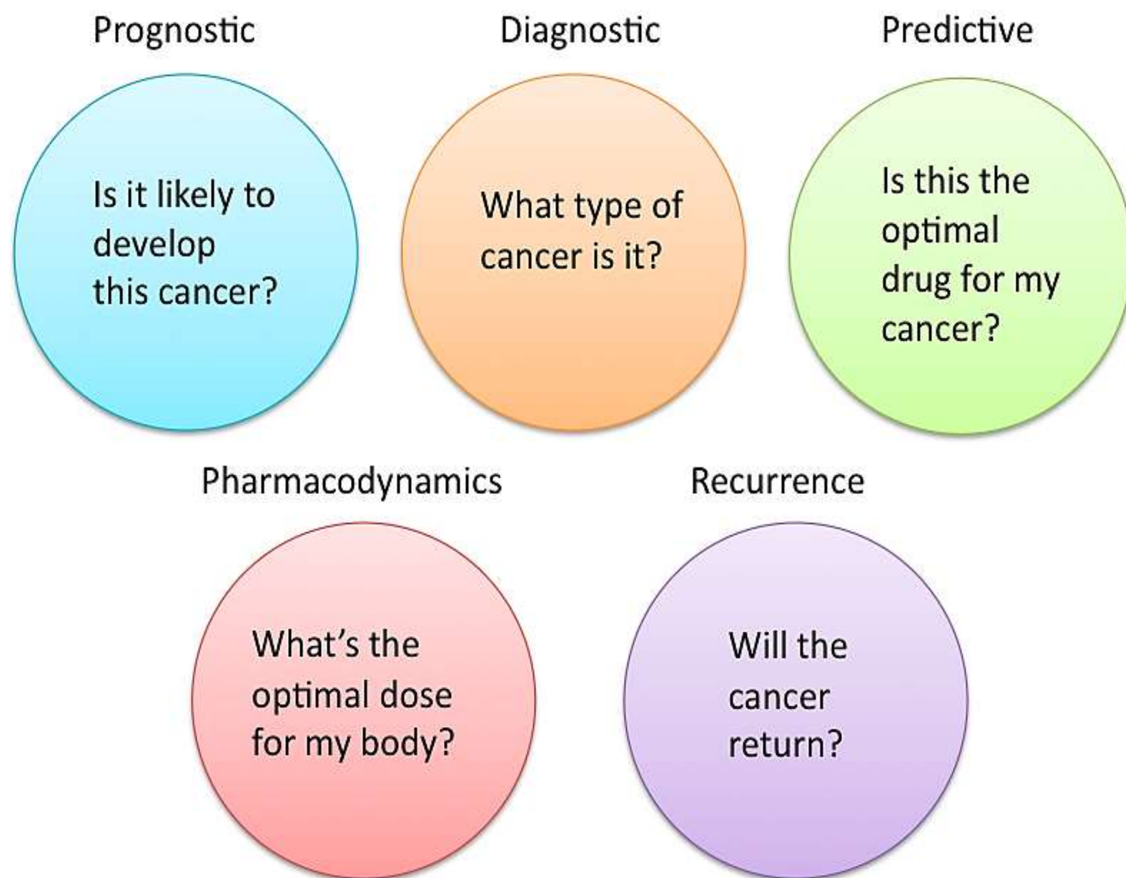


Figure 12: Questions which can be answered by Biomarkers

BIOMARKERS

There are several types of Breast cancer biomarkers. High Risk biomarkers are those associated with increased cancer risk[133-135]. Prognostic biomarker gives information regarding cancer outcomes irrespective of the therapy, whereas predictive biomarker gives information regarding response to the therapy (Figure - 12). Some prognostic, predictive biomarkers and biologic targets for breast cancer include[14];

a) Indices of proliferation – named as proliferating cell nuclear antigen (PCNA) and Ki-67.
b) Indices of apoptosis and apoptosis modulators – named as bcl-2 and the bax:bcl-2 ratio.
c) Indices of angiogenesis – such as vascular endothelial growth factor (VEGF) and the angiogenesis index.
d) Growth factors and growth factor receptors – named as platelet-derived growth factor, human epidermal growth factor receptor 2 (Her2/neu), transforming growth factor, epidermal growth factor (EGFR), and the insulin-like growth factor family.
e) Steroid hormone receptor pathway.
f) The Cyclins, and cyclin-dependent kinases.
g) The Proteasome.
h) The Cox-2enzyme.

i) The Peroxisome Proliferator-Activated Receptors (PPARs).

j) Tumour Suppressor Genes – such as p53.

k) The mammalian target of rapamycin (mTOR) signaling pathway. The breast cancer markers which are most important in determining the therapy are estrogen and progesterone receptors and Her- 2/neu.

GENE	ESTIMATED CANCER LIFETIME RISK %
BRCA1	55-65%
BRCA2	45-47%
TP53	49-60%
PTEN	25-50%
PALB2	33-58%
STK11	30-50%
CDH1	39-60%
ATM	15-52%
CHEK2	20-42%

Steroid Hormone Receptor Pathway

Steroid hormones play a very important role in the development and progression of breast cancers. Estrogens, Estrogen metabolites, and progesterone all have shown to have an effect.

Tumors which test positive for estrogen or progesterone receptors have very high response rate to endocrine therapy than those tumors that do not express the receptors. Tumors positive for both receptors have a positive response rate of $>50\%$, tumors negative for both receptors have a positive response rate of $<10\%$, and tumors positive for one receptor but not the other have a 33 percent intermediate response rate.

ER-a can be demonstrated using immunohistochemistry in the nuclei of both the ductal and lobular epithelial cells with a higher proportion in lobules most often ER-a positive cells in the lobules admixed with and surrounded by ER-a negative cells[41].

The proportion of proliferating ER-a positive cells increase with age[137]. ER-a expression varies with phase of the menstrual cycle being greater in the follicular than in the luteal phase in premenopausal women[38.] Expression of ER-b have been observed not only in the epithelial cells of duct and lobule, but also seen in myoepithelial, endothelial and stromal cells[41].

STAGING OF BREAST CANCER

The staging systems which we currently used for breast cancer are based on clinical size and to the extent of invasion of primary tumor (T), clinical absence or presence of palpable axillary lymph nodes and evidence of their local invasion (N), together with the clinical and imaging evidence of the distant metastasis (M). This is then translated into TNM classification which has thus been subdivided into Stage T_{IS} called carcinoma in situ (lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS) and four broad categories by Union International Centre for Cancer (UICC), which are as follows.

STAGE	TUMOR GRADE	CLINICAL EXTENT	NODE GRADE	CLINICAL EXTENT	DISTANT METASTASIS
T_{IS}	T_{IS}	NO PALPABLE TUMOR	N_0	NO NODAL METASTASIS	M_0- NO DISTANT METASTASIS
I	T_1	< 2 cm	N_0	NO NODAL METASTASIS	M_0- NO DISTANT METASTASIS
II	T_2	2 – 5 cm	N_1	MOBILE AXILLARY NODES	M_0- NO DISTANT METASTASIS
III a	T_3	>5 cm	N_2	FIXED AXILLARY NODES	M_0- NO DISTANT METASTASIS
III b	T_4	ANY SIZE INVADING SKIN OR CHEST WALL	N_3	SUPRACLAVICULAR IPSILATERAL NODES	M_0- NO DISTANT METASTASIS
IV	T4	ANY SIZE INVADING SKIN OR CHEST WALL	N_3	SUPRACLAVICULAR IPSILATERAL NODES	M_1- DISTANT METASTASIS

MICROSCOPIC GRADING OF BREAST CANCER

Histological grading is based on the assessment of three morphological features namely tubule formation, nuclear pleomorphism and mitotic counts.

Nottingham Modification of the Blood – Richardson System

Tubule Formation:

1 point -Tubule formation in more than 75% of the tumor.

2 points -Tubule formation in 10% - 75% of the tumor.

3 points –Tubule formation in less than 10% of the tumor.

(Note: For scoring of tubule formations, the overall appearance of tumor has to be taken into consideration.)

Nuclear Pleomorphism:

1. 1 point – Nuclei with minimal variation in size and shape.
2. 2 points – Nuclei with moderate variation in size and shape.
3. 3 points – Nuclei with marked variation in size and shape.

(Note: The tumor areas having cells with greatest atypia should be evaluated.)

Mitotic Count:

	40X	**25X**
1 point	0 – 5	0 -9
2 points	6 – 10	10 -19
3 points	>11	>20

(Note: Mitotic figures are to be counted only at periphery of the tumor. Counting should begin in the most mitotically active area; 10 HPF are to be counted in the same area. The fields should be filled with as much tumor as possible; cells in poorly conserved areas are to be avoided. Cells in prophase should be ignored.)

These three scores are added together to obtain the grade. Thus

a score of 3 – 5 = grade1

a score of 6 – 7 = grade2

a score of 8 – 9 = grade3

The higher the grade, the worse is the clinical behavior of the lesion.

Single Nucleotide Polymorphism and Cancer Susceptibility

The most common type of genetic variation in human genome is single nucleotide polymorphism[7]. SNPs regulate DNA mismatch repair cell cycle regulation, immunity and metabolism which are associated with genetics of cancer susceptibility[7]. The mechanism of cancer susceptibility due to SNPs is critical in understanding the cellular and molecular pathogenesis of various cancers. Recently SNPs are potential biomarkers in therapeutics and diagnostics of many cancer cell types. The location of SNP may be at different regions of gene such as exons, introns, promoters as well as 5'- and 3' UTRs. The effects of cancer susceptibility vary depending on the location of the SNP[7]. Alteration caused due to the SNP lead to altered gene expression[7].

There are various genetic and epigenetic mechanisms which underlie the cancer susceptibility and the utility of these SNPs as potential biomarkers[7].

Cancer susceptibility in association with Promoter region SNPS

Transcription Factors (TF) that regulate gene transcription are located abundantly in the promoter region. SNPs located in the promoter region affect the gene expression by altering transcription binding factor, promoter activity, histone modification and DNA methylation[7] (Figure-13). Polymorphisms located in non-coding regulatory sequence alter the histone modification by acetylation, phosphorylation,

