It is crucial to uncover whether an nsSNP could affect the protein function and contribute to the disease. The present study is based on identifying nsSNPs of GC-linked genes which might have the severely deleterious effect on its gene product. Initially, we have sorted out 44,000 nsSNPs from 1,45,342 SNPs of 40 genes of GC reported in available databases. We have then employed 7 tools namely, SIFT, PolyPhen2, PMut, MutPred, SNAP2, SNP&GO and PANTHER for the selection of nsSNPs with the most deleterious/damaging impact on the respective GC gene. At the end of this stringent filtering pipeline, only one nsSNP was chosen as the most damaging nsSNP for each gene and assumed to be involved in alteration of protein function. In total, we identified 11363 missense nsSNPs located within the 40 GC genes, 474 of whom were predicted to be damaging and 40 to be the most damaging.

The current study also set out to further a comprehensive framework for robust *in vitro* detection of association of our target nsSNP with 40 GC genes. Therefore, we have predicted a primer set (forward primer, reverse primer), allele specific primer and restriction enzyme to accelerate the association study where the human blood sample have to be utilized for DNA extraction. We have retrieved the gene sequence (length 500-100bp) of each gene where the nsSNP was included within this sequence. We also checked the similarity of the designed primers against Human Genome using the BLAST tool. We have scrutinized the primer to make sure that there would be no non-specific amplification. To identify the specific polymorphism (nsSNP) within the respective gene sequence from PCR product we have also selected the most suitable restriction enzyme. But we were unable to find out the specific restriction enzyme of each of the 40 GC genes for the specific location of polymorphism. For this reason, we also designed the allele specific primer by which PCR could confirm the polymorphisms of genes.