

Chapter 6:

**Summary and
Conclusion**

6. Summary and Conclusion

This section has been divided into two subcategories for better understanding, namely, population based study and RA based study..

6.1. Population Based Study

Analyses of the genotypic and haplotypic organization of the KIR loci among the five populations have deciphered vital information about the amount, pattern and distribution of genetic variation in different studied populations of India. The present study provided valuable information to comprehend the effect of socio-cultural barriers on the genetic makeup of Indian populations. Furthermore, the perspective correlation of the KIR genetic profile of studied populations with the past human movements and other historical records facilitated the determination of their genetic ancestry/origin. The results also provided an inference about the pattern and causes of genetic similarity and differences between different Indian and world populations.

The analyses of genome wide distributed KIR loci in all the populations have divulged surfeit of facts about the genetic sketch of studied populations. The similarities and differences in the KIR gene frequencies, measures of genetic distances, haplotype distribution and several other parameters, including LD analysis, have demonstrated that the contemporary studied populations are highly diverse population groups. The reason of such high diversity include both accumulation of large number of mutations during long term survival in a particular environment and the colossal gene flow from all over the world. Furthermore, genetic variations within and between the populations revealed a definite pattern of diversity distribution, wherein populations structured into socio-religious groups have more genetic similarity within their own group and are genetically more distant from populations of other groups.

Our study is a pioneering population-based analysis of KIR genes in the five populations, which we have selected from the sub-Himalayan region of West Bengal, India. It was evident from our KIR genotyping data, that among the studied populations, the

Rajbanshis showed the lowest genetic distance with the Rabha population. This could be due to the fact that Rajbanshis adopted the process of transformation of a tribe into a caste resulting in a Tribe-Caste continuum, and in between this process population like Rajbanshi who were once tribal, gradually adopted the attributes of caste system (Kumar, *et al.*, 2004). Interestingly, the Muslims are the second closest population to the Rajbanshis. Another interesting fact was that Muslims showed the lowest genetic distance with the heterogenous Bengali population. This may be because of the fact that although Muslims practiced consanguinity, they married outside their religion. Previously published reports also documented that a section of the present day Bengali Muslims are converts. They were once low cast Bengali Hindus before Muslim invasions, who were forcefully converted to Islam (Esposito, 2003). On the other hand, the Gurkhas showed lesser genetic distance with the Rabhas, compared to the Rajbanshis. These findings provoked us to hypothesize gene flow among the studied groups owing to the recent massive gene flow between the populations or possible identical recent common ancestor.

Finally, the phylogenetic assessment based on KIR loci revealed that four of the studied populations except the Rabhas, clustered with other Indian populations. In contrast, the Rabhas shared the same clade with the East Asians owing to their tribal ethnicity and strict endogamous nature, which helped them to retain their ancestral genotypic pattern. This may demonstrate substantial gene flow from the geographically neighboring populations owing to their settlements along the human migratory routes. Furthermore, proximity of the Muslims with the Bengali population may indicate the role of environmental selection on the KIR repertoire of a population in addition to influences from migratory and gene flow events. Thus, it can be said that the phylogeny of KIR polymorphisms studied in the five Indian populations generated a molecular narration of the population demography, relatedness, and genetic substrata reflecting possible signatures of ancestors and novel micro-evolutionary genetic differentiations.

In summary, our study based on KIR genotypic profiles, suggests the following statements:

- ❖ In spite of having Indian origin, the Indo-European speaking Rajbanshis from the Northern part of Bengal has Tibeto-Burman influence. However, this population is highly diversified with considerable admixtures from neighboring population. Furthermore, detailed analysis is required to characterize all the available KIR haplotypes.
- ❖ In the little known Rabha tribe, the influence of mongoloid element in Rabha gene pool was very much prominent, which was well maintained due to their strict endogamous character.
- ❖ The Indo-European speaking Bengalis from Northern part of Bengal share both Dravidian and Indo-Aryan gene pool with some Mongoloid and European influences, thus becoming one of the most heterogenetically diverse community of the Indian Sub-continent.
- ❖ The Gurkhas have shown prominent tendency cluster with the NEAs and the SEAs. This suggests strong connection with populations of East Asian lineage. However, sub-continental influence on their gene pool cannot be ignored. Furthermore, this study also suggests the continuous gene flow between Nepali speaking Gurkha population of India with the people of Nepal, whose gene pool has received considerable influence from both the South Asian and East Asian lineages.
- ❖ Genetic architecture of the Muslims from the Northern part of Bengal has received considerable influence not only from the neighboring populations of Bangladesh but also from the historical invasions of the Middle-Eastern populations. This study has also revealed the presence of Tibeto Burman element in the Muslim population of this region, which may differentiate them from other Muslim populations of India.

Conclusively, the combined picture represented by the empirical results of the analyses of KIR Loci infers that:

- ❖ The studied five populations are genetically highly diverse people with most of the variation scattered between individuals.

- ❖ The genetic differentiation does exist between numerous of endogamous groups across Indian mainland, but the differentiation is mainly configured geographically and linguistically.
- ❖ The influence of the Sino-Tibetan migration across the North Eastern Himalayan corridor is prominent on the gene pool of the populations in the Sub-Himalayan regions of the Eastern and North Eastern part of the country.

