

## Abstract

Noroviruses (NoVs) are a major causative agent of acute gastroenteritis in humans. Noroviruses are members of the *Caliciviridae* family and based on the genetic analysis of the RdRp and capsid regions, human NoVs are divided into three genogroups (Gs), GI, GII, and GIV, which further segregate into distinct lineages called genotypes. The NoV genus is genetically diverse and recombination of viral RNA is known to depend upon various immunological and intracellular constraints that may allow the emergence of viable recombinants.

Noroviruses (NoVs) are a major cause of epidemic acute gastroenteritis affecting 96% of people worldwide. Viral gastroenteritis is now well documented in most of the industrialized countries, in contrast to Greece where epidemiological data concerning NoVs are still lacking.

For this purpose, in the present PhD thesis, the detection and molecular characterisation of circulating recombinant Noroviruses was performed, in order to create important information on the evolution, pathogenesis and spread of these viruses in the population of central Greece. Sewage samples and fecal specimens were collected from central Greece and molecular techniques, that documented the genetic relationships between the circulating recombinant and non recombinant strains were assess.

The first Noroviral stain which was characterized during the present study was isolated from the clinical sample A6. The detection was carried out initially by enzyme-linked immunosorbent assay (ELISA) and the subsequent detection and molecular characterization of NoV strain was achieved by reverse transcription-PCR and sequencing. RT-PCR assay for GII NoVs that targets conserved regions of the genome, allowed the characterization of A6 strain at the molecular level, established its genetic relationship at the sub-genogroup level and classified A6 strain at the sub-genotype level by performing phylogenetic analyses with other GII NoVs that have previously been grouped into genotypes. Based on the sequence analysis, A6 strain was revealed to belong to the GII genogroup of NoVs. Partial ORF1 gene sequencing analysis and complete ORF2 gene sequencing revealed that ORF1 and ORF2 belonged to two distinct genotypes GII/9 and GII/6, respectively, making

obvious that A6 strain was a rare intergenotypic recombinant within the genogroup GII between GII.9 and GII.6 genotypes.

Subsequently, in an attempt to discern the circulation of the above intergenotypic recombinant GII.9/GII.6, we investigated NoVs from samples collected from raw sewages between the years 2006 to 2011 and compared the results with Noroviruses detected from clinical samples in the same area and in the same time period. Two specific primer pairs for NoVs were designed which amplified in a single PCR fragment from polymerase to capsid gene covering the widespread recombination point in ORF1/ORF2 junction. Based on the genetic analysis, recombinant NoV strains GII.9/GII.6 were identified. Fourteen out of 15 environmental and eight out of ten clinical samples that were used in the present study were positive, with both primer pairs, confirming that the intergenotypic recombinant GII.9/GII.6 was circulating in the population of central Greece from 2006 to 2011. The crossover point was identified to be within the overlapping region of ORF1/ORF2 (GII.9/GII.6, respectively) and was determined by Simplot at nucleotide position 5,032 bp.

Finally, three more Noroviral strains were detected in clinical samples collected in the University Hospital of Ioannina, Greece that revealed a hitherto unobserved recombination event between GII.9/GII.4 and GII.9/GI.7 genogroups. Moreover a novel strain was also detected in clinical sample AK1 belonging to GII.4 genocluster. Phylogenetic analysis classified AK1 strain as GII.4 genotype, but forming clearly a new cluster at the nucleotide level, according to BLAST clustering.