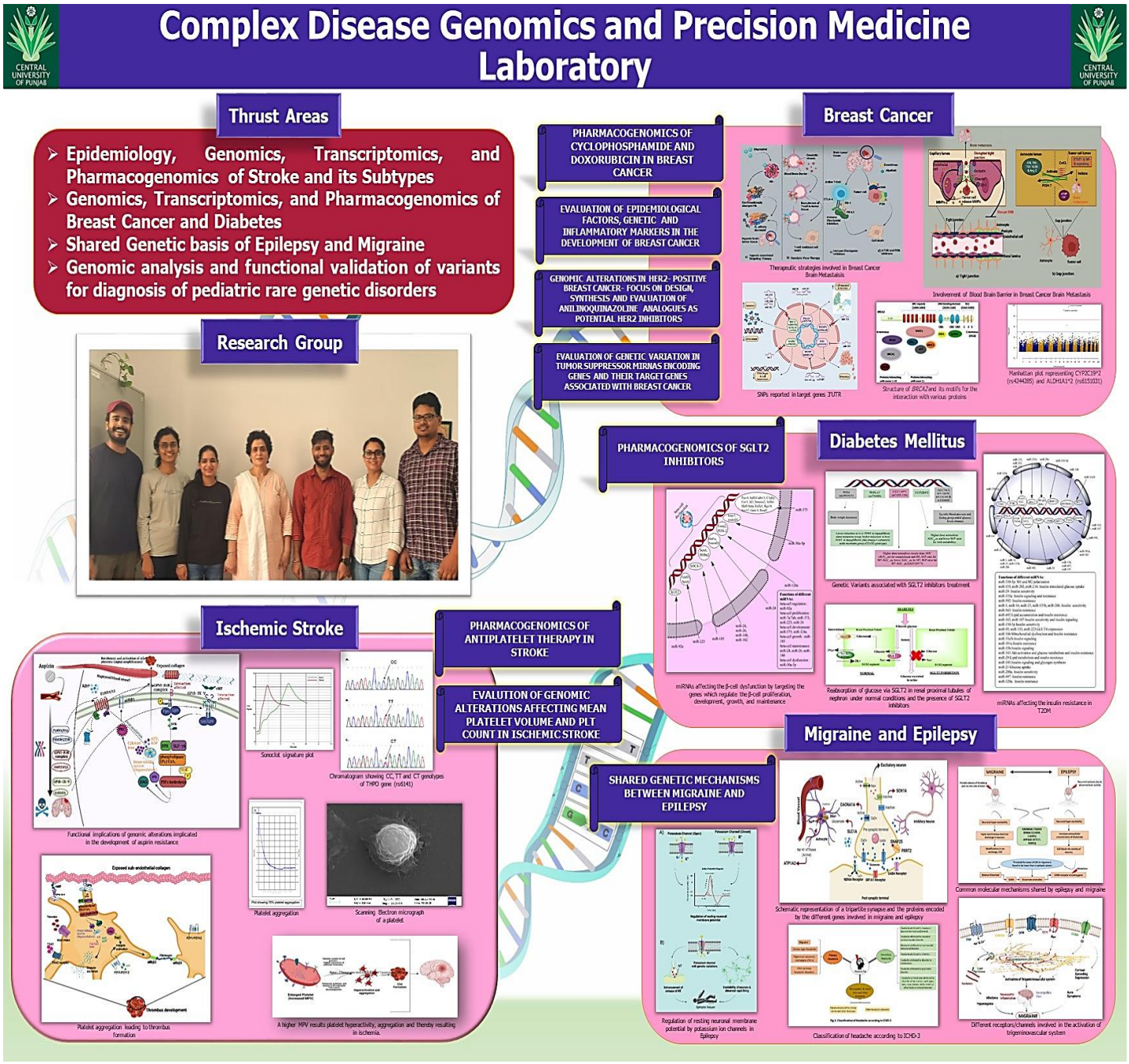
d. Details of research work

Prof. Anjana Munshi is currently working as a Professor and Director Research & Development Cell at the Central University of Punjab, Bathinda. Her research interests include multiple omics approaches to complex genetic diseases like Stroke, Diabetes and Breast Cancer, Hemoglobinopathies and modifier genes in Thalassemia and Sickle Cell Anaemia, shared genetic susceptibility between Migraine and Epilepsy; Paediatric rare diseases.



Areas of Research interest depicted grossly

CONTRIBUTION TO STROKE GENOMICS AND PHARMACOGENOMICS

Genetic predisposition to stroke occurs and has been documented in both animal models and human beings. Evidence has accumulated that variants of genes involved in the coagulation cascade, vascular remodeling, lipid metabolism, CAMP degradation and haemostasis pathways play an important role in the development of stroke. Prof Munshi has evaluated the role of various genes involved in these pathways including ACE, PDE4D, eNOS, CYP11b2, E-selectin, estrogen receptor α, IL-10, MMP3, CYP4F2 and LPL.

Some publications highlighting the association of genes with ischemic stroke and its subtypes:

GENETICS OF STROKE

ACE gene

Angiotensin-converting enzyme is an attractive candidate to play a role in the vascular diseases including stroke. Angiotensin-converting enzyme plays an important role in production of Angiotensin II and catabolism of bradykinin; involved in the modulation of vascular tone and the proliferation of smooth muscle cells. To the best of our knowledge this was the first study to investigate the association of ACE gene polymorphism and ischemic stroke in an Andhra Pradesh population from South India. Stroke patients in our study tended to have more ID and DD genotypes in comparison to controls which were statistically significant. There was a significant difference in the allelic frequency between the patients and healthy controls. An association between the polymorphism in ACE gene and the risk of lacunar infarction has been reported in Japanese stroke patients. However, it showed no significant effect on atherothrombotic and cardioembolic infarction. Similarly, Doi et al. reported a significant association between ACE gene polymorphism and thrombotic brain infarction in another Japanese population. Further, a significant association between the presence of D alleles and mortality following ischemic stroke was also found by these researchers. A positive correlation between ACE gene polymorphism and carotid artery stenosis and ischemic cerebrovascular disease has also been reported by Kostulas et al. It has already been reported that the DD genotype of ACE shows twice the levels of ACE compared to II genotypes. The increased level of the enzyme and the inactivation of bradykinin may increase vasoconstriction, cellular hypertrophy and thrombosis and thereby contribute to the risk of ischemic stroke.

The distribution of ACE genotype and allelic frequency within the stroke subtypes was also studied. D allele was found to be significantly associated with only intracranial large artery atherosclerosis, whereas insignificant associations were found in other subtypes. This might be because intra cranial large artery disease was the most frequent ischemic stroke subtype in our study. This is similar to previous report from the study hospital. Most notable difference of the stroke registry of Nizams Institute of Medical Sciences from Western registries was the predominance of intracranial rather than extra cranial location of the large artery atherosclerosis. In conclusion our data supports a significant association between the ACE insertion/deletion polymorphism and stroke in South Indian population [1].

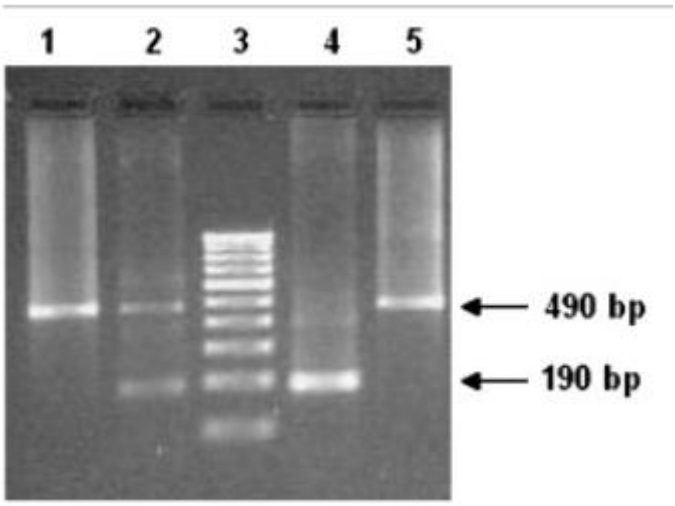


Fig 1: Agarose gel electrophoresis, showing the amplification for ACE/I D polymorphism. Lane 3 represents the 100 bp ladder. Lanes 1and 5 show II genotype (490 bp product), Lane 2 shows I D (490 and 190 bp products) and lane 4 shows DD genotype (190 bp product).

PDE4D gene

The identification of phosphodiesterase 4D gene as a risk factor for stroke caused a great deal of interest in stroke genetics. Many of the studies of PDE4D gene have focused on the original Icelandic findings but the association between specific SNPs and haplotypes has been inconsistent. The aim of this study was to investigate the association of three SNPs 32 (rs 456009), 83 (rs 966221) and 87 (rs 2910829), originally described by deCODE group; with stroke in a South Indian population from Andhra Pradesh. Two hundred and fifty ischemic stroke patients and two hundred and fifty controls were included in the study. The stroke patients were sub typed according to TOAST classification. SNP 83 showed significant

association with stroke in the population under study while SNPs 87 and 32 were monomorphic. Further SNP 83 was found to be significantly associated with two stroke subtypes, intracranial large artery atherosclerosis (the most frequent subtype in the population) and small artery occlusion. The association with other subtypes was found to be insignificant. Further, SNP 83 was found to be associated significantly with some conventional stroke risk factors like diabetes and smoking [2].

