The study aims at understanding the importance of the genes LIMD1, RBSP3 and CDC25A in the development of Head and neck squamous cell carcinoma. To this end, profiling of molecular signature (expression/ promoter methylation) was first performed in basal/ parabasal and spinous layers of normal epithelium, followed by deciphering the mechanism of alterations (expression/ promoter methylation/ deletion/ mutation) in dysplastic epithelium and head and neck tumours. Etiological factors (HPV, tobacco) were used to compare the different factors in the study. Clinico-pathological characteristics of patients were correlated with the results obtained. Different alleles of markers of RBSP3, LIMD1 and CDC25A were determined in normal specimens, followed by detection of different sized alleles undergoing deletion in HNSCC. Homozygous and heterozygous alleles of various sizes were correlated with expression of the proteins in normal epithelium. Finally, the importance of the genes with respect to the cell cycle was unravelled in pre- and post- neoadjuvant chemotherapy treated paired tumour/ adjacent normal specimens from the same patients to understand the importance of the genes in carcinogenesis of head and neck.

Profiling of the molecular signature of the proteins in different zones of normal epithelium, viz. proliferative basal/ parabasal and differentiated spinous zones through immunohistochemical expression revealed differential motifs of expression. While RBSP3 showed low expression in basal/parabasal and high cytoplasmic expression in spinous layers, LIMD1 and CDC25A showed high nuclear and moderate cytoplasmic expression throughout the epithelium. The mechanism of the above was explained on the basis of an inverse correlation between expressions and promoter methylation of RBSP3 and LIMD1. With progression of dysplastic expression and HNSCC, loss of expression of the genes was due to maintenance of promoter methylation/ additional deletion of RBSP3 and deletion/ deletion and methylation of CDC25A and LIMD1 respectively. Expression of the genes was Segregating HNSCC on the basis of a combination of etiological factors HPV and tobacco revealed significantly lower deletion of the genes in HPV- TOB+ (group II) compared to the other three groups, indicating the importance of HPV in preventing genomic instability. Alterations of all genes were directly and significantly correlated to the habit of tobacco with HPV infection producing lower alterations in tobacco positive patients. Overall survival of patients were significantly correlated to alterations of the genes in univariate analysis and additionally with HPV negativity, nodal status and stage in multivariate analysis.
Finally, survival of Group II patients was superior compared to Groups I, III or IV, irrespective of alterations of any gene, with similar pattern reflected in patients with/without alterations, indicating the importance of etiological factors in determining outcome of HNSCC patients.

Identification and characterization of susceptible alleles of the genes revealed differential presence of (CA) repeat lengths of RBSP3, LIMD1 and CDC25A in the normal population. For most markers, the most prevalent alleles underwent the maximum deletion in head and neck tumours, probably due to their predominant presence. Most markers showed loss of the upper allele in tumour, probably due to higher probability of allele of greater size being lost. There was however no difference in levels of expression of the genes with varying allele size, both in homozygous and heterozygous normal epithelium, either due to the rare presence of homozygous alleles, or due to distal location of the markers from the genes. Further studies might demonstrate the importance of allelic size with the development of HNSCC.

Using neoadjuvant chemotherapy as an in vitro model of cell cycle revealed the importance of the genes in the cell cycle. Compared to pre-therapeutic tumours, post-therapy tumours revealed diminished proliferation index and enhanced apoptotic index, indicating halting of the cell cycle. Compared to pre-therapy tumours, RBSP3 and LIMD1 showed increased expression in post-therapy tumours, validating their tumour suppressive role in the cell cycle, although no such difference existed for CDC25A. Similarly, cMYC showed reduced expression and CCND1 comparable expression in post-therapeutic tumours, with increases in RB/pRB ratio, further validating the role of these proteins associated with the candidate proteins in the cell cycle. Enhanced BAX/BCL2 ratio in post therapy tumours indicated induction of apoptosis. p53 similarly showed enhanced expression post therapy with comparable expression of MLH1, indicating upregulation of repair/ apoptosis pathways. Therefore, the candidate genes were important regulators of the cell cycle, as were their associated proteins. Therefore, the candidate genes were important, not only in development and progression of HNSCC, but also in inducing shrinkage of tumours during neoadjuvant chemotherapy. Alterations of these genes and their outcome to therapy might prove to be valuable in their use as diagnostic and prognostic tools in head and neck cancers.