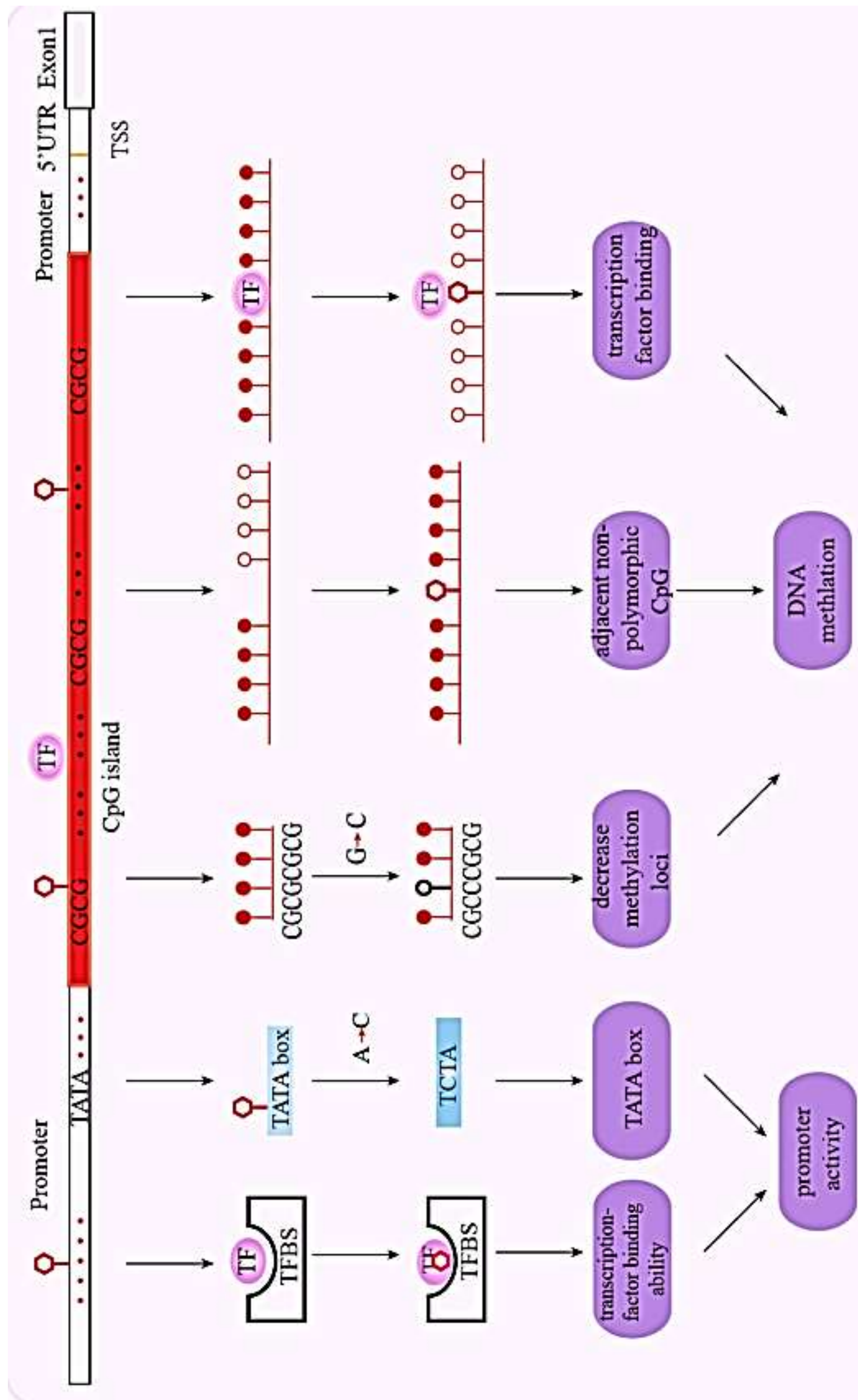


Fig: 13 Schematic representation of mechanism associated with promoter SNPs and cancer susceptibility. SNPs in transcription factor binding sites affect transcription factor binding to the gene promoter. SNPs in the TATA box affect promoter activity with A to C substitution decreasing the number of the TATA boxes. SNPs in the CpG islands decrease methylation,affecting adjacent non- polymorphic CpG and transcription factor binding. The red triangle represent SNP; red arrows show substitution of SNPs; red hollow circle represents unmethylated loci; red solid circle represents methylated loci

methylation, ubiquitination and glycosylation[7]. These histone modifications affect the translational rates to various extents. Regional histone modifications are also brought by promoter SNPs located in the transcription factor binding sites. SNPs inhibit the transcription factors by binding at the CDH1 promoter and vastly promote tumorigenesis of breast, prostrate, colon and pancreatic cancers thereby affecting gene transcription[7].

Promoter region SNPs alter Epigenetic mechanism

The SNPs located in the promoter region alter the DNA methylation by activating the methylation related enzymes. SNPs located in the promoter region of DNA methyl transferase (DNMT), methionine synthase (MS) and methylene tetra hydrofolate reductase (MTHFR) promote abnormal DNA methylation and inhibit DNA synthesis. Studies by Ogino et al have showed that the common MGMT promoter SNP affects the expression of the enzyme in colorectal tumours[7].

The binding between the transcription factors like SPI, c-Myb, E2F1, Ets and GATA-1with the corresponding binding sites is regulated based on the SNPs located in the promoter region. SNPs in the MICA promoter region (rs2596538) are associated with increase in the risk of Hepatitis-C associated liver cancer. SNPs in promoter region of COX -2

generates c-Myc binding site which results in increased COX -2 expression leading to increased risk of oesophageal cancer.

Genome wide association studies (GWAS) show that 38 SNPs located in 12 CpG loci are associated with methylation and expression of 10 genes. Studies done by Zhang et al showed that polymorphism located in CHEK 2 (rs2236141) gene lowers the risk of lung cancer due to transcriptional repression by eliminating methylation locus.

Non imprinted autosomal genes in normal human tissue is affected by CpGSNPs by allele specific gene expression (ASE), allele specific DNA Methylation (ASM) and allele specific transcription factor binding (ASTF). SNPs in the cis–acting elements such as GATA-1 TF binding site enhance the Survin gene expression in breast cancer patients.

SNPs located in the exon region affect gene transcription and translation, thereby affecting the cancer susceptibility. Exonal SNPs are classified based on the ability to alter the encoded amino acids as synonymous and non synonymous coding SNPs. These exonal SNPs alter and influence the cancer susceptibility by genetic mechanism (Figure-14).

SNPs located in the intronic region produce splice variants of transcript and promote or disrupt DNA binding (Figure-15). They also alter the functions of long non coding RNAs. SNPs located in 5' –UTR

and 3'-UTR affect the translation and micro RNA binding[7] (Figure-16). SNPs situated in the regions far from actual gene exert their effects by either enhancing or reducing gene transcription through long range cis effects[7].

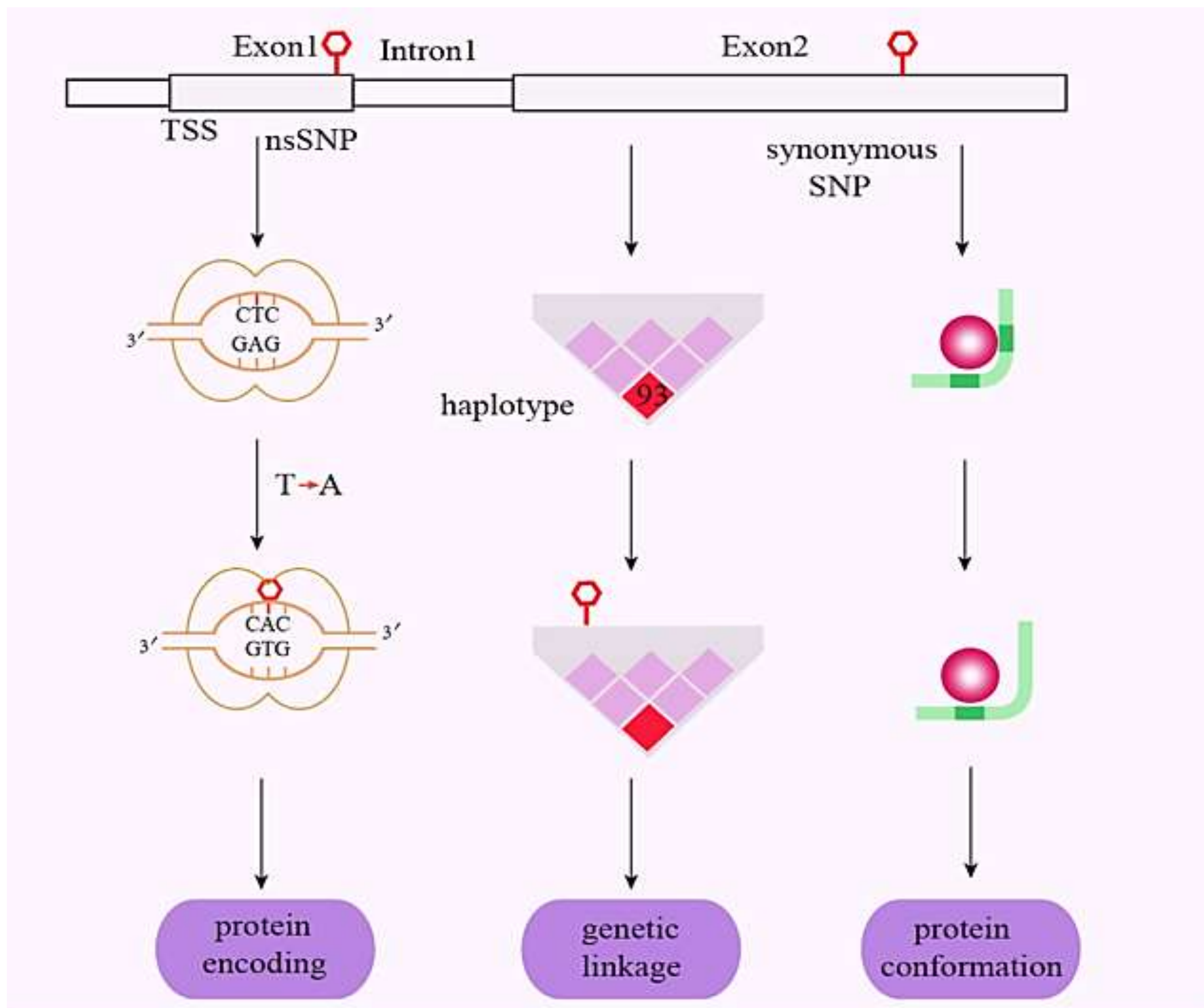


Figure:14 Schematic Representation of mechanism associated with exonal SNPs and cancer susceptibility. Non synonymous exonal cSNPs change the amino acid sequence of the encoded protein. Synonymous exonal cSNPs change protein conformation and function via genetic linkage

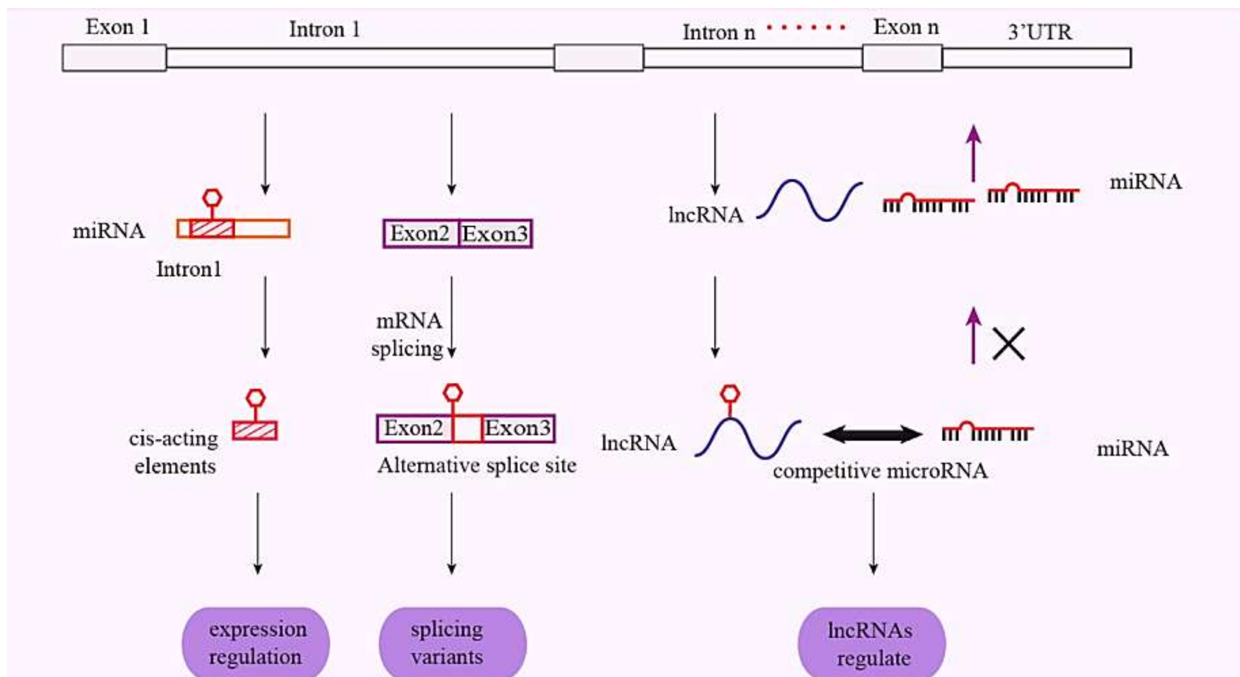


Figure 15: Schematic representation of mechanism associated with Intronal SNPs and cancer susceptibility. Intronal SNPs influence gene expression through cis –acting regulatory elements. Intronal SNPs influence protein synthesis by m RNA splicing and regulation of Inc RNA function.

