The present doctoral thesis entitled “Analysis of the natural evolution of the hepatitis B virus quasispecies in the preCore/Core regions and treatment effects using conventional techniques and ultra-deep pyrosequencing” is the research line on the study of the Hepatitis B Virus (HBV) quasispecies variability. In particular, the preCore/Core regions have been studied as the least overlapping regions of the HBV genome and due to its relation to the immune response: the PreCore encodes the "e" antigen (HBeAg) immunomodulator and the Core gene encodes the nucleocapsid, wtih abilities to stimulate the host immune system.

Three studies constitute the thesis. In the first study the Core gene of 185 samples was analyzed by conventional sequencing. Specifically, four regions were studied: the region between the aminoacids 1 to 11, the minor Th28-47 epitop and the main Th50-69 and B74-84 epitopes. Chronic hepatitis B patients and inactive HBV carriers were included in the study and samples were grouped attending the absence of treatment or the administration of interferon, lamividune or adefovir. The results confirmed the immunostimulating effect of interferon, basically in Th50-69 and B74-84 main epitopes. Besides, lamivudine or adefovir administration induced variability in minor Th28-47 epitop, indicating a possible immunoestimulator effect of these treatments different to the observed under interferon. Finally, differences in Core variability were observed between chronic cases and inactive HBV carriers, suggesting possible differences in the immune escape mechanisms.

The ultra-deep pyrosequencing was applied in the second study to analyse the *quasispecies* of the preCore region, in addition a technique was designed to analyze the YMDD motif of the polymerase of the same viral genome. The designed method of this work enabled to establish the sequencing error rate (0.03%), because an internal control sequence was included, which was also needed to join the preCore and the polymerase. The simultaneously presence of mutations in the preCore and YMDD motif of the Polymerase in the same viral genome was confirmed. In baseline samples without treatment, genomes containing mutations against treatment were detected in low proportions. Indeed, in HBeAg (+) samples, genomes with preCore mutations and codifying HBeAg (-) variants were also detected in low proportions. Also high conserved positions were described, indicating possible essential roles in HBV replication cycle. Finally, it was described that the estability of the secondary structure adopted by the preCore region of the pregenomic RNA limited the nucleotidic variability.

The third study was focused on the analysis of the Th50-69 and B 74-84 epitopes by methods of ultra-deep pyrosequencing. Due to the complexity and cost of the technique, just 4 baseline samples and 2 samples corresponding to one patient were included in the study (to complete a sequential analysis). However, the more than 200,000 sequences obtained suggested mechanisms of variability in the Core gene that might be genotype-dependant. High conserved positions were detected; some of them described as essential for the nucleocapsid surface antigens interactions. The sequential study of one patient demonstrated that on minor baseline variant (1.31%) was selected as the main poplation after a treatment-free period and it was also maintained after lamivudine treatment.

The results from the thesis are in accordance with the high HBV variability. The next-generation sequencing technologies showed the high complexity of the quasispecies, some essential positions and possible specific mechanisms of HBV replication.