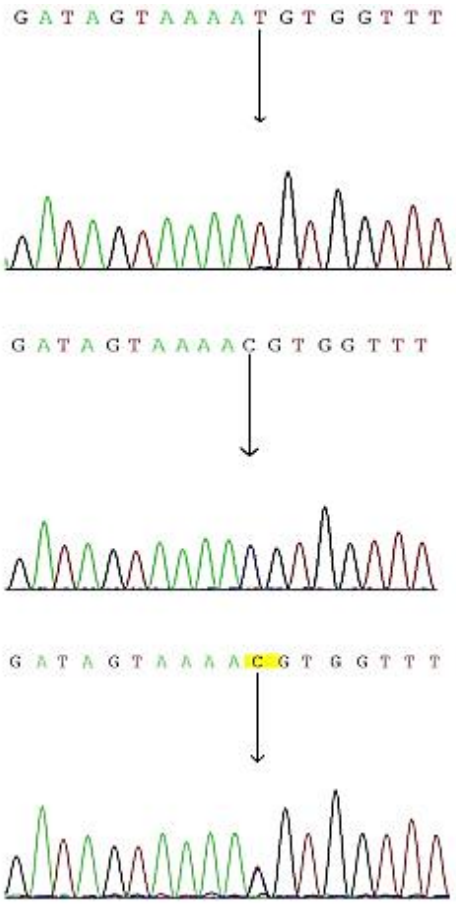


Fig 2: Sequenced PCR products of the region bearing SNP 83 showing TT homozygote, CC homozygote and CT heterozygote respectively.

eNOS gene

We investigated the association of 4b/a polymorphism in eNOS gene with ischemic stroke in a South Indian population from Andhra Pradesh. This was the first study to investigate this association from India. There was a significant difference in genotypic and allelic frequencies between the patients and healthy controls. Further examining the association of this polymorphism with stroke subtypes, we did not find significant association with any stroke subtype. Intracranial large artery atherosclerosis is the most frequent subtype in the study

population consistent with our previous study. Multiple logistic regression analysis of the 4a allele of eNOS gene and other risk factors revealed that the independent risk factors that best predicted the incidence of ischemic stroke were the 4a allele of eNOS gene and hypertension. Hypertensives and alcoholics bearing 4a allele in a high frequency are more predisposed to stroke. Our findings document a high prevalence of the 4a allele in ischemic stroke patients. Many studies have been taken up to study the relationship of 4b/a polymorphism in eNOS gene and the risk of ischemic stroke. An increased risk in individuals homozygous for 4a allele compared with 4b allele carriers has been shown [3].

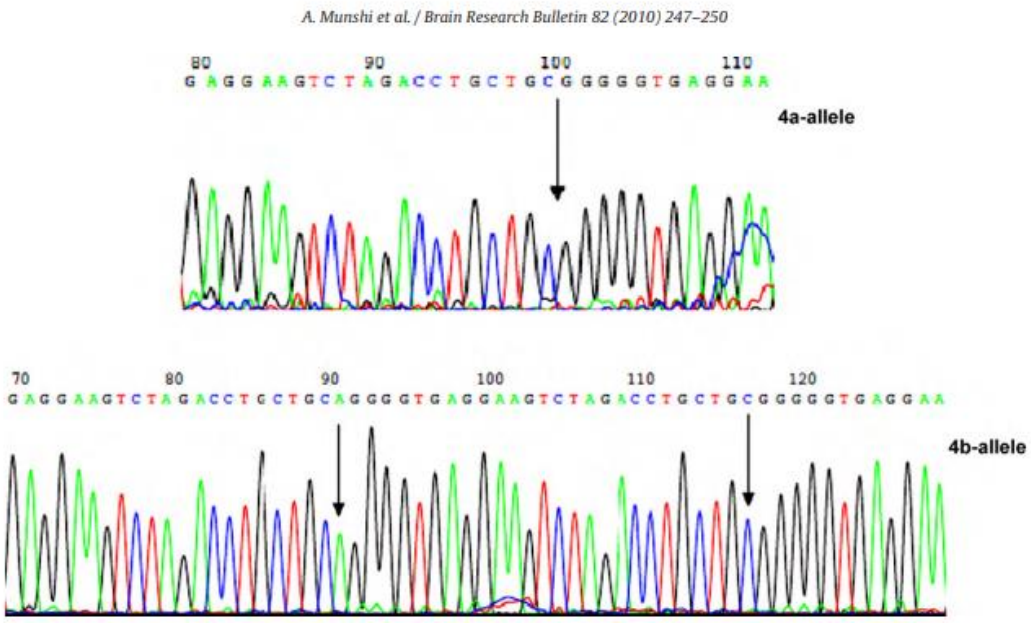


Fig 3: Sequenced PCR products of the region bearing 4b/a VNTR polymorphism. 4b-allele: The present repeat starts with 90–117 bp. 4a-allele: The 27 bp seen in the 4b-allele is missing.

MMP-3 & TNFα genes

There is increasing evidence that the genetic variation in the genes coding for pro-inflammatory markers and matrix metalloproteinase may play an important role in the pathogenesis of various human diseases including stroke. The aim of this study was to evaluate the association of genetic variants within the genes encoding tumor necrosis factor-a (TNF-α) and matrix metalloproteinase-3 (MMP-3), with stroke. Allelic and genotypic frequencies of TNF-a G/A polymorphism differed significantly between patients and healthy controls ($P < 0.001$). A stepwise logistic regression analysis confirmed these findings ($P < 0.001$). Further, evaluating the association of this polymorphism with stroke subtypes, we found significant association

with intracranial large artery atherosclerosis, extracranial large artery atherosclerosis, and stroke of undetermined etiology. As far as MMP-3 1612 5A/6A polymorphism is concerned, there was no significant difference in genotypic distribution and allelic frequency between the patients and healthy controls (P = 0.5 and 0.9 respectively). We tested the gene–gene interaction between TNF-α and MMP-3 genes using the logistic regression model but didn't find an interaction between the two. TNF-α +488 G/A variant was found to be an important risk factor for ischemic stroke in the South Indians from Andhra Pradesh, whereas MMP-3 1612 5A/6A polymorphism didn't associate with stroke in the same population [4].

CYP11B2 gene

This was the first study to investigate the association of CYP11B2 −344C/T polymorphism with stroke in an Indian population. The TT genotype and T allele was found to be significantly associated with stroke (p = 0.000 in each case). Based on multiple logistic regression analysis, we suggest that the T allele of CYP11B2 gene is a risk factor for ischemic stroke. The association of the polymorphism with hypertension was also studied by comparing the hypertensive subjects with normotensives. TT genotype and T allele was found to be significantly associated with hypertension (p = 0.000 in each case). Multiple logistic regression analysis (after controlling all other confounding variables) revealed that the T allele of CYP11B2 gene is significantly associated with hypertension. Further we evaluated the association of this polymorphism with stroke independent of hypertension. In this case also statistically significant differences were observed in genotypic distribution as well as allelic frequency between stroke patients without hypertension and controls without hypertension (p <0.01). Evaluating the association of this polymorphism with stroke subtypes classified according to TOAST classification, we found significant association of TT genotype and T allele with large artery atherosclerosis, lacunar stroke and cardio embolic stroke (p < 0.01 in each case) [5].

Estrogen Receptor α (ESR1 gene)

This study was carried out to investigate the role of ESR1 gene polymorphisms [PvuII (rs 2234693) and XbaI (rs 9340799)] with stroke in a South Indian population from Andhra Pradesh. The relationship between ESR1 genotypes with estradiol levels was also investigated in pre- and postmenopausal women. In case of PvuII polymorphism statistically significant difference was observed in the genotypic and allelic frequencies between patients and controls

(joint analysis of men and women) ($p = 0.003$ and 0.004 respectively). However, the XbaI genotypes and alleles did not show an association with stroke in the study population. When the analysis was carried out separately for men and women, the PvuII polymorphism did not show significant association with stroke in men; women showed a significant association. Further when women were grouped in to premenopausal and postmenopausal, the premenopausal group did not show a significant association with the polymorphism but significant association with stroke was found in postmenopausal women. A stepwise multiple logistic regression analysis confirmed these findings. Women with pp genotype had low estradiol levels in comparison with PP genotypic individuals ($p < 0.05$). Further evaluating the association of this polymorphism with stroke subtypes, we found significant association of PvuII polymorphism with extracranial atherosclerosis, lacunar and cardioembolic stroke. In conclusion our results suggest the PvuII gene polymorphism is significantly associated with stroke in postmenopausal women in a South Indian population from Andhra Pradesh. The pp genotypes have average 17β estradiol levels which are significantly low in comparison with PP genotypes. Therefore, postmenopausal women with a high frequency of pp genotype are more predisposed to ischemic stroke [6].

LPL gene

Lipoprotein lipase (LPL) plays an important role in lipid metabolism by hydrolyzing triglycerides in chylomicrons and very low-density lipoproteins. An increasing number of studies have suggested an association of LPL gene variants with the risk of cardiovascular and cerebrovascular diseases. The aim of this study was to test whether HindIII polymorphism of LPL gene is associated with ischemic stroke and its subtypes as well as plasma lipid levels in a South Indian population from Andhra Pradesh. Five hundred and twenty five ischemic stroke patients and 500 controls were enrolled in this case–control study. The LPL HindIII polymorphism was determined by PCR-RFLP technique and the lipid levels were estimated using commercially available kits. We found significant difference in the genotypic distribution between patients and controls [for HindIII (+/+) vs. HindIII (−/−), $\chi 2 = 4.916$; $p = 0.02$; Odds ratio = 1.59 (95%CI; 1.054–2.413); HindIII (+/+) vs. HindIII (−/−) and HindIII (+/−), $\chi 2 = 5.25$; $p = 0.02$; Odds ratio = 1.24 (95%CI; 1.03–1.503)]. A stepwise multiple logistic regression analysis confirmedthese findings. The relationship between HindIII genotypes and plasma levels of HDL, LDL, VLDL and triglycerides was analyzed using ANOVA and further confirmed by Post-hoc analysis. The levels of triglycerides were found to be elevated in

individuals bearing HindIII (+/+) genotype in comparison with HindIII (−/−) genotype. HDL levels were found to be significantly reduced and triglyceride levels significantly elevated in HindIII (+/+) genotype in comparison with HindIII (−/−). However, there was no difference in the levels of LDL and VLDL between the two genotypes. Examining the association of LPL gene HindIII polymorphism with stroke subtypes, we found significant association of HindIII polymorphism with Intracranial large artery atherosclerosis [Odds ratio = 2.12 955CI (1.656–2.848); p = 0.009]. Our results suggest that the HindIII polymorphism of LPL is significantly associated with ischemic stroke risk and elevated levels of plasma triglycerides and reduced HDL levels. Further, this polymorphism is significantly associated with intracranial large artery atherosclerosis which is the most frequent subtype in our region [7].