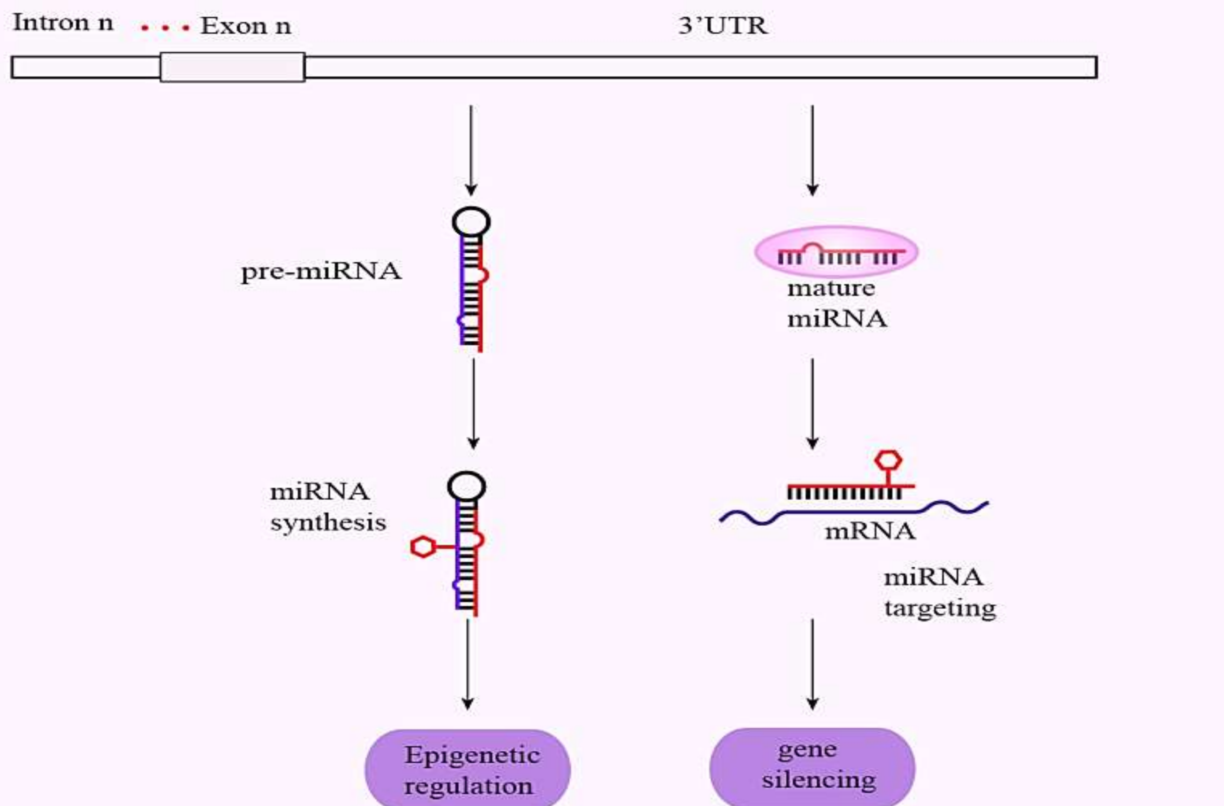


Figure 16: Schematic representation of mechanism associated with 3' UTR SNPs and cancer susceptibility. SNPs in the 3' UTR affect mi RNA synthesis and gene silencing by altering miRNA mediated translational repression

TABLE OF MOLECULAR MECHANISMS OF REGION BASED SNP ON CANCER

SNP regions	Possible molecular mechanism	Unclear issues
Promoter	Genetic regulation: promoter activity (TATA box, transcription-factor binding ability)	the interaction between genetic and epigenetic elements
	Epigenetic regulation: DNA methylation, histone modification	effect of SNPs on DNA methylation status
Exons	Non-synonymous cSNPs: coding protein structure and function	detail mechanism at biochemical and cellular level
	Synonymous cSNPs: secondary structure conformation translation dynamics	mechanism of kinetics of translation
Introns	cis-regulatory elements mRNA splicing genomic imprinting lncRNAs chromatin looping	detail functions of cis-regulatory elements and splicing
UTRs	5′-UTRs: protein translation and transcription activity.	how SNPs in the 5′-UTR affect the efficiency of translation
	3′- UTRs : Regulate mRNA degration and translation	how the 3′-UTR affects miRNA binding sites
Non definite regions	long range cis regulation tRNA and rRNA	the ways polymorphisms affect long range cis regulation, tRNA and rRNA

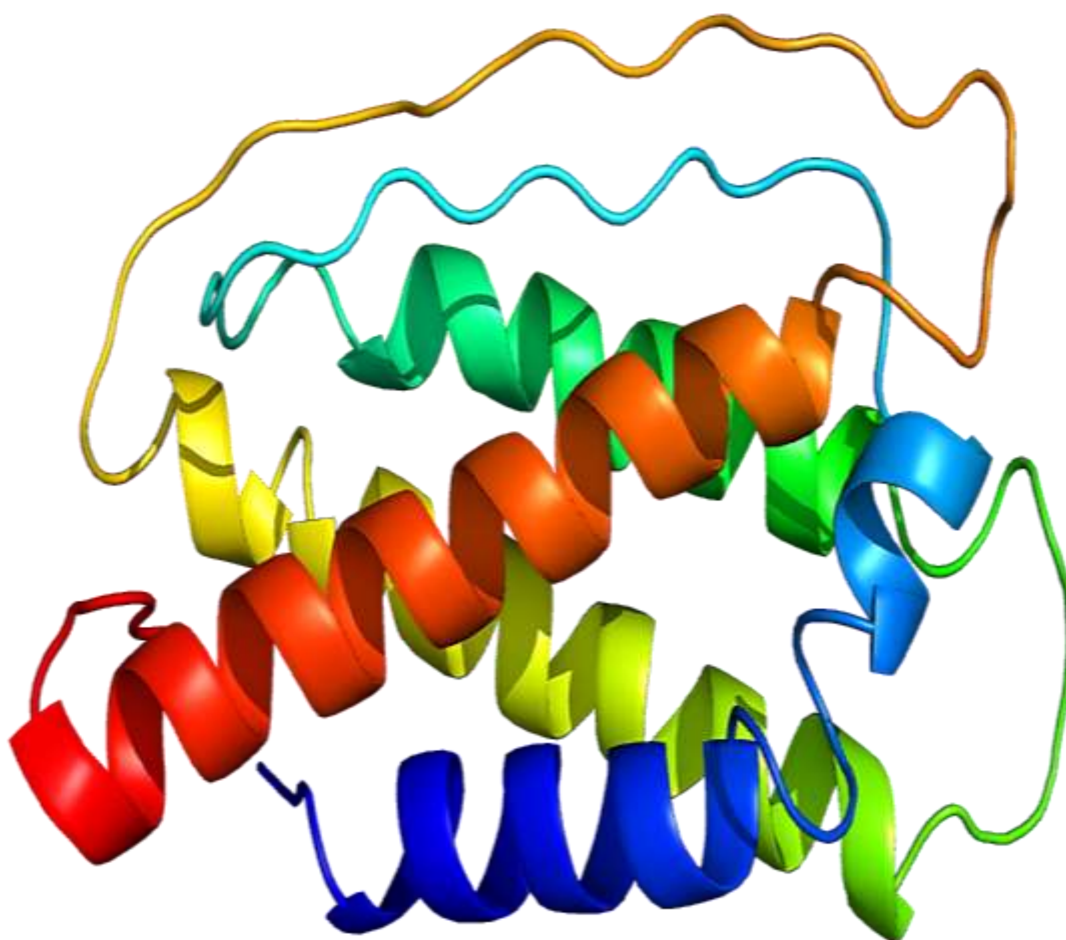


Figure 17: Structure of INTERLEUKIN - 7

Interleukin 7

Interleukin 7 is a potent immune regulatory protein (Figure-17). Interleukin -7 is important for development and survival of T and B cells (Figure-18). Fibroblastic reticular cells synthesize IL-7 in the T cell zone in lymphoid organs. IL – 7 is also produced by stromal cells and also by several different types of inflammatory cells[1].

IL 7 helps in development of lymphocytes and in regulation of peripheral T cell populations. While thymic regeneration of T cells in healthy adults experiences a 10 -100 fold decrease, homeostatic T cell proliferation remains stable throughout life (Figure-19). The production of interleukin-7 is also found in solid tumours. IL-7 is also found to be identified in lymphomas and leukaemias. The action of interleukin -7 on tumour cell proliferation is still not clear.

IL-7 has also been used in gene therapy method of treatment of non small cell lung cancer through tumour environmental immune modulation. It is shown that IL-7 levels are associated with poor prognosis of breast cancer.

In early stages of Prostate cancer the levels of IL-7 and IL-15 are increased. IL-7 levels are associated with metastatic bone disease and haematological malignancies. Various levels of IL-7 are found to be expressed in renal, colorectal, nervous system and lung cancers (Figure-20).

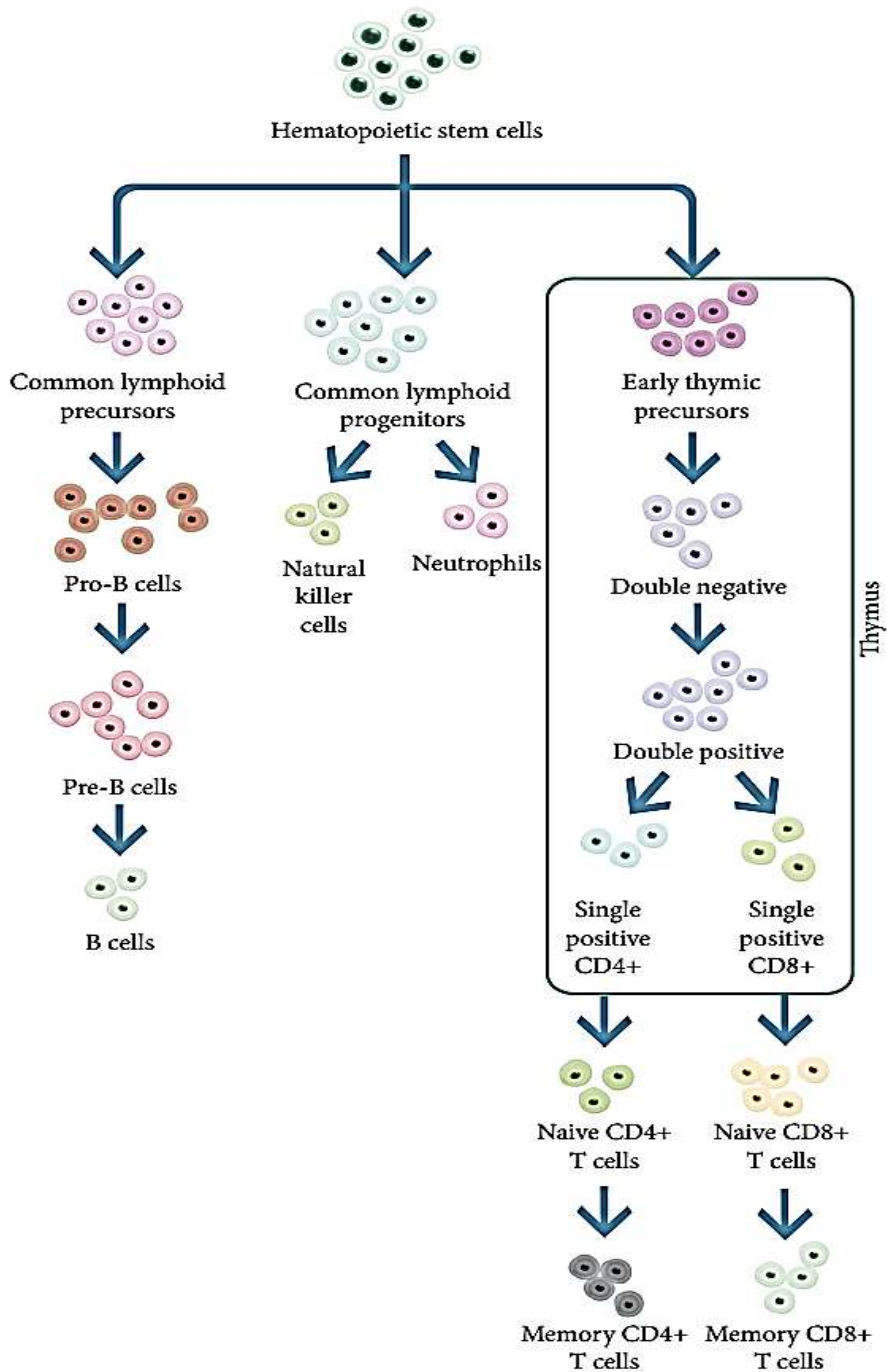


Figure 18: The development of lymphocytes.CLP cells are the earliest lymphoid progenitor cells derived from hematopoietic stem cells giving rise to T-Lineage, and natural killer (NK) cells. **IL-7 plays a significant role at specific stages in the development of these cells**

