b. 10 Important Publications

1. Ludhiadch, A., Sulena, S., Singh, S., Chakraborty, S., Sharma, D., Kulharia, M., ... & **Munshi, A.** (2023). Genomic Variation Affecting MPV and PLT count in association with development of Ischemic Stroke and its Subtypes. ***Molecular Neurobiology.*** **(Impact Factor - 5.686)**.

   We observed an association of ARHGEF3 (rs1354034) and THPO (rs6141) genes with higher MPV, higher rate of clot formation, and risk of developing IS. Further, we also observed that MPV and PLT count showed an inverse relationship with mutant alleles of both genes. Expression analysis of both THPO and ARHGEF3 genes revealed a higher expression of variant genotypes in the platelets. In silico analysis carried out for THPO (rs6141) gene showed that the mutated protein has reduced compactness in the protein structure in comparison with the wild type, which might be resulting in the higher expression of the THPO gene in the platelets. We used UNAFold to predict the changes caused by the variant ARHGEF3 (rs1354034) at the mRNA level because it is difcult to simulate the mutated ARHGEF3 (rs1354034) since it is an intronic variant. It showed that the mutant (rs1354034) variant RNA exhibits qualitatively greater stability with respect to the free energy associated with the secondary structure as compared to normal ARHGEF3 (rs1354034), which might lead to a higher expression in the platelets. Based on the STRING analysis, it was observed that these two signifcant proteins interact with other proteins which are involved in various pathways such as platelet activation, aggregation, erythropoiesis, megakaryocyte development, cytoskeleton organization, and cell adhesion. Cell migration, vascular development, apoptosis, cell proliferation, and cholesterol homeostasis. The current study is a step forward to establish MPV as a diagnostic or prognostic marker for IS. There is a need to develop specific treatment strategies that can particularly reduce MPV.

2. **Munshi, A.** (2012). Genetic variation in MDR1, LPL and eNOS genes and the response to atorvastatin treatment in ischemic stroke. ***Human genetics***, *131*(11), 1775-1781. **(Impact factor: 5.881).**

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 telephone interviews were conducted with patients post-event to determine stroke outcome. Blood samples were collected and genotypes determined by polymerase chain reaction-restriction digestion technique. 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 stratin 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.

3. **Munshi, A.,** Kaul, S., Aliya, N., Shafi, G., Alladi, S., & Jyothy, A. (2009). Prothombin gene G20210A mutation is not a risk factor for ischemic stroke in a South Indian Hyderabadi Population. ***Thrombosis research***, *124*(2), 245-247. **(Impact factor: 10.409).**

In this study, we investigated the association between the G20210A prothrombin gene variant and ischemic stroke in a South Indian Hyderabadi population. The study was approved by the Institutional Review Board. The ethical clearance was also obtained from the study hospital. The study consisted of one hundred and sixty two ischemic stroke patients (Males: Females= 120: 42) presenting with new or recurrent stroke evaluated in the stroke clinic of Nizams Institute of Medical Sciences, Hyderabad (A.P., India) between July 2007- March 2008. Informed consent was obtained from all the subjects included in the study. Each patient with acute stroke was examined by a

qualified stroke neurologist to confirm the diagnosis. Ischemic strokes were differentiated by computed tomography scans and magnetic resonance imaging and classified according to TOAST classification [16].

When the factor G202010A polymorphism was analyzed, none of the study subjects were either heterozygous or homozygous for this gene mutation as indicated by PCR analysis followed by restriction digestion. Even SSCP analysis of PCR products did not show any mobility shift of bands further confirming the absence of any mutation (data not shown). Various studies have indicated that PT 20210A is uncommon in indigenous population from north and western India. The identification of various candidate genes associated with stroke gives some hope that genetic studies might have a direct impact on the treatment of the disease. However, a separate study is necessary for each population with a distinct ethnic background. In conclusion, our results suggest that prothrombin G20210A polymorphism is either absent or very rare among the Hyderabadi population from South India and therefore, should not be included in the diagnostic panel of cerebral ischemia. However, patients and normal individuals from other parts of the state need to be studied before evaluating the exact role of this mutation in stroke patients from this region

4. **Munshi, A.,** Sharma, V., Kaul, S., Al-Hazzani, A., Alshatwi, A. A., Manohar, V. R., & Jyothy, A. (2010). Estrogen receptor α genetic variants and the risk of stroke in a South Indian population from Andhra Pradesh. ***Clinica Chimica Acta***, *411*(21-22), 1817-1821. **(Impact factor: 6.315).**

The present 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. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

5. **Munshi, A.,** Anandraj, M. P. J. S., Joseph, J., Shafi, G., Anila, A. N., & Jyothy, A. (2009). Inherited hemoglobin disorders in Andhra Pradesh, India: a population study. ***Clinica Chimica Acta***, *400*(1-2), 117-119. **(Impact factor: 6.315).**

   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. 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 fetus at risk.