PHARMACOGENOMICS OF STROKE

Statins

Statins reduce the risk of cardiovascular events by lowering the blood cholesterol. Many genes involved in the pharmacodynamic pathway of statins have been part of pharmacogenetic research in patients with hypercholesterolemia, with an emphasis on genes involved in the cholesterol pathway. The present study was carried out with an aim to evaluate the association between the genetic variants of lipoprotein lipase gene [HindIII (+/+)/HindIII (−/−)], multiple drug resistance gene (C3435T) and endothelial nitric oxide synthase gene (4a/4b) with clinical outcome including an increased risk of recurrent stroke or death in ischemic stroke patients on atorvastatin therapy. 525 stroke patients and 500 healthy controls were involved in the study. Follow-up telephonic interviews were conducted with patients post-event to determine stroke outcome. A significant association of MDR1 and LPL gene variants with bad outcome in stroke patients on atorvastatin therapy was found. However, there was no significant association of 27 bp VNTR polymorphism of eNOS gene with outcome. MDR analysis was carried out to analyze gene–gene interaction involving these gene variants contributing to clinical outcome of patients on statin therapy but no significant interaction between these variants was observed. In conclusion the individuals with HindIII (−/−) genotype of LPL and CC genotype of MDR1 gene would benefit more from atorvastatin therapy [8].

Aspirin

ALOX5AP gene

The important role of genetic variants in the etiology and pathophysiology of stroke is being increasingly recognized. Simultaneously, the influence of genetic factors in the clinical outcome of drug therapy cannot be ignored. 5-lipoxygenase activating (ALOX5AP) gene involved in the synthesis of leukotrienes, has been recognized as an important gene contributing towards susceptibility of stroke risk. Leukotrienes are involved in the physiological mechanism of atherosclerotic events and inflammation. The present study was designed to identify the association of SG13S114T/A polymorphism in ALOX5AP1 gene with risk of stroke, its subtypes and aspirin resistance. We studied six hundred and ten patients with ischemic stroke and six hundred and ten age and sex matched healthy controls. The ischemic stroke was classified according to Trial of Org 10172 in Acute stroke Treatment. Follow-up was done for all the patients for a period of 3 months, 6 months and 12 months. The patients were classified into two groups responders and non-responders. The non-responders were identified to have a poor clinical outcome defined as a score of more than 2 on modified Rankin Scale Score and less than 5 on extended Glassgow Outcome Scale from stroke onset. We found statistically significant difference in the genotypic distribution between patients and controls (for AA vs TT, $\chi 2 = 9.894$; $p = 0.001$, odds ratio = 1.68 (95% confidence interval (CI); 1.215, 2.326). Significant difference was observed in the frequency of A and T alleles in patients and controls (A vs T $\chi 2 = 10.23$; $p = 0.001$, odds ratio = 1.301 (95% CI; 1.107, 1.528). Multiple logistic regression analysis revealed, the most predictive risk factor for stroke was AA genotype [adjusted odds ratio = 1.660 (95% CI; 1.167–2.361) and $p = 0.005$], hypertension, smoking and diabetes ($p < 0.001$ in each case). We also found a significant association of AA genotype with intracranial large artery atherosclerosis ($p = 0.002$, odds ratio = 2.04, (95%CI; 1.279–3.275) and cardioembolism ($p < 0.001$, odds ratio = 4.73 (95% CI; 2.661–8.439). The risk of aspirin resistance was significantly higher among patients with AA genotype in comparison to carriers of homozygous TT genotype (AA vs TT, $\chi 2 = 22.25$, odds ratio = 2.983, 95% CI; 1.884- 4.723, $p < 0.001$). The frequency of recurrence and death events was more in non-responders. We didn't find a significant association of the aspirin dose with outcome. Our results indicate that the individuals bearing AA genotype of ALOX5AP1 SG13S114T/A polymorphism are more prone to stroke and bad outcome as well as with aspirin resistance than TA and TT genotypes [9].

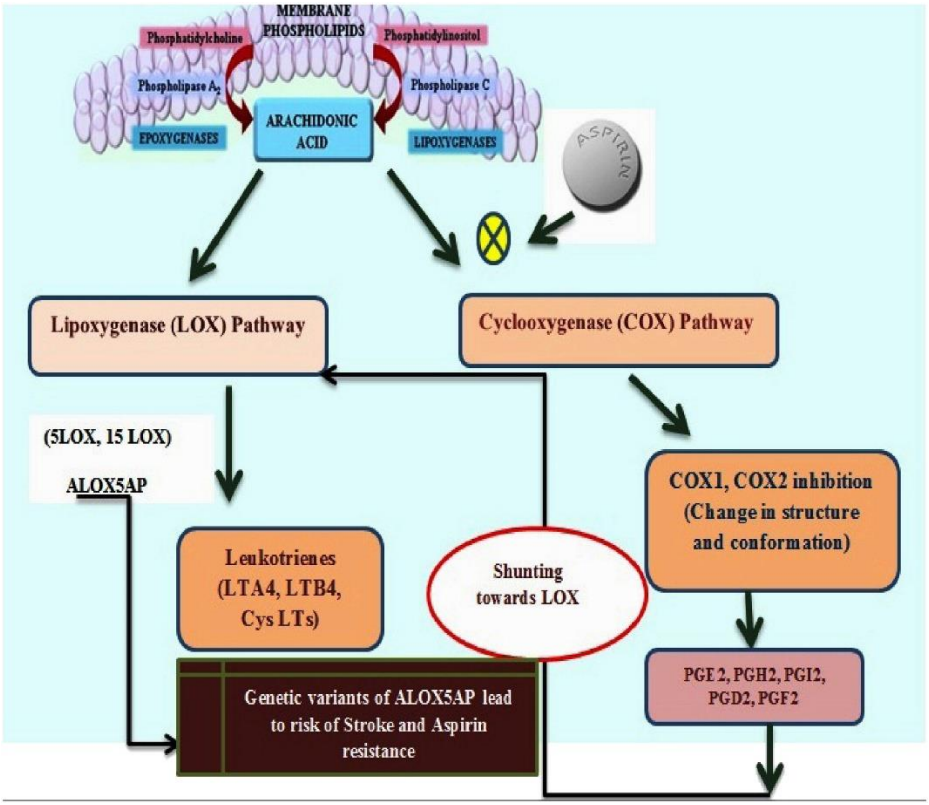


Fig 4: Pathway showing metabolism of arachidonic acid, shunting of COX pathway towards LOX pathway after inhibition COX-1 and COX-2 by aspirin, affecting the production of Cys LT, and compromising anti-aggregating and anti-inflammatory effect of aspirin due to genetic variants of ALOX5AP1.

PLATELET PARAMETERS IN ISCHEMIC STROKE

Platelet traits such as mean platelet volume (MPV) and platelet count (PLT) and pathways involved in the recruitment of platelets have also been implicated in the disease pathophysiology. Platelets are known to play an important role in the pathophysiology of IS by virtue of their capability in the formation of intravascular thrombus after the erosion or rupture of atherosclerotic plaques. We have already established the association of increased MPV with a degree of disability and rate of clot formation in IS patients. PLT count and MPV are markers of platelet function and activation and are positively associated with platelet reactivity and aggregation. An increase in MPV occurs when platelets become activated and swollen spheres instead of quiescent discs. Large platelets are more adhesive and likely to aggregate more than smaller ones. These traits and other platelet functions have been reported to be highly influenced by genetic variation. Various genome-wide association studies (GWAS) involving

different populations have demonstrated that genomic alterations are associated with PLT count and MPV. Variation involved in the genes taking part in significant processes such as megakaryopoiesis, megakaryocyte/platelet adhesion, platelet formation, and cell cycle regulation has been reported to influence platelet physiology. PLT count and MPV altered by genetic profile have not been evaluated in association with the development of IS and its subtypes. Therefore, the current study has been carried out with an aim to explore the alterations in genes affecting MPV and PLT count and their functional implications associated with IS and its subtypes.

MPV and PLT count was evaluated using flow cytometry and a cell counter. SonoClot analysis was carried out to evaluate activated clot timing (ACT), clot rate (CR), and platelet function (PF). Genotyping was carried out using GSA and Sanger sequencing, and expression analysis was performed using RT-PCR. In silico analysis was carried out using the GROMACS tool and UNAFold. The interaction of significant proteins with other proteins was predicted using the STRING database. Ninety-six genes were analyzed, and a significant association of THPO (rs6141) and ARHGEF3 (rs1354034) was observed with the disease and its subtypes. Altered genotypes were associated significantly with increased MPV, decreased PLT count, and CR. Expression analysis revealed a higher expression in patients bearing the variant genotypes of both genes. In silico analysis revealed that mutation in the THPO gene leads to the reduced compactness of protein structure. mRNA encoded by mutated ARHGEF3 gene increases the half-life of mRNA. The two significant proteins interact with many other proteins, especially the ones involved in platelet activation, aggregation, erythropoiesis, megakaryocyte maturation, and cytoskeleton rearrangements, suggesting that they could be important players in the determination of MPV values. In conclusion, the current study demonstrated the role of higher MPV affected by genetic variation in the development of IS and its subtypes. The results of this study also indicate that higher MPV can be used as a biomarker for the disease and altered genotypes, and higher MPV can be targeted for better therapeutic outcomes [10].