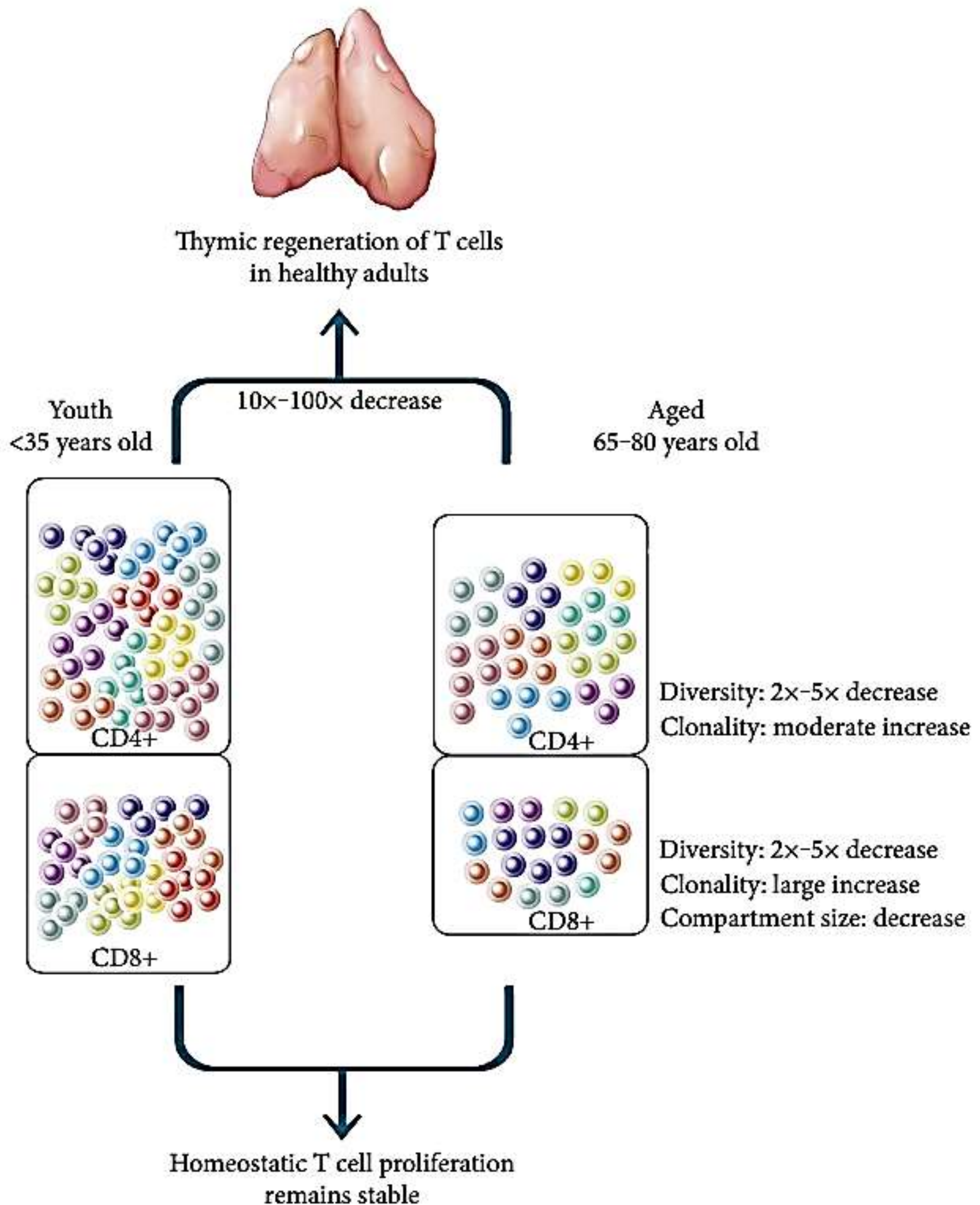


Figure 19: Thymic regeneration of T cells in healthy adults experiences a 10 -100 fold decrease, homeostatic T cell proliferation remains stable throughout life. Both T cell lineages decrease by 2- 5 fold in diversity. However, differences in the homeostatic proliferation of CD4+ and CD8+ cells with a concomitant decline in the size of the CD8+ compartment

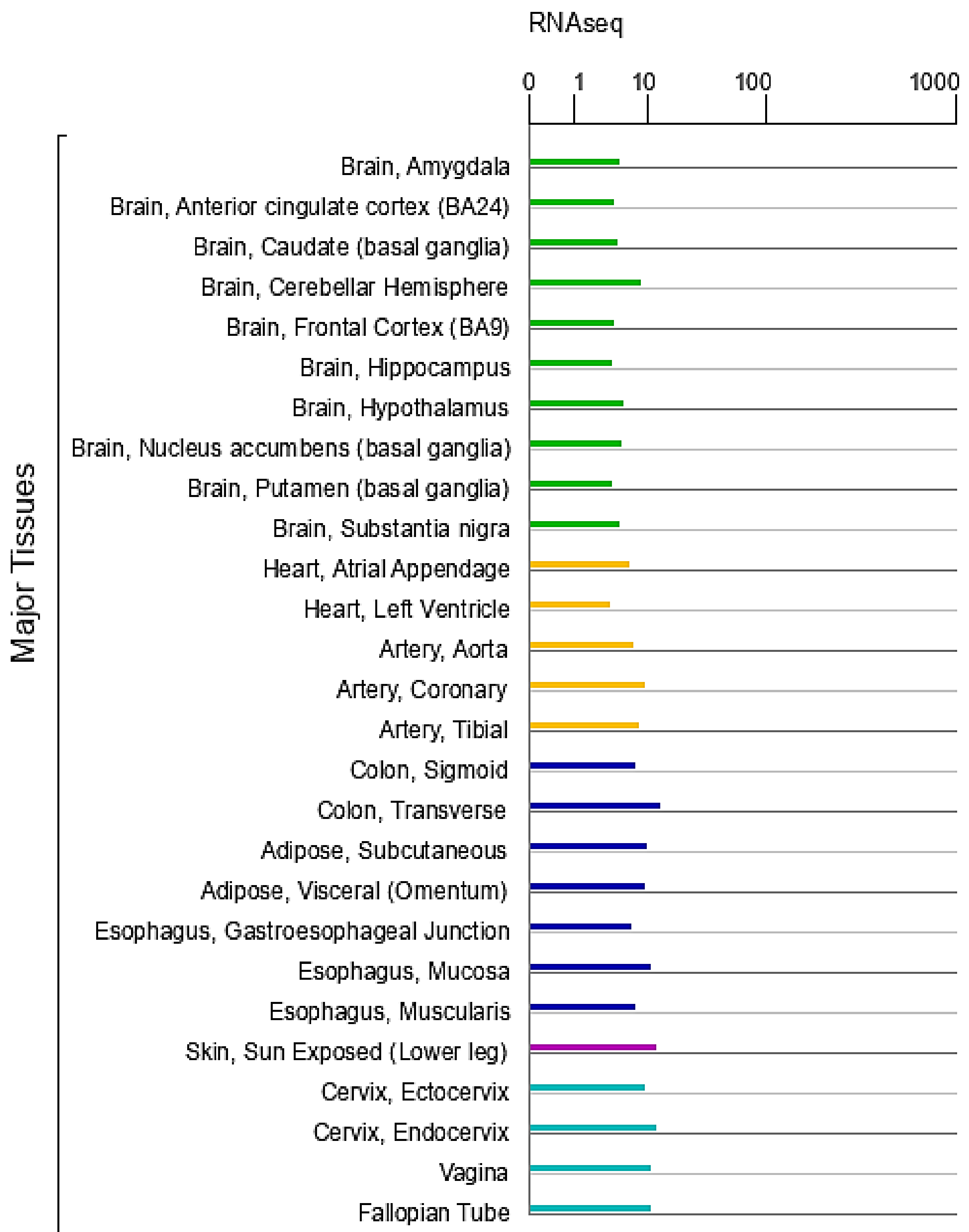


Figure 20: Percentage of expression of IL-7 in various tissues in the body

Interleukin-7 and Immunosenescence

Degenerative pathological processes play a major role in dysfunction of multicellular organism. They include calcification, non-enzymatic glycation, stem cell drop out, fibrosis, degradation of cellular matrix, uncontrolled inflammation and compromised mitochondrial biogenesis. Lifelong reduction in immunological reserve and homeostasis is called Immunosenescence. This process contributes to decreased resistance to infectious diseases, increased propensity to autoimmune diseases and cancer. Immunosenescence limits the body's ability to anti-tumour immunity.

IL-7 Receptor & Signal Transduction

IL-7 belongs to the common ϒ chain (ϒc-CD 132) interleukin family. This comprises of interleukin -2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. The signalling cascade mechanism initiated by ϒc interleukins and receptors plays a important regulatory role in homeostasis of B, T and Natural killer (NK) cells of the immune system.

IL-7 signals via its unique α- receptor IL-7 Rα (CD 127). The interaction between IL-7 Rα (CD 127) snf common ϒc receptor stimulates the Janus Kinase (JAK),signal transducers and activation of transcription proteins (STAT). This subsequently activates

Phosphoinositol 3–Kinase (PI3K)/Akt or Src pathways leading to gene transcription (Figure-21)

There are two forms of IL-7 R namely membrane bound and soluble IL-7 R. Both exhibit different functions. Membrane bound IL-7R mediates signal transduction, while soluble IL-7 R function as modulatory control mechanism. Disruption of IL-7 signalling pathways plays an important role in immunosenescence.

The series of signalling cascade initiated by ϒc interleukins and their receptors play a major regulatory role in homeostasis of B,T and natural killer (NK) cells of the immune system.

Disruption of signalling pathways and receptors associated with IL-7 plays a major central role in deterioration of immune system with age.

IL-7 regulates T cell homeostasis through three immune modulation pathway namely Thymic differentiation, Peripheral expansion, and extrathymic differentiation (Figure-22). IL-7 directs T cell differentiation and maturation in the thymus to regenerate peripheral T cells. As age –related decline in thymic function becomes apparent, IL-7 contributes to the maintenance of the T cell pool through expansion of existing peripheral Tcells.In all three scenarios,IL-7 is known to have an important signalling effect.

