Thalassemia is an autosomal recessive disorder as a result of mutations in $\alpha$ or $\beta$-globin gene. Inspite of possessing apparently the same mutations, the phenotypic manifestation of the disease varies widely among thalassemia patients. Patients with low, intermediate and high degree of severity of the disease are readily found and the Indian population is no exception in this respect. The objective of this thesis "Studies on Thalassemia in Eastern India" is an attempt to reveal some of the underlying molecular events in order to explain this difference in severity of the disease in the eastern Indian population. This study was carried out in the Department of Biophysics, Molecular Biology & Genetics, University of Calcutta.

The first chapter of the thesis briefly summarizes our present knowledge about $\beta$-thalassemias and the molecular mechanisms known to affect the severity in $\beta$-thalassemias. Hereditary persistence of fetal hemoglobin (HPFH) syndromes and conditions leading to high fetal hemoglobin (HbF) were emphasized on as these conditions are well known to affect the severity. Co-inheritance of $\alpha$-thalassemia and $\alpha$-triplication also affect the severity in $\beta$-thalassemia and these topics have also been described briefly.

The second chapter takes into consideration three groups of $\beta$ and $\varepsilon\beta$ thalassemia patients with low, intermediate and high clinical severity. The $\beta$-gene mutations were determined by the Amplification Refractory Mutation System (ARMS). The same patients were investigated for factors that modulate the severity of the disease, i.e., mutation of $\beta$-globin gene, presence of $\alpha$-deletion or $\alpha$-triplication and
the presence/absence of an Xmnl site at the -158 position of the Gγ-gene. Presence of α-deletion and/or homozygosity for the presence of the Xmnl site was in general associated with less severe disease. About 12% of the patients harbored single α-gene deletion and the ratio of Xmnl (+) allele to Xmnl (-) allele was 0.94.

The third chapter deals with the specific configurations of an AT-rich polymorphic motif (AT)_x T_y at the -540 region of the β-globin gene cap site in two selected groups of patients, one group being severely affected with regular transfusion dependency and the other with low severity and no transfusions. This polymorphic motif, considered in conjunction with the -158 Gγ (C→T) Xmnl polymorphism has been reported to influence HbF levels. Ten different configurations of the (AT)_x T_y motif were identified in this study and 7 of them carried a single -C deletion, which, to the best of our knowledge is hitherto unreported. The individual sequences of the motifs were determined by allele specific sequencing and cloning the motifs into plasmids followed by PCR based sequencing. The data presented in this chapter suggest that the presence of the Xmnl site at -158 Gγ may be important for less clinical severity and higher HbF response in thalassemics. The effect of (AT)_x T_y motif on clinical severity was also investigated.

A case study has also been carried out on a family where two brothers inherited similar β-gene mutations from the parents, yet one of them were clinically very severely affected while the other enjoyed a milder clinical course. It was determined after PCR based microsatellite marker studies that the brothers inherited similar chromosomes 6 but different X-chromosomes. Notably, both these
chromosomes harbor F-cell loci on them and an X-linked locus might be responsible
here for the clinically milder phenotype in one of the brothers.

The fourth chapter describes the detection of two rare mutations in the
Eastern Indian population. Part A of this chapter highlights a case of the rare Asian –
Indian Gγ (Aγδβ)° thalassemia mutation in a female patient which was associated with
a high HbF phenotype. In Part B of this chapter, the detection of a rare β°(IVS-I-130
G→C) splice acceptor site mutation has been elucidated. It was shown that this
mutation could be detected by a restriction enzyme Saul. Mutation specific ARMS –
primer could also be designed which detected the mutation with ease and accuracy.

The fifth and last chapter describes the solutions, chemicals and buffers used
throughout this study.