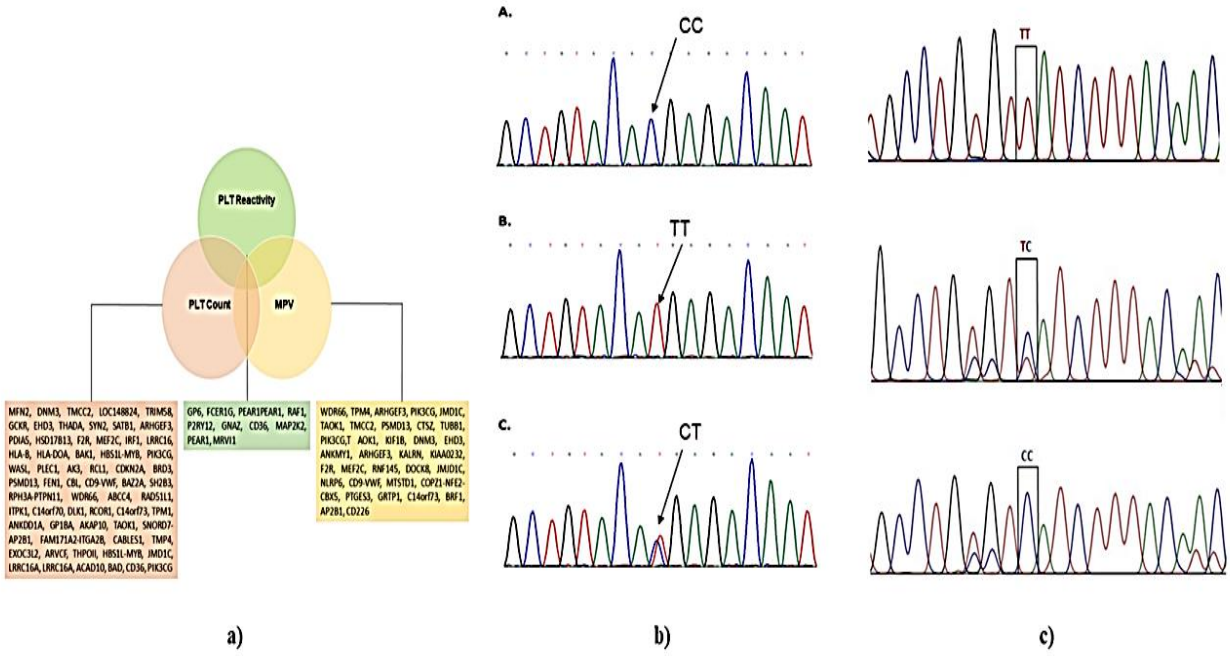


Fig 5: a) Genes involved in MPV, PLT Count, and Platelet Reactivity analysed by GSA. b) Chromatogram showing CC TT and CT genotypes of THPO gene (rs6141). c) Chromatogram showing TT, TC and CC genotypes of ARHGEF3 gene (rs1354034) [10].

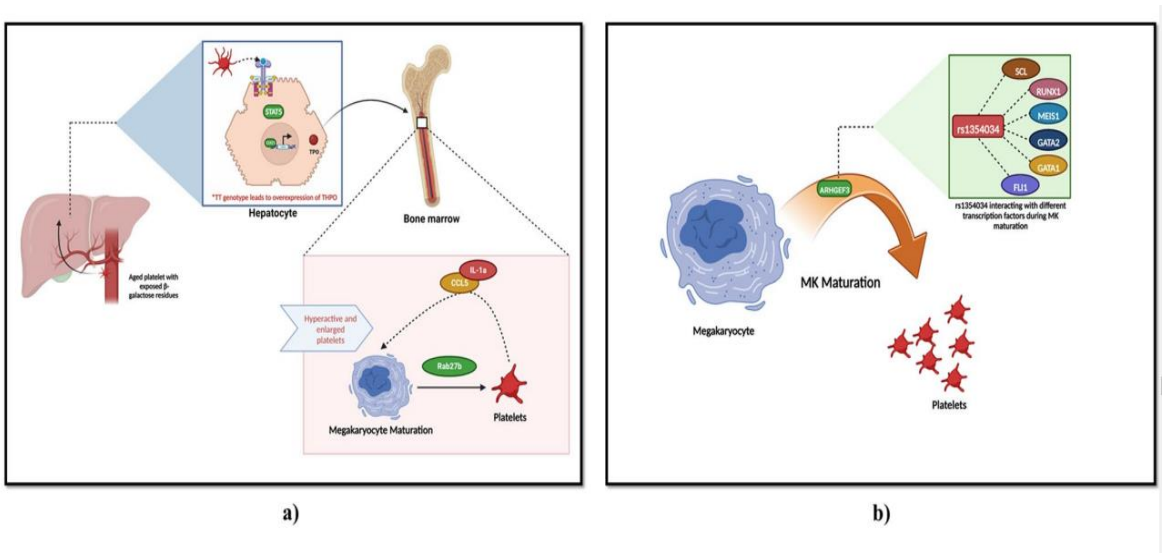


Fig 6: a) Desialylated platelets from circulation activates the JAK-STAT cascade in the hepatocytes leading to the activation of THPO gene, which further induces the MKs to produce platelets through c-mpl receptor in the bone marrow. b) ARHGEF3 upregulated during MK maturation, genomic region of ARHGEF3 where rs1354034 located may influence the binding of certain transcription factors promoting MK maturation to form platelets [10]

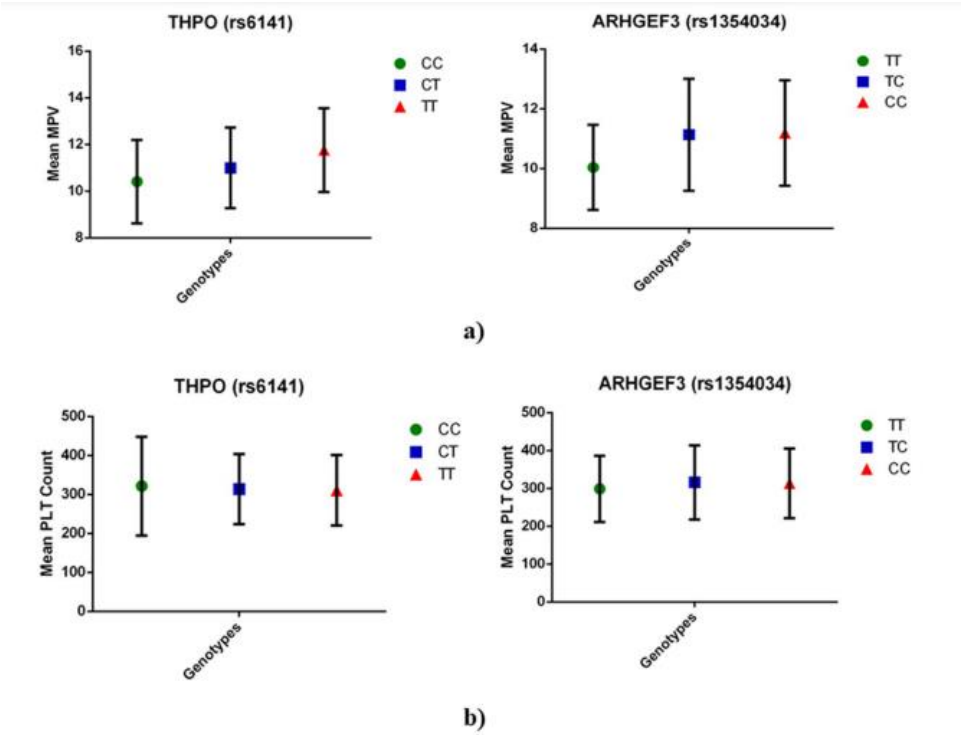


Fig 7: a) Association of Mean MPV with diferent genotypes of THPO (rs6141) and ARHGEF3 (rs1354034) genes. b) Association of Mean PLT count, and diferent genotypes of THPO (rs6141) and ARHGEF3 (rs1354034) genes [10].

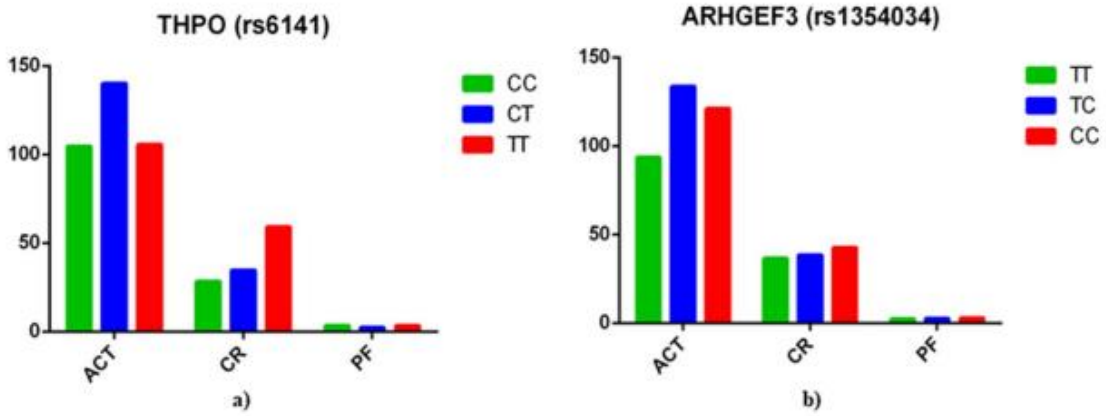


Fig 8: a) ACT, CR and PF values in patients bearing CC, CT and TT genotypes of THPO gene. b) ACT, CR, and PF values in patients bearing TT TC and CC genotypes of ARHGEF3 [10].

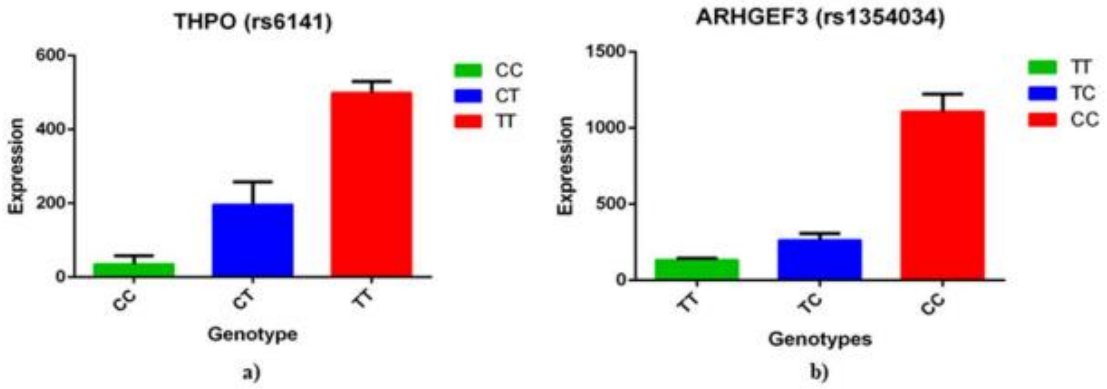


Fig 9: Expression analysis of different genotypes of a) THPO (rs6141) and b) ARHGEF3 (rs1354034) genes [10].