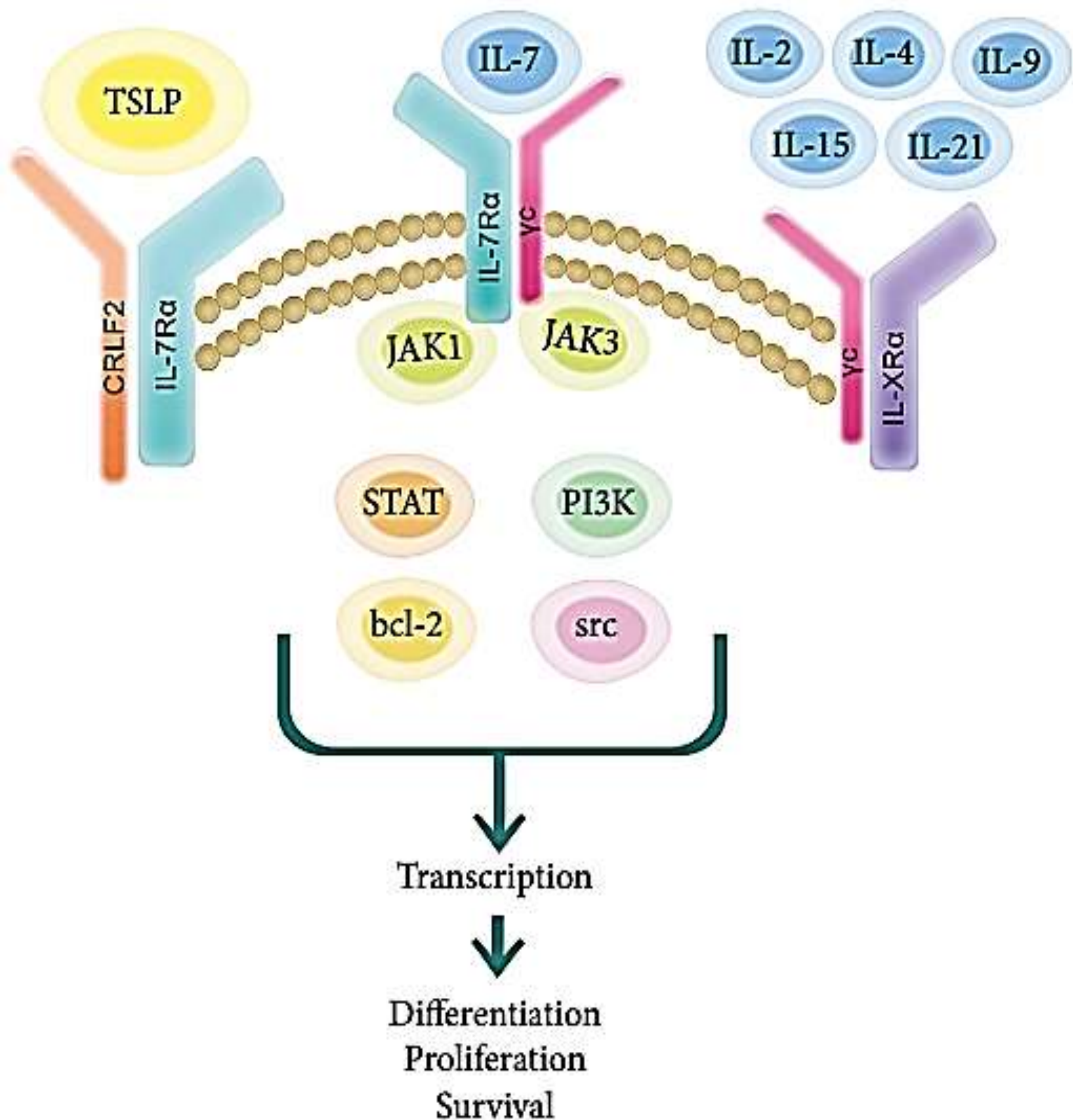


Figure 21: Schematic of IL-7r signal transduction. IL-7 and its cell surface receptor- a heterodimer consisting of the IL-7 Rα and the common ϒ chains(ϒc)- form a ternary complex that engages the JAK STAT pathway. The subsequent downstream activation of PI3K, bcl-2 and src kinases lead to gene transcription. IL-7R signal transduction is important in directing the differentiation ,proliferation, and survival of immune cells including B,T and natural killer (NK) cells. The IL-7Rα chain is shared with another receptor recognizing thymic stromal lymphopoietin (TSLP). In this scenario, the IL-7Rα non-covalently associates with the cytokine receptor like factor 2(CRLF2). Likewise the ϒc is shared with other receptors specifically recognizing IL-2, IL-4, IL-9, IL-15 and IL-21.

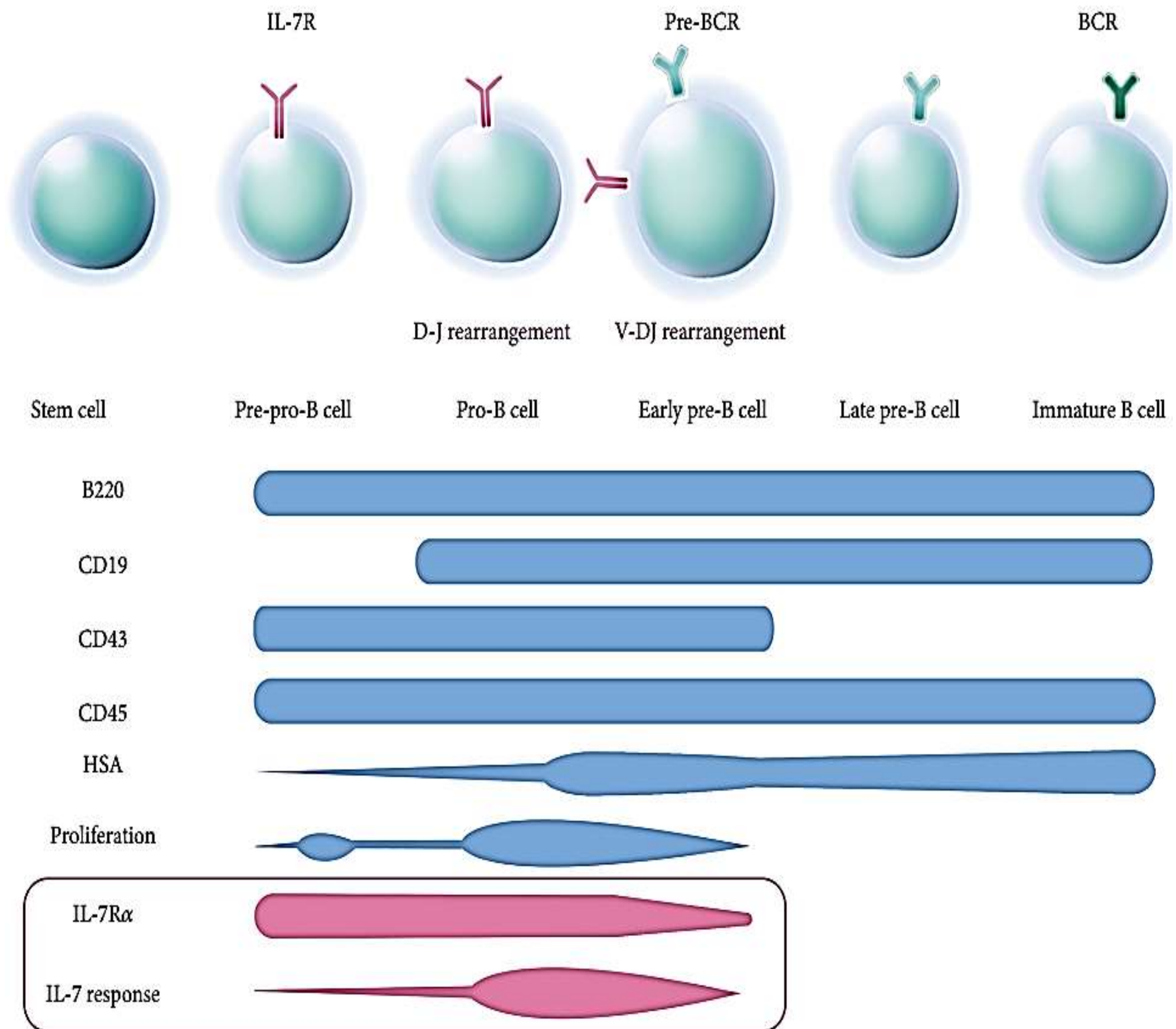


Figure 23: Schematic of B lymphopoiesis in the bone marrow. The presence of various cell surface markers is indicated in parallel with each stage in the development of B cells . Among these, IL-7 is important in early B cell differentiation because it promotes the commitment of CLP to the B-lineage. It also acts in concert with transcription factors to regulate immunoglobulin gene rearrangement in the pro B cell and early pre B cell stages. Early pre B cells express IL-7 Rα until V-DJ rearrangement is complete. Successfully rearranged cells then proliferate in response to IL-7 and other cytokines.

HEMATOPOETIC STEM CELLS:

The cells of immune system are derived from bone marrow hematopoietic stem cells (HSC) as shown in Figure-23.The renewal of HSC prevents the clonal exhaustion and differentiation along multiple lineages.

Recent studies show that IL-7 and associated proteins plays a critical role in maintaining the cell life span. IL-7 network is a significant biomarker of successful ageing. Aging affects the vitality of immune system.

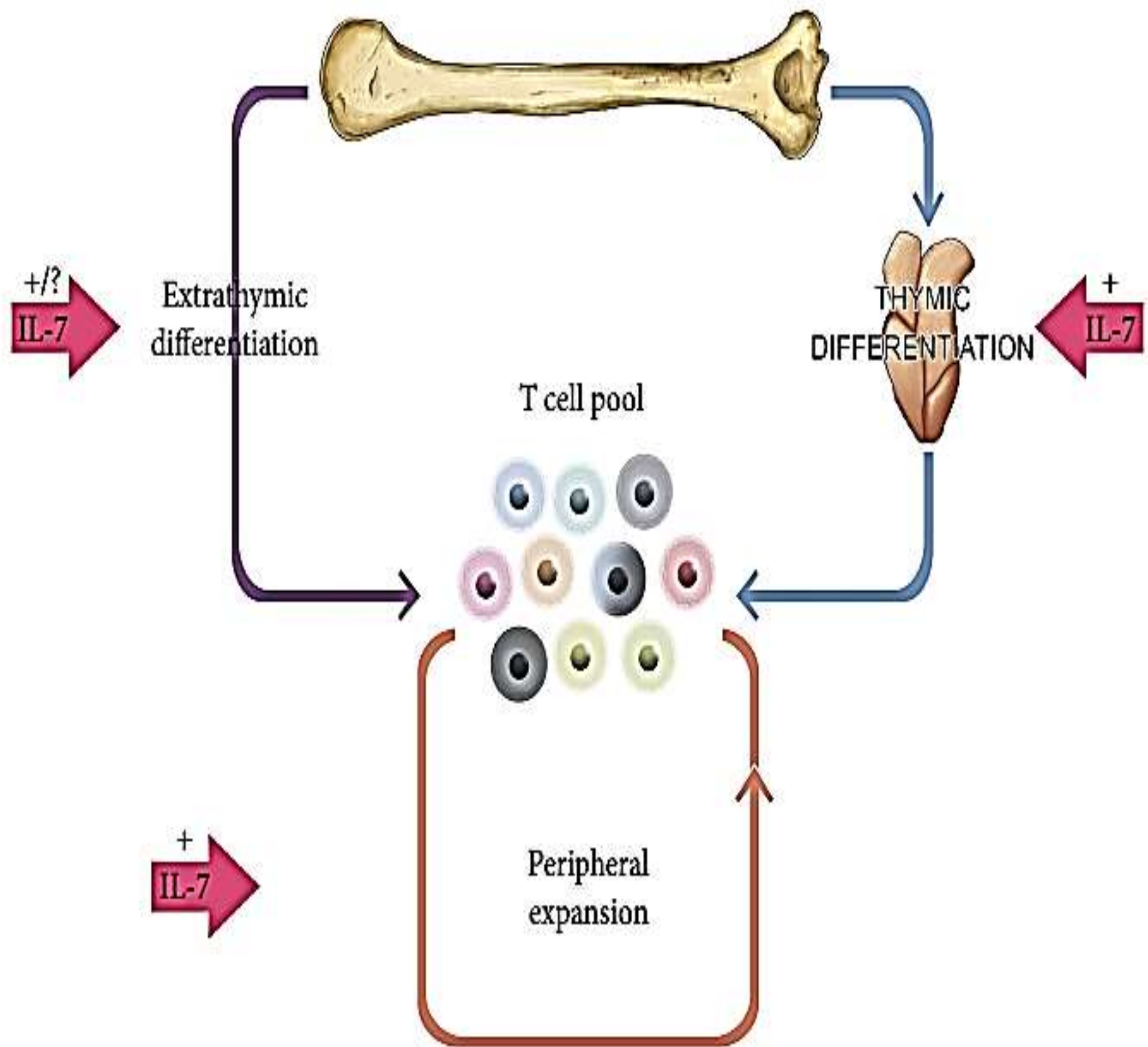


Figure 22: Effect of IL-7 on T cell Regeneration. IL-7 regulates T cell homeostasis through three immune modulation pathways: Thymic differentiation, Peripheral expansion, and extrathymic differentiation. To regenerate peripheral T cells, IL-7 directs T cell differentiation and maturation in the thymus. As age –related decline in thymic function becomes apparent, IL-7 contributes to the maintenance of the T cell pool through the expansion of existing peripheral T cells. Extrathymic differentiation from CLP cells is possible but is only a minor pathway. In all three scenarios, IL-7 is known to have an important signalling effect.

MATERIALS & METHODS

MATERIALS AND METHODS

The population selected for the study group includes patients attending the Regional Cancer Centre, Coimbatore Medical College Hospital. Patients were from Coimbatore, Tirupur, Pollachi, Valparai, Udumalpet, Mettupalayam, Niligiris and also from bordering districts of Kerala like Palaghat. Thus this group clearly represents the South Indian population minimising the potential selection bias. Thus this cohort was genotyped to identify the prevalence of Interleukin 7 Receptor alpha Thr244Ile genetic polymorphism.

Statistical Study Design : Observational study

Study Place : Government Medical College Hospital, Coimbatore.

Study Sample : Blood sample of Breast Carcinoma patients attending Regional Cancer Centre at Coimbatore Medical College Hospital.

No of Samples : 70

Study period : 1 year (March 2018 till May 2019).

Inclusion criteria:

1) Age > 18 years and < 65 years
2) Diagnosed breast cancer patients.