Overall, the genetic configuration of Indians is extremely complex, interwoven in numerous threads of unknown facts. More genetic data from the North Eastern part of the country is highly necessitated in tracing the missing blocks of the causes and consequences of human genetic variation. The present study has provided some clues that might help in unwinding the complex interwoven threads of Indian genetic composition. However, further studies on other available genetic markers may fine-tune the present knowledge of the genetic background of both the populations.

6.2. RA-Based Study

Rheumatoid Arthritis (RA) is one of the most common autoimmune diseases affecting nearly 1% of the population worldwide and is characterized by the progressive articular damage leading to joint deformities and disability. The multi-factorial nature of RA provides high disease heterogeneity with the specific combinations of a genetic background and environmental factors that influence the susceptibility, severity and outcome of the disease. RA heterogeneity is marked by the presence of distinct autoantibodies, such as rheumatoid factor and anti-CCP antibodies. In addition, genetic contribution has been estimated to be 50–60% towards the etiology of RA. In the present study, the aim was to study the association of the KIR genes with the prevalence of RA and to estimate the levels of clinical parameters like RF and anti CCP in the RA patients in comparison to the control group.

The finding from this study can be summarized as follows:

- ❖ The sensitivity and specificity of RF titre and anti-CCP antibodies in case of RA patients of this region was more or less consistent with the previously published data.

Lower to moderate specificity of RF titre assay may be one of the underlying reasons behind selecting anti-CCP antibodies as another important serological marker in RA diagnosis.

- ❖ The Pearson correlation coefficient between anti-CCP and RF titres were found to be 0.716 and 0.356 respectively, among the patient and the control groups, thereby signifying that the presence of both the factors i.e. anti-CCP and RF are relevant as far as the diagnosis of the disease is concerned.
- ❖ Although, it was observed from the ROC curve that the AUC for anti-CCP was slightly greater than that of RF titre, but there were several crossovers, suggesting that neither of the serological markers is superior to the other in all circumstances of the study and performing both the tests in combination may be helpful in proper diagnosis of RA.
- ❖ Both anti-CCP and RF titre in combination with ESR can predict shared variance in Das28, which is an important diagnostic criteria for RA.
- ❖ In a small section of the study, it was shown that CRP and ESR are the two crucial measures of RA prognosis as they are the determinant of inflammation, which in turn play the central role in the pathogenesis of RA.
- ❖ This study also reported ASO titre as another essential clinical parameter of RA pathogenesis especially, during the occurrence of acute phase reactions and rheumatic fever.
- ❖ This study further demonstrated that serum ceruloplasmin has shown significant differences between the RA patients and the control groups. Thus, the study predicts the active role of ceruloplasmin in governing the serum antioxidant activity which when increased may be an important component of the systemic inflammatory response during RA. This study further documented the moderate differences in serum creatinine level between RA patients and control blood samples. Hence, it is predicted that although, serum creatine is not a very potent tool for early diagnosis of RA but can be useful for detection and treatment of nephropathy during late Rheumatoid Arthritis.
- ❖ Molecular typing of KIR genes was also performed in the RA patient group and the control group. The results demonstrated the significant associations of *KIR2DL3*,

KIR2DS2 and *KIR2DL2* with RA when compared with control samples. Thus, it can be said that, decreased frequency of *KIR2DL3* among the RA patient group may indicate the protective role of the gene against the incidence of RA. On the other hand, increased frequencies of *KIR2DS2* and *KIR2DL2* may imply their role in enhancing the odds of the disease occurrence. No significant differences were observed in the proportions of AA and Bx genotypes between the patient and the control group, but when the Bx genotypes was divided into two groups namely AB and BB, it was found that the frequency of the BB genotypes were significantly higher among the patients compared to the control.

- ❖ Further detailed analyses of the KIR genotypes have revealed that genotype ID 3 and 81 showed significant association with RA, suggesting their role as susceptibility factors of the disease.
- ❖ Based on the two LD parameters, which have biological interpretations, namely D' and r^2 , it was found that five such two-locus KIR haplotypic combinations were reported having both the values high namely *KIR2DL2-KIR2DS2*, *KIR3DL1-KIR2DS4*, *KIR2DS1-KIR2DL3*, *KIR2DS1-KIR3DS1* and *KIR2DS1-KIR2DS5*. Among them, highest values for these two parameters were reported for *KIR3DL1-KIR2DS4* followed by *KIR2DL2-KIR2DS2*. Since, both *KIR2DS2* and *KIR2DL2* were found to be significantly associated with Rheumatoid Arthritis, hence it can be inferred that both *KIR2DL2* and *KIR2DS2* are good predictors of each other and are co-inherited roughly 87% of the time.

In this work, the main goal was to determine the genetic predisposition to RA and the role of KIR genes in the disease pathogenesis in a multi-ethnic population residing in Siliguri and adjoining areas of North Bengal, India. Taken together, the current findings on RA risk based on KIR genetic profile may provide a bird-eye picture of the genetic basis of the disease. In addition, further detailed studies are required on allelic polymorphism of the KIR genes, which may illuminate the role of KIR molecules in RA pathogenesis in these important communities of the sub-Himalayan region of Eastern India.

In conclusion, the present work has demonstrated the genotyping of KIR genes and made an effort to construct a database for five population groups in the sub-Himalayan region of West Bengal, India. Our investigation highlighted the polymorphic nature of KIR genes and their role in constructing the genetic structure of a population. This work further demonstrated the role of KIR genes in the pathogenesis of RA and added to the current understanding and comprehension of the central role of NK cells in immunity.