HEMOGLOBINOPATHIES

The inherited disorders of hemoglobin synthesis are the most common monogenic disorders worldwide. These disorders have been receiving increasing attention in India. About 10,000 children are born annually inheriting a major hemoglobin disorder. Though, there are many variants of thalassemia, β-thalassemia is the most common variant found in the Indian sub-continent. There are nearly 30 million thalassemia carriers in India with a wide geographic variation in the frequency. Asian Indian Gγ(Aγδβ)0 thalassemia and E-thalassemia (HbEE) have been reported from North India and the Eastern region of the country respectively. Since thalassemia is difficult to cure, it becomes a priority to prevent this disorder. The only cure for affected children is bone marrow transplantation which is expensive, risky and difficult to perform with discouraging success rates. The only treatment to sustain life is regular blood transfusion with iron chelation therapy, but the cost of desferal for iron chelation is very high. The procedure is painful and premature deaths result from inadequate chelation. Till date, > 300 β-thalassemia alleles have been characterized in and around the β-globin gene cluster. In most of the populations studied, it has been found that 5–6 mutations account for > 90% of the cases in a specific geographic/ethnic area. The most common effective approach for developing countries like India is preventing disorders associated with β-globin gene cluster and major efforts need to be directed at applying simple and well defined strategy to control these disorders by carrier detection, genetic counseling and prenatal diagnosis. Early detection of anemia in these disorders allows timely intervention, preventing serious consequences.

MOLECULAR CHARACTERIZATION OF THALASSEMIA

The hemoglobinopathies are a very heterogeneous group of congenital hemolytic anemias. They include thalassemias, hemoglobin variants and hereditary persistence of fetal hemoglobin. β-thalassemia is the most common monogenic disorder in India. Molecular characterization of this disease has revealed an extremely heterogeneous picture. 1592 blood samples from suspected cases were studied using high performance liquid chromatography, amplification refractory mutation system polymerase chain reaction and reverse dot blot techniques. Out of 1592 cases, we found 119 cases of β-thalassemia major, and 347 cases of β-thalassemia trait. In addition to this, cases with structural variants like sickle cell anemia, sickle cell trait, D-thalassemia (Hb DD), E-thalassemia (Hb EE), double heterozygotes and the hereditary persistence of fetal hemoglobin were also found. Molecular analysis revealed the presence of different β-thalassemia mutations in the population under study. Molecular analysis revealed that IVS1-5(G–C) and 619 bp deletion are the most common mutations in the population under study. The knowledge about the frequency of predominant mutations in the present population helps in offering prenatal diagnosis to the families having foetus at risk [11].

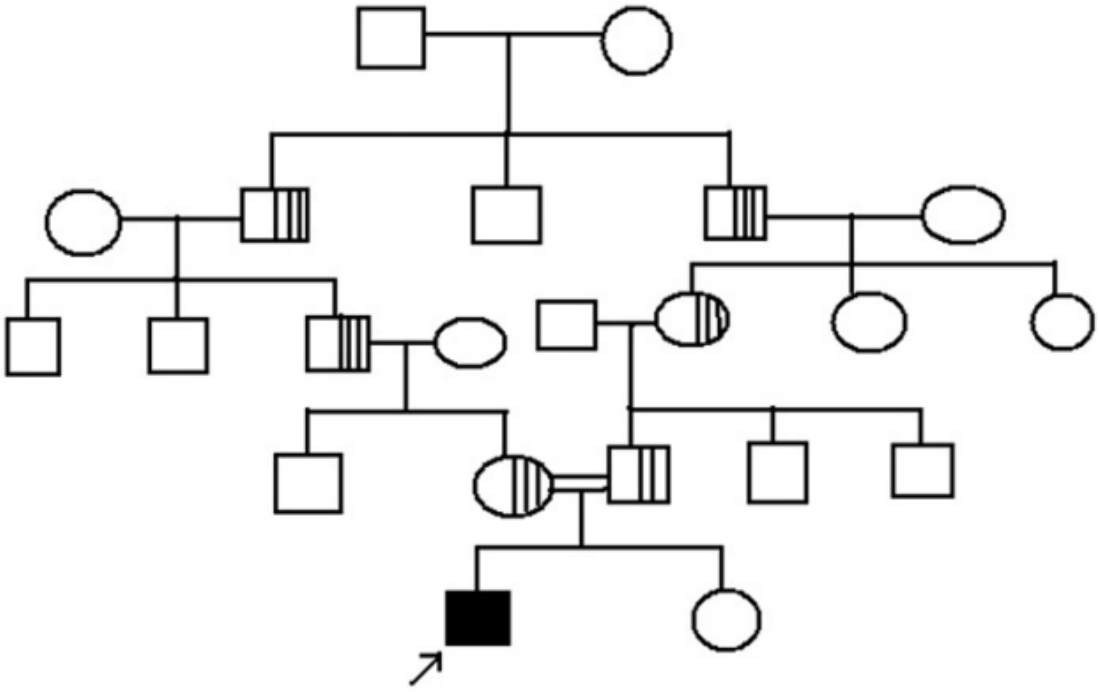


Fig 10: An extensive pedigree showing second cousin marriage. Parents are carriers for β-thalassemia. One child is affected [11].

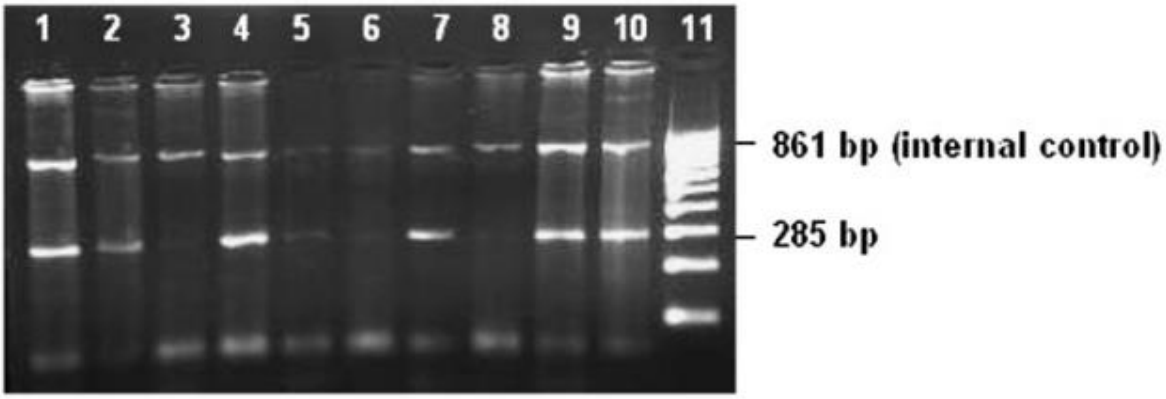


Fig 11: Ethidium bromide stained agarose gel analysis of the ARMS-PCR reactions amplified with primers specific for IVS 1–5 (G–C) normal/mutant (genotyping) and internal control primers. Lanes 1 & 2 and 9 & 10: Heterozygotes along with internal controls (representing parents of the affected child shown in the pedigree, Fig. 2). Lanes 3 & 4: Homozygous mutant along with internal control product (representing the affected male child in the pedigree). Lanes 5 & 6: Heterozygote along with the internal control product (representing the paternal grandmother of the affected child). Lanes 7 & 8: Homozygous normal along with internal control product (representing the sibling of the affected individual). Lane 11: 100 bb DNA ladder [11].

MODIFIER GENES

XMN1 restriction site polymorphism

There is considerable clinical variability between patients inheriting identical β-globin mutations. The reasons for this variability are not well understood. Previous studies have suggested that a variety of genetic determents influence different clinical phenotypes. The genetic variants that modulate HbF levels have a very strong impact on ameliorating the clinical phenotype. In this study 6,500 blood samples from suspected cases were analysed using HPLC, ARMS-PCR, RDB techniques. Patients with β-thalassemia and SCA were classified into mild, moderate, severe according to the severity score based on Hb levels, age of onset, age at which patients received their first blood transfusion, the degree of growth retardation and splenectomy. Patients with β-thalassemia and SCA were analysed for Xmn1 polymorphism and association between this polymorphism and severity of β-thalassemia and SCA was evaluated. We found a significant difference in genotypic and allelic frequencies of Xmn1

polymorphism between mild and moderate and mild and severe cases. There was a significant difference in high and low percentage of HbF in CC, CT and TT bearing individuals. The TT bearing individuals were found to have a high percentage of HbF in β-thalassemia as well as SCA. This study confirms that increased γG-globin expression associated with Xmn1 polymorphism ameliorates the clinical severity in β-thalassemia as well as SCA in the study population [12].

BCL11A gene

The amount of foetal haemoglobin that persists in adulthood affects the clinical severity of haemoglobinopathies including β-thalassaemia major and sickle cell anaemia (SCA). This study was undertaken to analyse β-thalassaemia as well as SCA patients for the single nucleotide polymorphism (SNP), rs11886868 (T/C) in BCL11A gene and to evaluate the association between this polymorphism and severity of β-thalassaemia major and SCA. There was a significant difference in genotypic and allelic frequencies of BCL11A gene polymorphism between mild and moderate and mild and severe cases in both the groups. A significant ($P<0.001$) difference was observed in the mean HbF levels between the three genotypes in different severity groups. HbF levels were found to be high in CC genotype bearing individuals followed by TC and TT in β-thalassaemia major as well as SCA. This study confirms that the T/C variant (rs11886868) of the BCL11A gene causing downregulation of BCL11A gene expression in adult erythroid precursors results in the induction of HbF and ameliorates the severity of β-thalassaemia as well as SCA [13].