Exclusion criteria:

1) Patients with autoimmune disorders
2) Patients with familial history of breast cancer
3) Patients who developed secondary breast cancer.

METHODOLOGY

The above study was done during the period from March 2018 till May 2019. The study subjects are selected from patients attending Regional Cancer Center. The study was carried out among confirmed cases which are diagnosed as carcinoma breast. The study was approved by the Institutional Ethics Committee of Coimbatore Medical College.

STUDY POPULATION

The study sample comprised of 70 breast cancer patients. The Cases for study were chosen from the Regional Cancer Center, Medical Oncology and Surgical Oncology, Coimbatore Medical College Hospital. The confirmed diagnosis of breast cancer was established by histopathological examination and FNAC by Department of Pathology at Coimbatore Medical College. The patients were screened for past clinical history such as autoimmune status, familial history of breast cancer, previous history of the tumor and also regarding the metastasis, stage and about type of the breast cancer. The details of prognosis of the disease and chemotherapy status were also collected.

SAMPLE COLLECTION

5ml of venous blood from peripheral vein was withdrawn under sterile aseptic conditions with disposable syringes from all the study cases and was carefully transferred to EDTA blood collection tube and mixed thoroughly. EDTA tube is then centrifuged at 2000 rpm for twenty minutes to get the buffy coat layer for DNA extraction.

BUFFY COAT SEPARATION

Buffy coat separated by centrifugation of EDTA tubes at 2000 revolutions for 20 minutes in REMI centrifuge at department of Biochemistry. Buffy coat thus obtained was transferred to 2mL micro centrifuge tube and was used for DNA extraction. DNA extraction of the samples was done by chemical method.

DNA EXTRACTION BY CHEMICAL METHOD:

The method described below involves separating the white blood cells from the whole blood after lysis of erythrocytes, digesting the pelleted leukocytes with proteinase K in the presence of EDTA (to seize the divalent cations and thereby inhibit DNAases) and solubilizing membranes and denaturing proteins with a detergent such as SDS. The nucleic acids are then purified by phase extractions with organic solvents. Contaminating RNA is eliminated with digestion with RNAase

Buffers and solutions

RBC LYSIS BUFFER

Ammonium chloride	150 mM	8.0235g
Potassium chloride	10 mM	0.7455g
EDTA	0.1 mM	0.0372g

Dissolve the contents in distilled water and make up the volume up to 1000ml. store at 4 degree Celsius after filtration and autoclaving.

TRIS BUFFERED SALINE (TBS) PH 7.4

Sodium Chloride	140mM	8.18g
Potassium chloride	0.5mM	0.0373g
Tris base	0.25 mM	0.0303g

Dissolve in 900 ml distilled water and adjust PH to 7.4. Make up the volume to 100 ml.filter, autoclave and store at 4 degree Celsius.

PHOSPHATE BUFFERED SALINE (PBS) PH 7.4

Sodium chloride	137mM	8g
Potassium chloride	2.7 mM	0.2g
Disodium hydrogen Phosphate	10 mM	1.44g
Potassium Dihydrogen Phosphate	2 mM	0.24g

Dissolve 8g of Nacl and 0.2g of Kcl, 1.44g of Disodium hydrogen Phosphate and 0.24 g of Potassium Dihydrogen Phosphate 800 ml of distilled water. Adjust the pH to 7.4 with Hcl. Add water to 1000 ml, dispense in aliquots and sterilize by autoclaving. Store at room temperature (RT).

Tris EDTA (TE) Buffer (pH8.0)

Tris Base	10mM	1.2114g
EDTA	0.1mM	0.3722g

Dissolve in 900 ml of distilled water and adjust the pH to 8.0. Make up the volume to 1000 ml. Autoclave and store at 4 degree Celsius.

Tris 1M (pH8.0)

Tris base 121.14 g

Add distilled water up to 1000 ml, adjust pH to 8.0, filter and store at room temperature

Phenol (Saturated, pH 7.8)

Melt the commercially available crystalline Phenol at 65 degree Celsius in a water bath. Add 8-hydrooxyquinolone to a final concentration of 0.1 percent. To the molten phenol, add 0.5 M Tris HCL (pH 8.0) in equal volume and stir for 30 minutes in a magnetic stirrer. Transfer the contents into a separating funnel. Collect the lower phenolic phase and mix with equal volumes of 0.1 M TrisHcl (pH 8.0) and stir again for 30 minutes. Collect the phenolic phase and repeat the extraction with 0.1 M Tris HCL (pH 8.0) until the pH of phenolic phase is more than 7.8. Finally add 0.1 volume of 0.01 M TrisHcl (pH8.0) and store in a dark bottle at 4 degree Celsius.

Proteinase –K (20 mg/ml in water)

Sodium Dodecyl Sulphate (SDS-20 %)

SDS 20 g

Distilled water make up to 1000 ml, stirred, filtered and stored at room temperature.

Sodium chloride (5M)

Dissolve 29.22 g of sodium chloride in 80 ml distilled water and make up the volume to 100 ml. Filter the solution and store at room temperature

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Chloroform: Isoamyl alcohol (24:1)

Isopropanol

Ethanol 70 %

Tris EDTA (TE buffer (pH 8.0)

Tris base 10mM 1.2114g

EDTA 0.1mM 0.3722g

Dissolve in 900 ml distilled water and adjust the pH to 8.0.Make up the volume to 1000 ml, filter, autoclave and store at 4 degree Celsius.

Sodium Acetate (3M)

Dissolve 40.824 g of sodium acetate in 70 ml of distilled water and adjust the pH to 5.5 with glacial acetic acid. Make up the volume up to 100ml, autoclave and store at room temperature.

PROTOCOL

1) Collect 2 ml of blood in EDTA vacutainer .Centrifuge at 2000 rpm for 20 minutes and discard the plasma leaving the erythrocytes and leukocytes.
2) Add two to three volumes of ice cold RBC lysis buffer and mix well. Incubate the mixture in ice with occasional shaking for 10 minutes for the complete lysis of erythrocytes.
3) Centrifuge at 4500 rpm for 10 minutes to pellet the contents. Recover the pellet of leukocytes after decanting the red colour supernatant, containing the lysed erythrocytes and hemoglobin.
4) Repeat steps 2 & 3, till the pellet is clear without any unlysed erythrocytes.
5) Wash the white cell pellet twice in 10 ml of tris buffered saline by vigorous vortexing followed by centrifugation at 4000 rpm for 10 minutes.
6) Resuspend the white cell pellet by vortexing in 10 ml of saline EDTA buffer so that no clumps remain. To this suspension , add 50 uL of proteinase –K (20 mg/ml in water) and 0.50 ml of 20 percent SDS, mix gently and incubate at 50 degree Celsius in a water bath with occasional shaking for period of at least three hours.
7) Cool the digested samples to room temperature and add 600 uL of

5 M sodium chloride solution and mix well by vortexing .Add an equal volume of tris saturated phenol(pH7.8),Mix gently by inversion of the tubes for 10 minutes and centrifuge at 4500 rpm for 15 minutes .

8) Collect the aqueous phase containing DNA in fresh tubes and add an equal volume of saturated phenol: chloroform :isoamyl alcohol (25:24:1). Mix the contents thoroughly by inversion of the tube and centrifuge at 4500 rpm for 15 minutes.
9) Collect the aqueous phase in fresh tubes , add an equal volume of chloroform: isoamyl alcohol (24:1). Mix and centrifuge at 4500 rpm for 15 minutes, repeat this step once more.
10) Transfer the upper aqueous phase carefully to a sterile 50 ml beaker and add $1/10^{th}$ volume of 3 M sodium acetate (pH 5.5) and mix well.
11) Add an equal volume of chilled isopropyl alcohol and spool out the precipitated DNA using fresh micropipette tip , wash in 70 percent ethanol and air dry.
12) Resuspend the dried DNA in 0.5ml of Tris EDTA buffer and store at -20 degree Celsius.

DETERMINATION OF YIELD AND PURITY OF DNA

Dilute 20 uL of the DNA stock solution to 2 mL with sterile distilled water giving a dilution of 100 times. Measure optical densities (OD) at 260 nm and 280 nm using a 2 ml cuvette in a spectrophotometer using sterile distilled water as a blank.

YIELD OF DNA SAMPLES

An OD of one at 260 nm wavelength corresponds to the volume of double stranded DNA obtained. Concentration of DNA stock solution is calculated as follows.

Concentration of DNA stock solution (ug/mL) = OD_{260} X dilution factor X 50

PURITY OF SAMPLES

The purity of DNA samples is assessed by estimating the ratio between the readings at 260nm and 280 nm. Pure DNA samples have OD 260/OD 280 ratios of 1.8 and above.

PRIMER DESIGNING FOR PCR

The polymerase chain reaction (PCR) is a technique for selective amplification of nucleic acid molecules that are initially present in minute quantities. Primer designing is an important step for the efficiency and sensitivity of PCR. The primers which are unique for the target sequence to be amplified should fulfill certain criteria such as primer length, GC %, annealing and melting temperature. 5 ‘end stability, 3’ end specificity etc. Many primer designing softwares are now available for designing PCR primers. The use of open source online software PRIMER 3 for designing PCR primers is detailed below.

Step1. Go to internet and select Google. Type PRIMER 3 in the search box and click.

Step2. Click PRIMER 3 input (version 0.040).

Step3. Copy the gene sequence and paste in the box in the PRIMER 3 worksheet.

Step4. Enter other details of the primers such as size range of PCR Product, Primer size, Tm Value etc..,or select the default values.

Step5. Click Pick Primers

The set of forward and reverse primers will appear in seconds. The designed primers need to be further validated for their specificity and other primer qualities before sending it for synthesis.

Step6. Check the specificity of primers (forward and reverse primers separately) by BLAST analysis. The sequence of designed primers should be 100 % homologous to the gene of interest and should not be homologous to other genes to avoid no specific amplification during PCR.

Step7. Check the primers for GC%, primer dimer effects, self annealing and hairpin formation etc. using free online softwares.

REAL TIME QUANTITATIVE PCR

In molecular biology, real time polymerase chain reaction, also called quantitative real time polymerase chain reaction (qPCR/qrt-PCR) or kinetic polymerase chain reaction (KPCR),is a laboratory technique based on the PCR, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification of one or more specific sequences in a DNA sample.

The procedure follows the general principle of polymerase chain reaction. Its key feature is that the amplified DNA is detected as the reaction progresses in real time. It is a new approach compared to standard PCR where the product of the reaction is detected at its end. Two common methods for detection of the products in real time PCR are (1) non specific fluorescent dyes that intercalate with any double stranded DNA, and (2) sequence specific DNA probes consisting of oligonucleotide that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary DNA target.

Real time PCR with double stranded binding dyes as reporters:

A DNA binding dye binds to all double stranded (ds) DNA in PCR, causing fluorescence of the dye. An increase in DNA product during PCR therefore leads to an increase in fluorescent intensity and is

measured at each cycle, thus allowing DNA concentrations to be quantified. However, dsDNA dyes such as SYBR Green will bind to all dsDNA PCR products, including non specific PCR products (such as primer dimer) necessitating the need for perfect standardization of PCR without the amplification of non specific products.

Fluorescent reporter probe method:

Fluorescent reporter probes detect only the DNA containing the probe sequence; therefore, use of the reporter probe significantly increases specificity and enables quantification even in the presence of non specific DNA amplification. Fluorescent probes can be used in multiplex assays for detection of several genes in the same reaction, based on specific probes with different colour labels, provided that all targeted genes are amplified with similar efficiency. The specificity of fluorescent reporter probes also prevents interference of measurements caused by primer dimmers, which are undesirable potential by-products in PCR. However, fluorescent reporter probes do not prevent the inhibitory effect of the primer dimmers, which may depress accumulation of the desired products in the reaction.

The method relies on a DNA based probe with a fluorescent reporter at one end and a quencher of fluorescence at the opposite end of

the probe. The close proximity of the reporter to the quencher prevents detection of its fluorescence; breakdown of the probe by the 5’ to 3’ exonuclease activity of the taq polymerase breaks the reporter – quencher proximity and thus allows unquenched emission of fluorescence, which can be detected after excitation with a laser. An increase in the product targeted by the reporter probe at each PCR cycle therefore causes proportional increase in fluorescence due to the breakdown of the probe and release of the reporter.