   Our cases came from the neighbouring district of Hyderabad and showed geographical variation in terms of mutations. For example HbE families in our study came from

Adilabad, whereas all HbE/β-thalassemia were from East Godavari. Majority of the HbS cases were from Visakhapatnam District. The occurrence of HbE gene in this State has been reported previously showing that this gene is not restricted to Eastern region of the country only where its prevalence is high. In Andhra Pradesh, the conservative families marry their children within the same caste and community and also perform consanguineous marriages, therefore, certain communities/castes/tribes show high incidence of these disorders. Therefore, counseling for screening before marriage needs to be encouraged in order to avoid the mental and physical trauma along with financial burden of an affected child. Though prenatal diagnosis is available for the fetus at risk, it is advisable to prevent the conception rather than having an affected fetus.

6. Dadheech, S., Rao, A. V., Shaheen, U., Hussien, M. D., Jain, S., Jyothy, A., & **Munshi, A.** (2013). Three most common nonsynonymous UGT1A6* 2 polymorphisms (Thr181Ala, Arg184Serand Ser7Ala) and therapeutic response to deferiprone in β-thalassemia major patients. ***Gene,*** *531*(2), 301-305. **(Impact factor: 3.919).**

Deferiprone is used as a chelation agent in chronic iron overload in β-thalassemia patients. Patients on deferiprone therapy show variable response to this drug in terms of reduction in iron overload as well as adverse drug reactions (ADRs). The pharmacogenetic studies on deferiprone have not carried out in patients with blood disorders in India. Therefore, this study was carried out to evaluate the three most common nonsynonymous UGT1A6 polymorphisms Thr181Ala (541 A/G), Arg184Ser (552 A/C) and Ser7A8. la (19 T/G) and therapeutic response to deferiprone in β-thalassemia major patients. Two hundred and eighty six (286) β-thalassemia major patients were involved in the study. Serum ferritin levels were estimated periodically to assess the status of the iron overload and the patients were grouped into responders and non-responders depending on the ferritin levels. The UGT1A6*2 polymorphisms were detected by PCR-RFLP methods. The association between the genotypes and outcome as well as ADRs was evaluated by Open EPI software. A significant difference was observed in the genotypic distribution of UGT1A6*2 Thr181Ala polymorphism in responders and non-responders. However, there was no difference in the genotypic distribution between patients with and without ADRs. As far as the UGT1A6*2 Arg184Ser polymorphism is concerned, no significant difference was observed between responders and non-responders. Further, evaluating the association of

UGT1A6*2 Ser7Ala polymorphism with drug response, there was no significant difference in the genotypic distribution between responders and non-responders. However, there was a significant difference between responders with and without ADRs and non-responders with and without ADRs. In addition to this haplotype analysis was also carried out. However, we did not find any specific haplotype to be significantly associated with the deferiprone response in β-thalassemia major patients.

7. Kaur, R. P., Vasudeva, K., Singla, H., Benipal, R. P. S., Khetarpal, P., & **Munshi, A.** (2018). Analysis of pro-and anti-inflammatory cytokine gene variants and serum cytokine levels as prognostic markers in breast cancer. ***Journal of Cellular Physiology***, *233*(12), 9716-9723. **(Impact factor - 6.513).**

   The aim of this study was to evaluate the genetic variation in all the genes encoding pro- and anti-inflammatory cytokines in association with breast cancer development in patients from Malwa region of Punjab. 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 and PCR-RFLP. 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.

8. Kalra, S., Kaur, R. P., Ludhiadch, A., Shafi, G., Vashista, R., Kumar, R., & **Munshi, A.** (2018). Association of CYP2C19* 2 and ALDH1A1* 1/* 2 variants with disease outcome

in breast cancer patients: results of a global screening array. ***European Journal of Clinical Pharmacology***, *74*(10), 1291-1298. (**Impact factor -3.064)**

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.

9. Kaur, R. P., Kumar, V., Shafi, G., Vashistha, R., Kulharia, M., & **Munshi, A.** (2019). A study of mechanistic mapping of novel SNPs to male breast cancer. ***Medical Oncology***, *36*(8), 1-7. **(Impact factor - 3.738).**

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. This 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. Surprisingly, these alterations were observed in all the patients from this region in MBC cases, which is a rare disease in comparison with FBC. These alterations if confirmed in more MBC patients from this region might guide us in terms of management of the disease in this region. No doubt MBC, like other multifactorial rare diseases, does suffer from the absence of comprehensive studies, thereby restricting the translation of these research finding into a personalized management of the disease. However, studies investigating the appropriate screening and risk management tools for MBC patients might lead to appropriate treatment strategies for this rare disease in future.

10. Singla, H., Kaur, R. P., Shafi, G., Vashistha, R., Banipal, R. P. S., Kumar, V., & **Munshi, A.** (2019). Genomic alterations associated with HER2+ breast cancer risk and clinical outcome in response to trastuzumab. ***Molecular biology reports***, *46*(1), 823-831. **(Impact factor - 2.742).**

    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