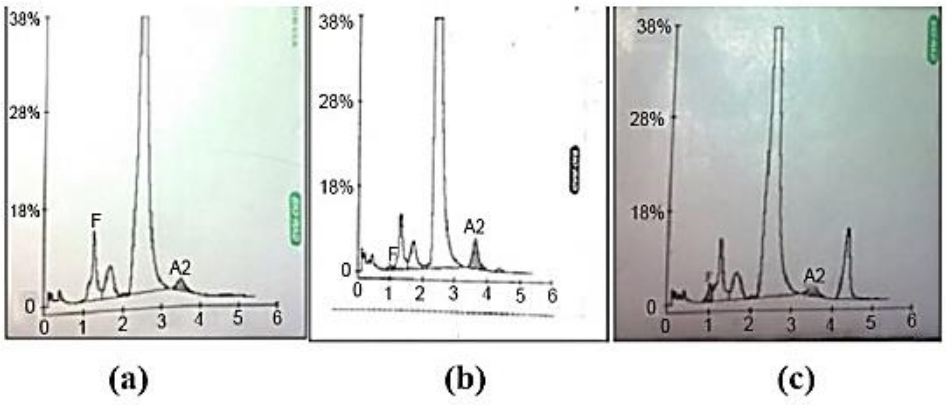


Fig 12: (a). HPLC chromatogram of a normal individual (A2- 2.1%; HbF-0.0% both A2 and HbF are in the normal range), (b) HPLC chromatogram of a β-thalassaemia carrier (A2-4.3%;

HbF-0.4% A2 is high), (c) HPLC chromatogram of a sickle cell carrier (HbS- 8.8%; HbF-1.7% HbS in high range).[13]

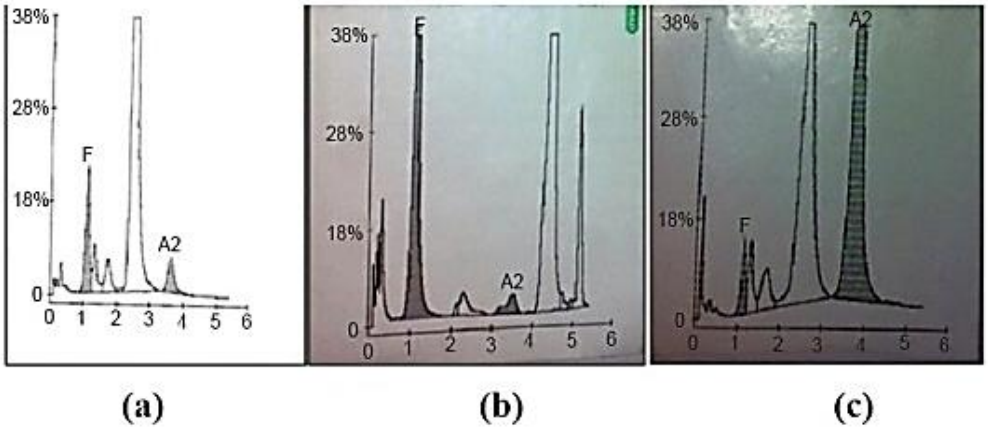


Fig 13: (a). HPLC chromatogram of a β-thalassaemia major (the chromatorgram is of a transfused beta thalassaemia major (A2-7.5%; HbF-3.7% both A2 and HbF are high), (b) HPLC chromatogram of a sickle cell anaemia (HbS-39.4%; HbF-10.6% both HbS and HbF are in high range), (c) HPLC chromatogram of a E-disease (A2-48.3%; hbF-3.9% A2 is too high HbF also high). [13].

BREAST CANCER GENOMICS AND PHARMACOGENOMICS

Alterations in BRCA2, PALB2, CHEK2, and p53 genes have been identified for their association with male breast cancer in various studies. The incidence of male breast cancer in India is consistent with its global rate. A study was carried out with an aim to evaluate the genetic alterations in male breast cancer patients from Malwa region of Punjab, India. Four male breast cancer patients belonging to different families were recruited from Guru Gobind Singh Medical College and Hospital, Faridkot, India. A total of 51 genes reported with implications in the pathogenesis of breast cancer were screened using next generation sequencing. Germline variations were found in BRCA1, BRCA2, PMS2, p53, and PALB2 genes, previously reported to be associated with MBC as well as FBC. In addition to these, 13 novel missense alterations were detected in eight genes including STK11, FZR1, PALB2, BRCA2, NF2, BAP1, BARD1, and CHEK2. Impact of these missense alterations on structure and function of protein was also analyzed through molecular dynamics simulation. Structural analysis of these single nucleotide polymorphisms (SNPs) revealed significant impact on the encoded protein functioning [14].

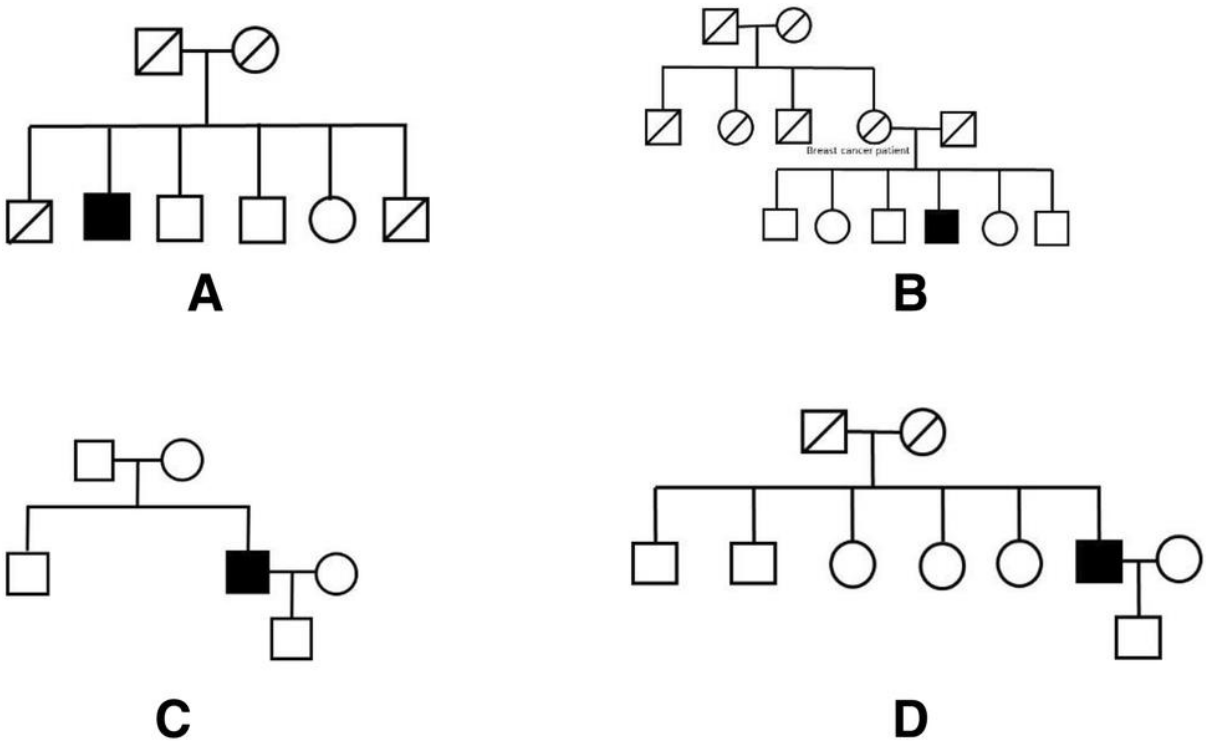


Fig 14: Pedigrees of MBC patients [14].

INFLAMMATORY MARKERS IN BREAST CANCER

Inflammation has a very strong association with different types of cancer. Tumor cells are highly proliferative in nature, and this proliferation is facilitated by many secretory factors, especially the inflammatory molecules released by tumor cells themselves or by other cells in the tumor microenvironment.

Role of pro and anti-inflammatory cytokines

The importance of the levels of interleukin (IL)-17, tumor necrosis factor, interferon γ, IL-10, IL-6, IL-4, and IL-2 with respect to clinicopathological data, prognosis, and disease-free survival was also determined in these patients. Two hundred and fifty female breast cancer patients and 250 age-matched controls were screened for variations in cytokine-encoding genes using global screening array microchip. The level of cytokines was estimated in 150 patients and 60 age-matched controls using BD™ Cytometric Bead Array (CBA) Human Th1/Th2/Th17 cytokine kit by BD Accuri flow cytometer. The difference in cytokine levels was evaluated by Mann–Whitney test. No significant variation in the genes encoding various cytokines was found between patients and controls. Out of the seven cytokines evaluated, the levels of IL-6 and IL-17a were found to be significantly high in patients in comparison with

controls (p = 0.001 and 0.02, respectively). The elevated levels of these cytokines are also associated significantly with poor outcome. We did not find any specific variation in the genes encoding various cytokines between patients and controls. However, there was a significant difference in the serum levels of IL-6 and IL-17a between patients and controls, and the elevated levels of these two cytokines associated significantly with poor outcome in breast cancer patients and, therefore, can be used as prognostic markers [15].