POLYMERASE CHAIN REACTION

Fragment of IL-7 gene was amplified using forward and reverse primers (from Bioserve Biotechnologies (INDIA) Pvt .Ltd.Hyderabad).

Primer Reconstitution

The primers are supplied in lyophilized form. Autoclaved double distilled water is used to prepare 100× concentrations i.e. the volume of water required to prepare 100× concentrations is 10 times the molecular weight of primer is which is 100 µmolar solution.

From the above stock solution 10× concentration is prepared as the working standard solution for PCR.

MASTER MIX:

2×PCR Master mix in the following composition was prepared and used.

Master Mix consists of basic components necessary for polymerase chain reaction.

1) Reaction buffer consisted of TrisHcl - 10mM at pH 8.3KCl – 50 mM
2) $MgCl2$ - 1.5 mM acts as catalyst.
3) dNTP's were used in a concentration of 2.5m Meach.
4) Taq polymerase in a concentration of 1.5 U.

Primers were used in a concentration of 10 picomol and DNA was used in a concentration of 200 ng.

PCR was carried out in a reaction volume of 25 µL with the following components.

PCR Master mix	12.5 µL
Forward primer	1.0 µL
Reverse primer	1.0 µL
C primer	1.5 µL
T Primer	2 µL
DNA	1.0 µL
Distilled water	6 µL
Total	25. 0 µL

Amplification was carried out in, Biorad CFX96 real time PCR with the following cycling conditions.

- Initial denaturation- 94^0 C - 5min

➢ 35 cycles of		
Denaturation	94^0 C	30 sec
Annealing	94^0 C 94^0 C	30 s 30 s
Extension	72^0 C	5 min

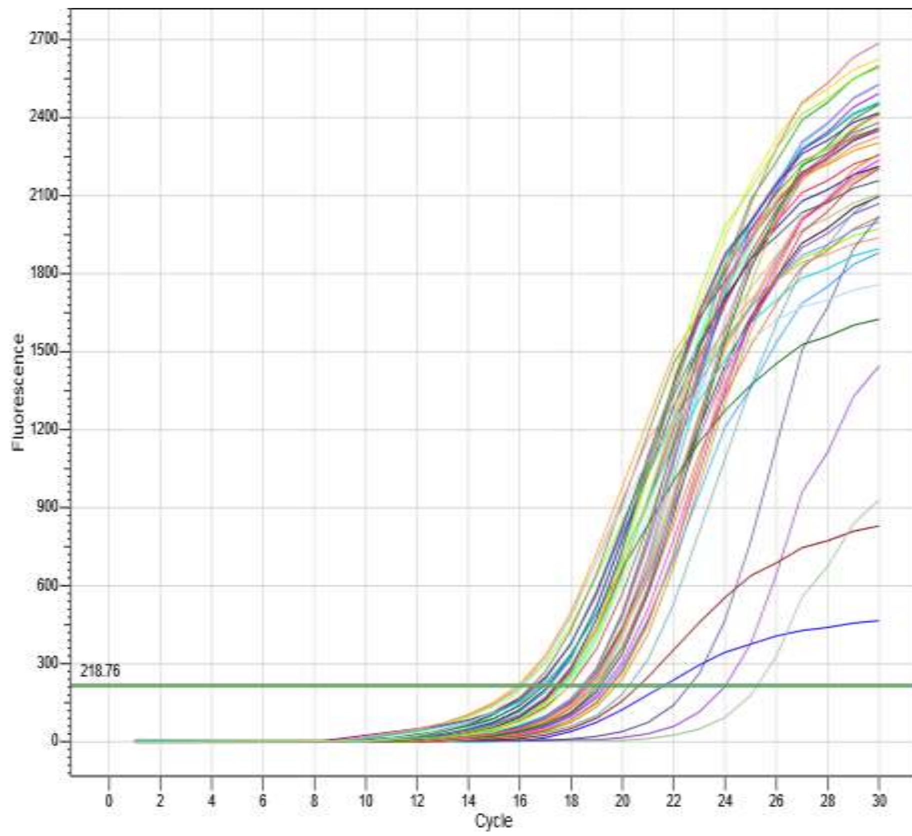
- Final extension at 72^0 C – 5 min.

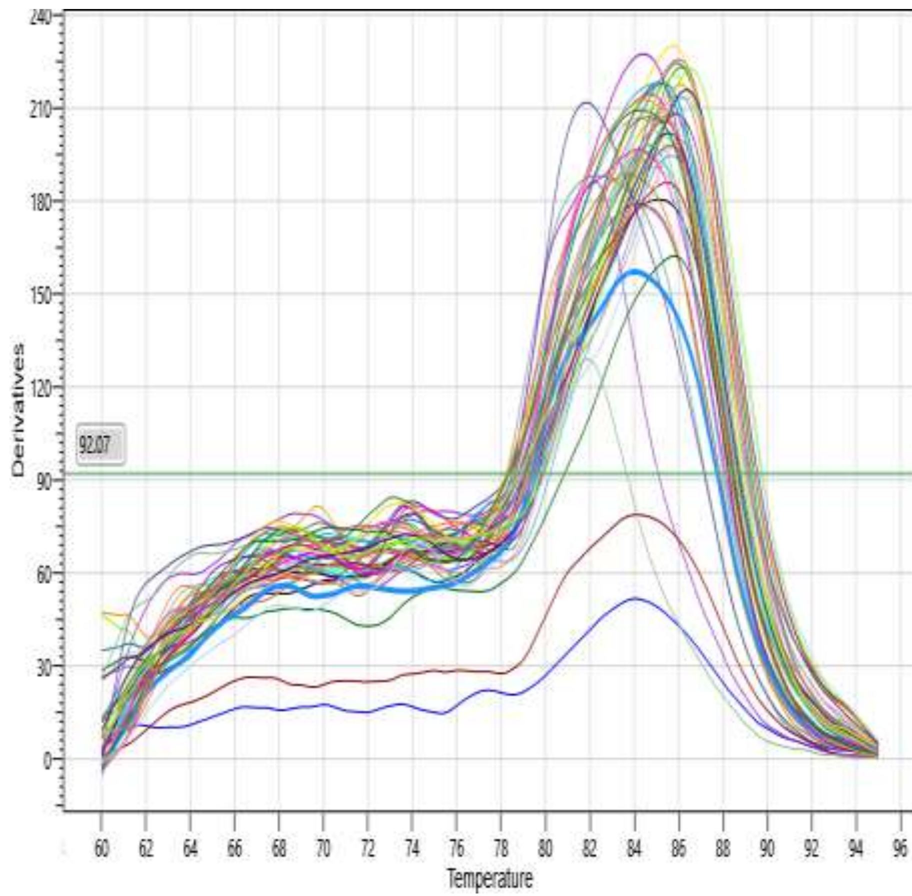
RESULTS

RESULTS

The results of Real Time Polymerase Chain Reaction, also called Quantitative Real Time Polymerase Chain Reaction (qPCR/qrt-PCR) or Kinetic Polymerase Chain Reaction (KPCR), is obtained as shown below after the amplification was carried out in Biorad CFX96 Real Time PCR with the following cycling conditions mentioned in methodology.

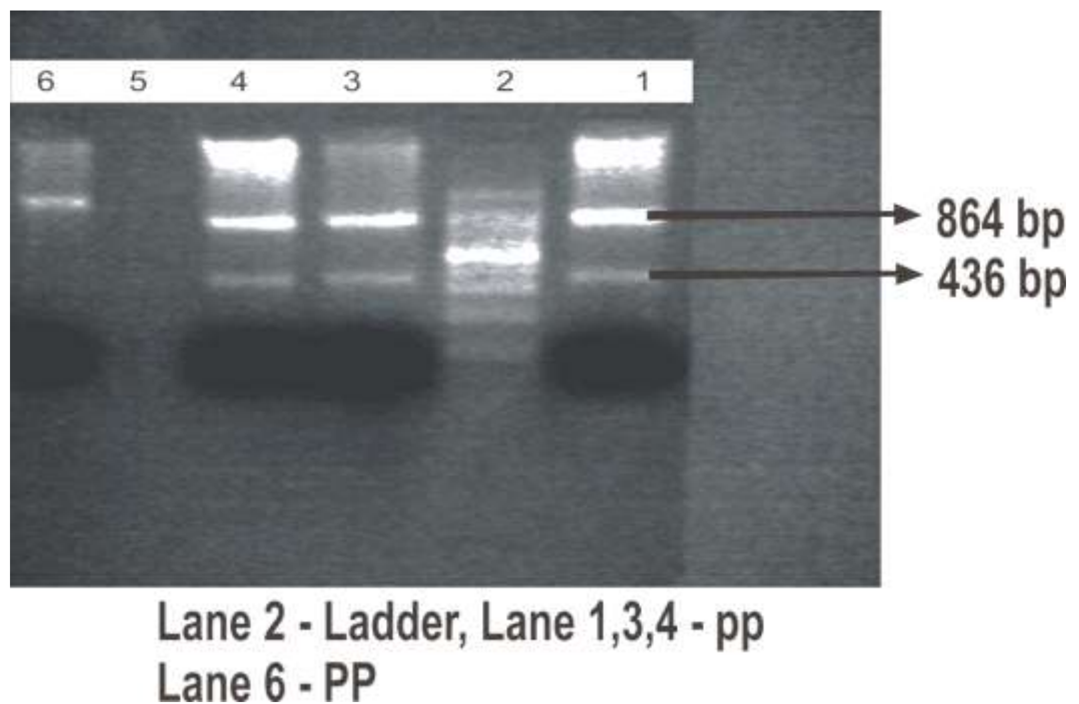
The melt curve showed the presence of the Interleukin 7 Receptor Alpha Thr244ile polymorphism in all the patients in the range of temperatures between 78 till 88. There were other insignificant polymorphisms identified in nearby regions which can be precisely detected only through gene sequencing.





The Melt Curve obtained by the analysis is shown above.

The product obtained was employed in gel electrophoresis to confirm the presence of bands with control as known DNA ladder and thus the specific single nucleotide polymorphism of 864 base pairs and 436 base pairs was confirmed.



Lane 2 - Ladder, Lane 1,3,4 - pp
Lane 6 - PP

Result:

All the 70 breast cancer patients showed the presence of the Interleukin 7 Receptor Alpha Thr244ile polymorphism.

DISCUSSION

DISCUSSION

Breast cancer is the most commonly diagnosed & leading cause of mortality and morbidity among women in India and world. A possible genetic and environmental contribution in breast cancer risk is indicated by the increased incidence among patients with a family history and increasing trend in recent times in our population.

In general, the incidence and mortality of breast cancer are comparatively lower among the Asian and African populations, which are relatively underdeveloped nations that have not adopted the westernized dietary and reproductive patterns. In contrast, women from heavily industrialized or westernized countries like European and North American have a significantly higher breast cancer burden[15].

From the statistical point of view it clearly shows that the incidence of cancer among the patients attending regional cancer centre in our population is on the rise every year. Genetic evaluation has to be done for our population because of huge variation in ethnic and geographic shift among our Indian population.

Cancers are now widely recognised as failure of immune cells to identify and kill these altered pathological cells at an early stage and this

is the concept used behind a treatment strategy called Immunotherapy in which patients own immune cells are used to identify and destroy the cancerous cells.

Currently immunotherapy is considered as an adjuvant to conventional cancer chemotherapy methods which use the body's own defence mechanism to fight against the cancerous cells. FDA has approved immunotherapy for melanoma (2014) and for lung and renal cancers in 2015 and third phase clinical trial is on the way for various other cancers. The results are astounding as it has completely cured the patients of cancer. Hence scientists around the world are considering whether immunotherapy could be considered as first line in aggressive tumour pathologies.

The gene for human IL-7 is located on chromosome 8q12-13, which spans for 6 exons and has open reading frame of 534 base pairs (177 amino acids). This also include 25 amino acid signal peptide. IL-7 belongs to family of cytokine and signals through common gamma chain. The pleotropic action of IL-7 is not only as a regulator of T cell homeostasis but also acts on immune modulation, Dendritic cells, Thymic ageing. Recombinant IL-7 is ideal solution for immune

reconstitution by promoting peripheral T cell expansion in lymphopenia patients and can prolong the survival of tumour bearing hosts.

A few studies done abroad have shown that IL7RA Thr244lle polymorphism is associated with increased susceptibility and this could act as prognostic marker in breast cancer (Vitello et al 2017). Even though IL-7 has been extensively studied in haematological malignancies, its mode of action in cancers had been least studied in our population with no such direct studies establishing the role of IL-7 polymorphism with breast cancer.