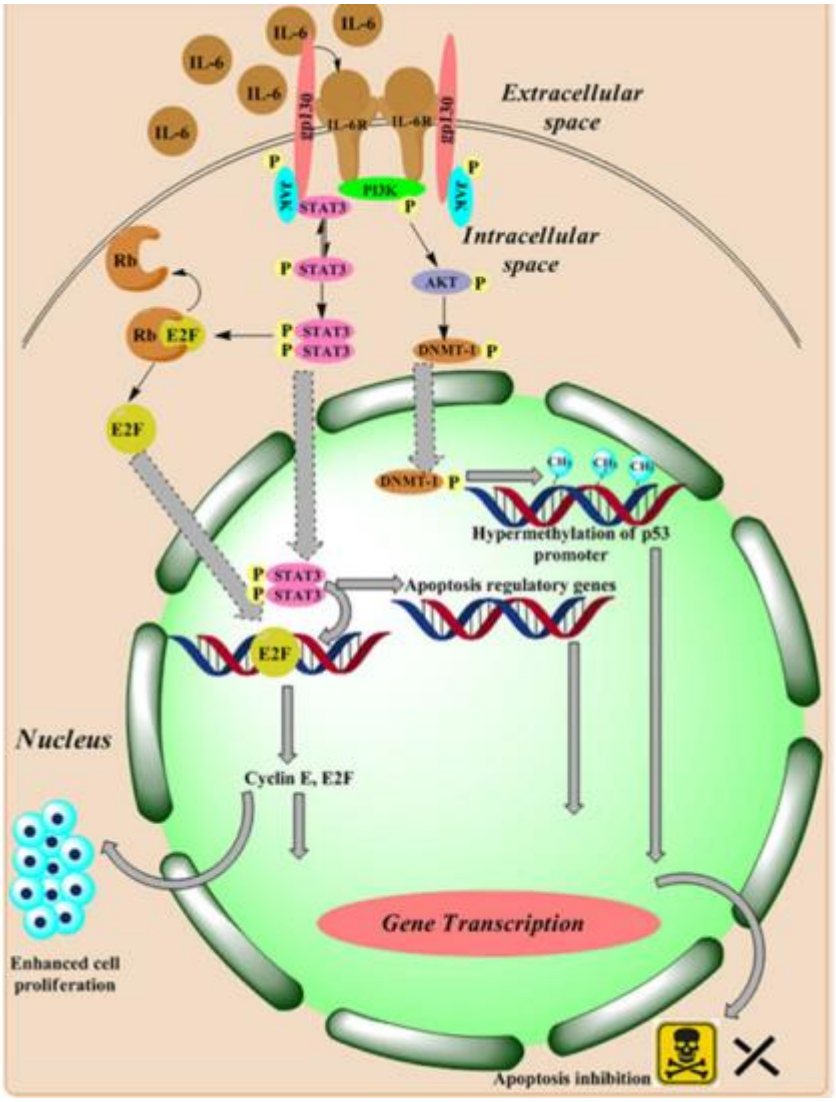


Fig 15: Involvement of IL-6 in tumor progression. IL-6 binds to IL-6 receptor and gp130 activates downstream signaling pathway and thereby promoting antiapoptosis, cell proliferation, and inhibits p53. DNMT: DNA methyltransferase; gp130: glycoprotein 130; IL: interleukin; JAK: Janus kinase; PI3K: phosphoinositide 3-kinase; STAT: signal transducer and activator of transcription [Color figure can be viewed at wileyonlinelibrary.com] [15].

PHARMACOGENOMICS OF CHEMOTHERAPEUTIC DRUGS IN BREAST CANCER

We have carried out a study with an aim to evaluate the variation in all the genes involved in pharmacokinetic and pharmacodynamics pathways of cyclophosphamide and doxorubicin, and correlate specific variants with disease outcome in breast cancer patients from the Malwa region of Punjab. Two gene variants, CYP2C19 (G681A) and ALDH1A1*2 (17 bp deletion), were found to be significantly associated with the disease outcome, including overall survival, recurrence and metastasis, in breast cancer patients on adjuvant therapy. Both these genes are involved in the pharmacokinetics of cyclophosphamide. However, none of the variants in the genes involved in pharmacokinetics and pharmacodynamics of doxorubicin were found to have any significant impact on disease outcome in the studied group. CYP2C19 (G681A) variant and ALDH1A1*2 emerged as two important biomarkers associated with bad outcome in breast cancer patients on adjuvant therapy [16].

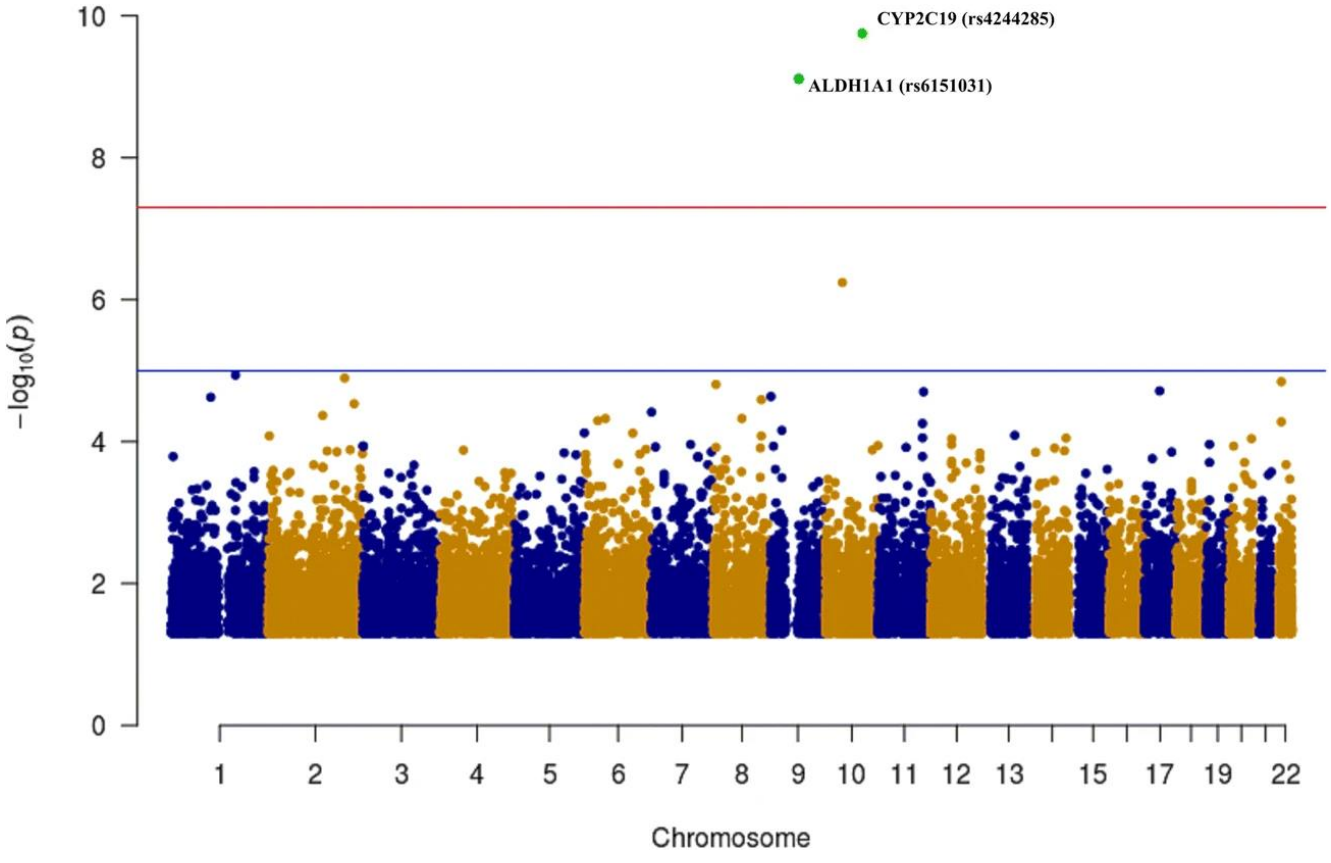


Fig 16: Manhattan plot representing CYP2C19*2 (rs4244285) and ALDH1A1*2 (rs6151031) SNPs plotted between chromosome number (location) and –log10p values

HER2+ Breast Cancer

Human epidermal growth factor receptor 2 positive (HER2+) breast cancer (BC) is an aggressive BC subtype characterized by HER2 overexpression/amplification. Genomic alterations of HER2 and others have been reported to be associated with, HER2 overexpression and prediction of trastuzumab-response. Here, we aimed at identifying germline and somatic alterations associated with HER2+ BC and evaluating their association with clinical outcome in response to trastuzumab therapy given to HER2+ BC patients. Global Sequencing Array (GSA) and polymerase chain reaction-restriction length polymorphism (PCR-RFLP) techniques were used to determine alterations in HER2 and other HER2-interacting as well as signaling-related genes in HER2+ BC. In addition, 20 formalin fixed paraffin-embedded tissue samples were also evaluated by GSA for identifying significant variations associated with HER + BC as well as response to trastuzumab therapy. A germline variant in HER2 (I655V) was found to be significantly associated with the risk of the disease ($p < 0.01$). A nonsense mutation in PTPN11 (K99X), a pathogenic CCND1 splice site variant (P241P), a hotspot missense mutation in PIK3CA (E542K) and a hotspot missense mutation in TP53 (R249S); were observed in 25%, 75%, 30% and 40% of the HER2+ BC tissue samples, respectively. Mutant CCND1 (P241P) and PIK3CA (E542K) were found to be significantly associated with reduced disease-free survival (DFS) in patients treated with trastuzumab (p: 0.018 and 0.005, respectively). These results indicate that HER2, PTPN11, CCND1 and PIK3CA genes are important biomarkers in HER2+ BC. Moreover, the patients harbouring mutant CCND1 and PIK3CA exhibit a poorer clinical outcome as compared to those carrying wild-type CCND1 and PIK3CA [17].

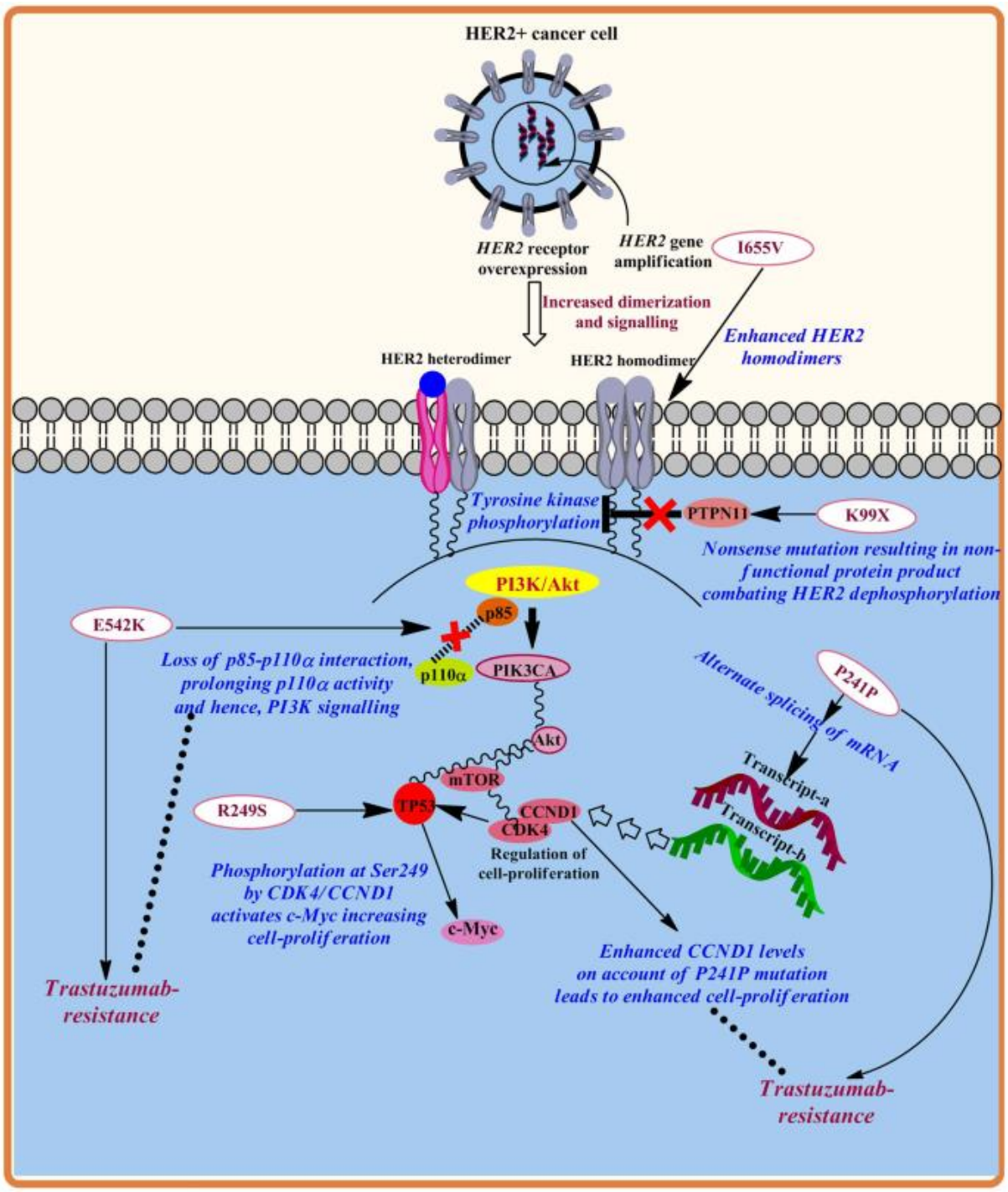


Fig 17: Functional implications of genomic alterations in *HER2* signal transduction. *HER2* germline variant along with *PTPN11* nonsense mutation exponentially multiplies *HER2*-mediated cell signaling. *CCND1*, *PIK3CA*, and *TP53* mutations lead to uncontrolled cell division and hence, elevated tumourigenesis. Mutant *CCND1* and *PIK3CA* confer resistance towards trastuzumab therapy [17]

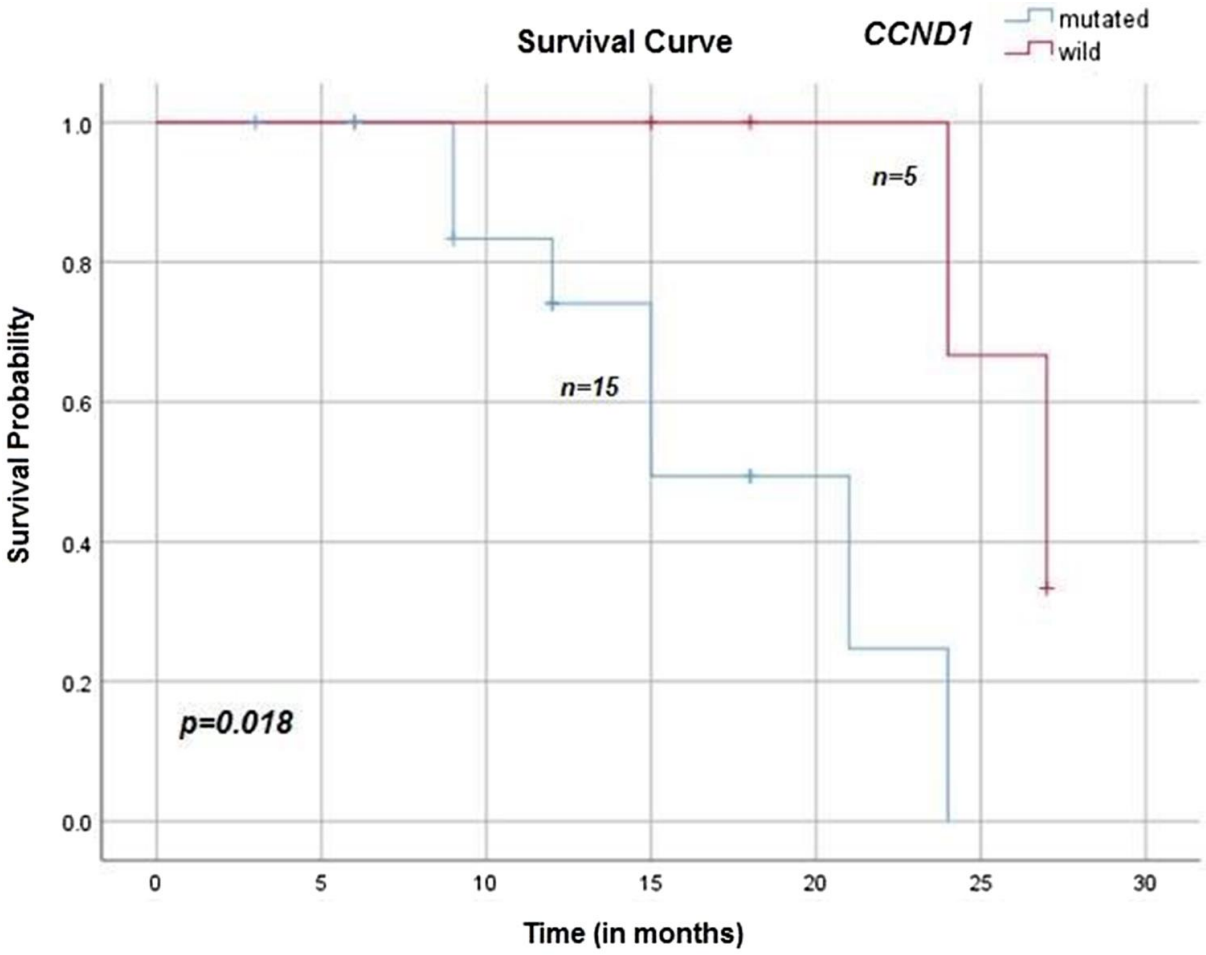


Fig 18: Kaplan–Meier plot showing DFS according to CCND1 (P241P) mutation status in HER2+ BC patients treated with trastuzumab [17]

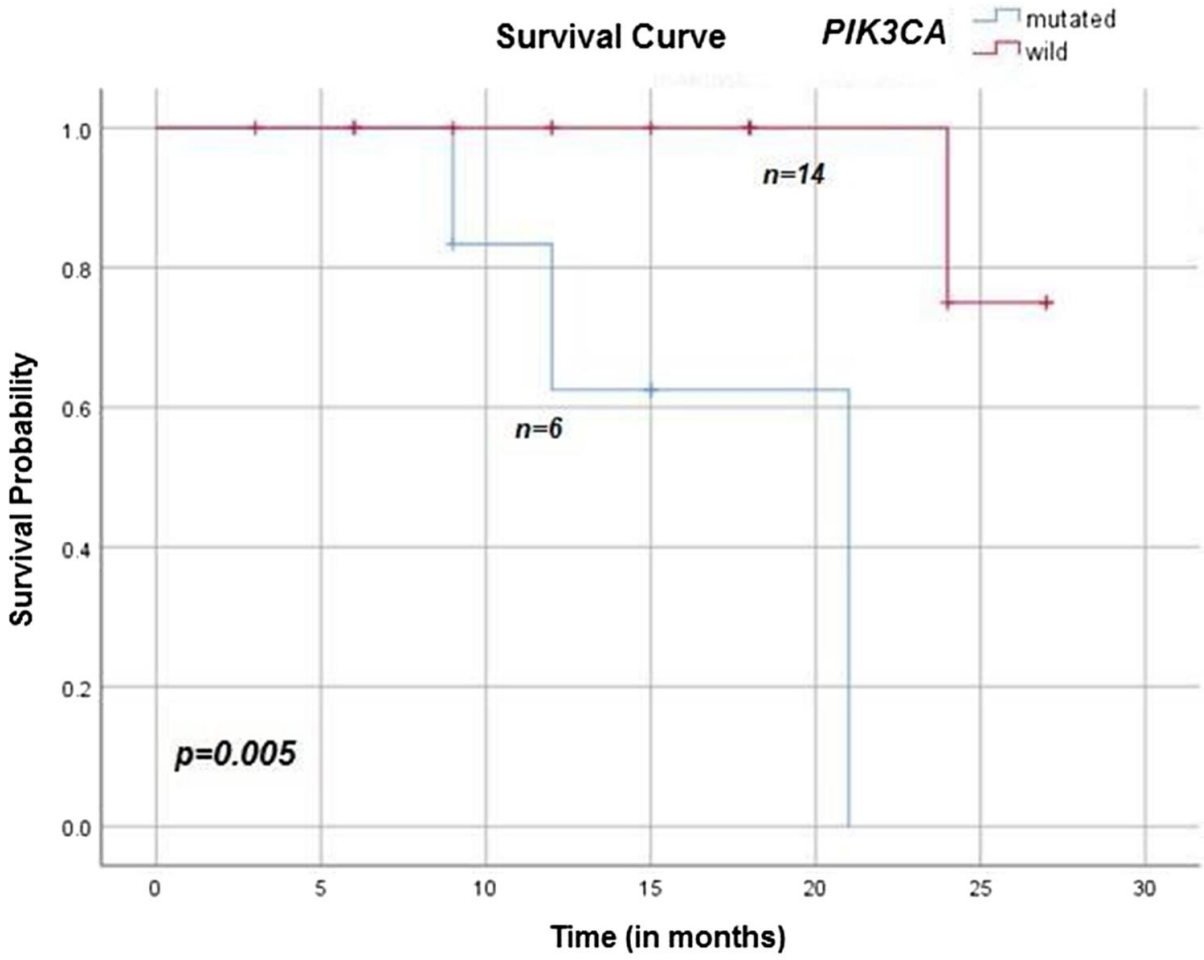


Fig 19: Kaplan–Meier plot showing DFS according to PIK3CA (E542K) mutation status in HER2+ BC patients treated with trastuzumab [17]

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