The data sheds light on autoimmunity associated genetic polymorphism at IL-7 playing an important role in breast cancer depending on molecular pathways activated in each tumour microenvironment.

The IL7RA Thr244lle polymorphism was found in all the patients attending the Coimbatore regional cancer except the gene expression varied among these patients and this strongly suggests that the molecular pathways in the onset of breast cancer works by suppressing the immune system. By identifying the polymorphic regions responsible for this, patients' own immune cells can be effectively used to treat them.

Churchmen et al have shown that IL-7 is involved in the chronic inflammation process linking to adaptive immunity and in explaining the molecular pathways in rheumatoid arthritis patients.

Various articles are reviewed by Jianbao et al and they have explained the mechanisms of action of IL-7 in cancer and the potential application and limitations in cancer immunotherapy

Numerous studies have shown that IL-7 and IL-15 are associated with molecular pathogenesis in various cancers and are considered as powerful pro inflammatory cytokines. They have the ability to destabilize chromosomes and induce tumorigenesis. These studies have also shown possible malignant proliferation control of these cytokines and their influence in tumour microenvironment and immune system. This favours the proposal of immunotherapy as treatment modality for which the exact mechanism and pathways are still under investigation.

Breast cancer secondary to treatment with checkpoint inhibitors is a new entity. Given the rapid course of the condition’s development, it is suggested that autoimmunity has some major role in evolution of breast cancers. The exact pathophysiologic mechanism and predictive biomarkers have not yet been established. Future treatment evolves

towards genetic screening of breast cancer at molecular level enabling to edit the defective disease causing gene using CRISPR technology.

CRISPR cas 9 technique promises the hope of precision and personalised gene editing technology using next generation medicine. Proteomic technologies provide us with improved understanding and discovery of biomarkers specific for cancers.

Genome Wide Association Studies (GWAS) were limited due to the poor relationship between mutant variants and the risk. The association among hormones and various metabolic factors in cellular milieu play an important role in breast cancer pathogenesis.

Major attributes for personalization namely mutation, gene, gene-gene interaction, epigenetic, environmental and, metagenomic factors and drug response and resistance have to be evaluated population wise (Octapodial Approach).

Western studies cannot be taken directly as reference in our population as there is huge variation in dietary patterns, epigenetic factors and polymorphisms. These differences make every ethnic group and population unique and hence genetic polymorphism studies have to

be done in our south Indian population to evaluate the impact of the above discussed factors in the causation of cancer in this population.

Cancer immunotherapy has been one of the highlights in the advancement of cancer care. Despite early failures, the application of immunotherapeutic strategies to the treatment of breast cancer holds promise.

Certain immune checkpoint inhibitors bind to PD-1 on T cells and mediate an antitumour immune response. Immune checkpoint inhibitors are becoming part of standard care, a new class of adverse events "Immune-related adverse events" has emerged. They are the usual side effects of fatigue, fever, diarrhoea, skin rash and itching and uncontrolled inflammation. Fewer than 10 percent of the patients have these side effects. This clearly shows that the road ahead is finding the right biomarker in our population so as to understand the benefits, efficacies and molecular pathways underlying these newer treatment options in our south Indian population

Among them is endocrine toxicity, most commonly targeting the thyroid, pituitary, or adrenal glands. New-onset diabetes mellitus has been reported in less than 1% of patients.

As various clinical trials including cancer vaccines, immune check point blockade (Nobel Prize 2018), antibodies, adoptive cell therapy, cytokines and oncolytic therapy are under trial in western population. Immunotherapy is being tried in breast cancers in western population. Further geno-profiling of our population in various autoimmune loci and identifying their polymorphisms will throw light on the role of immunotherapy as treatment option for our south Indian population too.

Precision medicine allows tailor made treatment for patients according to the genetic background. Different people respond differently to the cancer treatment and the adverse effects can be greatly reduced and the outcomes can be greatly improved by this approach and this greatly saves lives and time and money spent. Thus the quality of patient care and treatment are greatly improved. But for the above approach to be feasible we need a complete database of genetic variations and polymorphisms in our population.

No study has dwelled upon the integrated approach of autoimmunity loci and breast cancer in our Indian and south Indian population.

Hence the results from this study definitely support the immunotherapeutic strategies as new treatment option for breast cancer

in the sample population. If we could establish these in much larger groups and in all subtypes of breast cancer, then it would be a breakthrough in treating breast cancers in era of Precision Medicine.

CONCLUSION

CONCLUSION

This is a novel study done in our population showing that there is some association between autoimmunity coding genes with breast cancer. No other studies other than this have dwelled upon in this concept in India and especially in South Indian population. It is also pertinent to note at this juncture that 2018 Nobel Prize for Medicine was jointly awarded to James P Allison and Tasuku Honjo for their discovery of cancer therapy by negative immune regulation.

Despite vast and extensive research regarding the role of SNPs in the genetic predisposition of cancer, the underlying mechanism still remains complex. Moreover genetic evidence for association of IL7 genetic polymorphism in breast cancer and also in other cancers needs to be extensively studied in our population. The role of immune suppression genes is least studied in relation to breast cancer. Understanding the driving mechanism of SNPs in various cancers can have a global impact and could help identifying a potential biomarker. The identification of SNPs related biomarker could help us go a step ahead towards precision medicine by formulating tailor made protocols based on the genetic background which could reduce the healthcare cost,

drug wastage, complications and side effects of potent chemotherapy drugs and chemicals.

Complex mechanisms are involved in gene polymorphisms and cancer susceptibility. Gene polymorphisms change the spatial structure affecting the mRNA stability, methylation and allele–specific expression. Tissue specific gene expression due to polymorphism needs to be studied in detail for various cancers in our population and data base need to be created.

IL – 7 axis modulation acts as an important tool for repairing damaged or weak immune systems particularly in cancer patients receiving chemotherapy. IL-7 promotes reconstitution both from thymopoiesis and also from thymus independent homeostatic expansion of peripheral T cells. It helps in high immune effector cell infiltration for better up regulated killing and improved cell survival. It also improves the overall immune function by suppressing the immunosuppressive network of the body. The presence of IL-7 and its receptor in some solid tumour still needs further research. Therefore IL-7 is a definite target to enhance immune system, though the efficacy of IL-7 administration in cancer patients remains to be proved by further studies.

LIMITATIONS

LIMITATION

- The selected single SNP may not give a detailed comprehensive view of genetic variation as genomic sequencing does.
- Role of autoimmunity in the women with breast cancer patients would have been better understood and associated if the levels of IL-7 were estimated quantitatively.
- The expression of IL-7 receptor also needs to be assessed in tissues or plasma to correlate with the concentration of IL-7 to find out any feedback inhibition governing the levels on IL-7 in these patients.
- Categories of breast cancer SNPs were not separately analyzed to evaluate the role of autoimmunity and to study the molecular effects of autoimmunity coding regions.
- The effectiveness of the study would be more if the patients with different subtypes of breast cancer were followed up and their prognosis and response to treatment were compared with controls to effectively assess the expression patterns.
- This SNP could be a low penetrant allele and the function could be probably exerted by their effects through complex gene – gene interaction or by gene environment interactions. Such effects were not investigated by epigenetic mechanisms in the study.

SCOPE OF STUDY

SCOPE FOR FURTHER STUDY

- Further research can be targeted at identifying the association of breast cancer with autoimmunity in our population.

- Role of polymorphisms associated with immunotherapy in treatment resistant Breast cancer can be assessed to understand the underlying molecular mechanisms by pathway analysis studies.

- Studies to determine whether immunotherapy may be included as a first line treatment or as an adjuvant in treatment of late or in advanced cases of breast cancer.

- Future research may be aimed at tailor made gene therapy or immunotherapy as adjuvant with conventional treatment modalities aiming for cure.

- Data base for our population should be done region wise and should be identified for specific polymorphisms and variations among the ethnic groups in all prevalent diseases and cancers are to be studied in detail.

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ANNEXURES

INSTITUTIONAL HUMAN ETHICS COMMITTEE
COIMBATORE MEDICAL COLLEGE, COIMBATOR – 14

EC Reg No. ECR/892/Inst/TN/2016
Telephone No: 0422 – 2574375/76
Fax : 0422 – 2574377

CERTIFICATE OF APPROVAL

To
Dr.Rajesh Wilson,
Post Graduate,
Department of Biochemistry
Coimbatore Medical College,
Coimbatore -14.

Dear Dr.Rajesh Wilson,

The Institutional Ethics Committee of Coimbatore Medical College, reviewed and discussed your application for approval of the proposal entitled **"Prevalance of Interleukin 7 Receptor Alpha Thr244ile Genetic Polymorphism Among Breast Cancer Patients."** No.023/2017.

The following members of Ethics Committee were present in the meeting held on 21.11.2017.conducted at MM - II Seminar Hall, Coimbatore Medical College Hospital Coimbatore-18.

1	Dr.S.Ramalingam MD, Dean, PSG IMS&R, Cbe	Chairman
2	Dr.Usha MD., Professor of General Medicine, CMCH, Cbe	Member Secretary
3	Dr.R.Manonmani MD., Professor of O&G, CMCH, Cbe	Clinicians
4	Dr.N.Renganathan MS., Professor of General Surgery, CMCH,Cbe	Clinicians
5	Dr.Sudha Ramalingam MD., Professor of SPM, PSG IMS&R, Cbe	Clinicians
6	Dr.R. Shanmugavadivu MD., Professor of Physiology, CMC, Cbe	Basic Medical Scientist
7	Dr.N. Shanthi MD., Professor of Pharmacology, CMC, Cbe	Basic Medical Scientist
8	Dr.A.Dhanalakshmi MD., Assoc. Professor of Pathology, CMC,Cbe	Basic Medical Scientist
9	Dr.L.Madhan MD., Professor of Pharmacology, CMC, Cbe	Basic Medical Scientist
10	Dr.N.Paramasivan MD., Professor of Pharmacology, Sri Ramakrishna Dental College, Coimbatore	Basic Medical Scientist
11	Mrs.A.Sharmila BA., BL., Advocate	Legal Expert
12	Dr.K.P.Sampath Kumar M.Pharm, Ph.D., Asst. Prof. of Pharmacy, CMC, Cbe	Scientific Member
13	Dr.G.Vani Ganesh M.Sc.,Ph.D., Tutor in Medical Surgical Nursing, CMCH, Cbe	Scientific Member
14	Mr.V. Balasubramani MA,MA,MBA,LLB,M.Phil,PG.D.M, DLLAL, Chief Executive, Avinashilingam JSS Self Finance Courses, Cbe	Social Worker
15	Mr.V.A.Shahul Hameed, +2	Lay-Person

We approve the Proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL HUMAN ETHICS COMMITTE
COIMBATORE MEDICAL COLLEGE
COIMBATORE - 641 014.

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death 2 .The risk of developing the disease is 100 fold higher in women than men. Breast cancer is the second most common cancer in the world with overall estimate of 1.7 million cases. Breast cancer remains the most frequent cause of death among women as per GLOBOCON 2012 review 2 (Figure 1) As per 2018 WHO estimate approximately 6,27,000 women died from breast cancer which is 15% of all cancer related death among women 3 . The life time risk of BC is 3.4%. BC is the second most common cause of person years (19.3 years) of life lost to cancer among women 4 . One third of newly diagnosed cancers are Breast cancer and 1 in 8 women are affected by it 4 . The incidence of BC is rare in women younger than age 25 but the incidence increases rapidly after age 30.

2 Cancers have a multi-factorial genetic susceptibility. Breast cancers may be hereditary; however environmental factors clearly influence the penetrance of hereditary forms of breast cancer, and both genetic and environmental factors contribute to sporadic forms of Breast cancer. The identification of BC susceptibility genes (BRCA 1 and BRCA 2) has provided important insights into Breast Cancer. Mutation in tumour suppressor genes BRCA 1

and BRCA 2 are responsible for 80% to 90 % of single gene familial breast cancers and about 3 % of all breast cancers(

Figure: 2). The major risk factors for sporadic Breast Cancers are related to hormone exposure, gender, age at menarche and menopause, reproductive history, breastfeeding, exogenous estrogens, diet(Pala et al.,2009;Huet al .,2012), physical activity (Peters et al.,2009; Elissen et al.,2010),alcohol use (Zhang et