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Phylogenetic Analyses of the RpRd&Vp1 Overlap Region C and RpRd Region A of the Detected Norovirus Genotypes in Baghdad Province

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Abstract:

Noroviruse (NoV) are the most commonly recognized foodborne viral infection and second only to Rotavirus (RV) as a cause of severe diarrhea in children. The high burden of infection because of their stability in the extreme environment condition, diversity of strains, and low infectious dose ranging between 10–100 virus particles which is enough to infect individual Nucleotide sequence and phylogenetic analysis of the junction of Open Reading Frame one (ORF) and the open reading frame two (ORF2)and fragments of 60/81 (74.07%) positive samples results found that the appearance of four genotypes: GGII.4, GGII.2, GGII.17, GGI.3 ACCESSION KU291998.1 ; KU292999.1 ; KU292001.1 : ACCESSION KU292002.1 ; KU292003.1 ; KU292004.1 ; KU292005.1 ;KU744839.1 . The NVGII.4 was the most dominant strain with frequency percentage 61.6% and the higher frequency percentage 50% was belongs to the recombinant. It has been suggested that recombination could be an important mechanism by which GII.4 remains persistent in human population.

Keywords: Noroviruse, Phylogenetic tree, NVGI, NVGII, Sequence

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Introduction

Human Noroviruses(NoVs) is consider as the leading cause of acute gastroenteritis (AGE) and chronic gastroenteritis (CGE) [1], affected people of all ages, and about 10% of children may infected with it [2; 3]. The virus genome sized 7.5 kbp contain a positive-sense RNA genome, encoding two main components: a major structural protein (VP1) of about 58~60 kDa and a minor capsid protein (VP2) [4]. Noroviruses are genetically diverse; there are 35 different genotypes classified within five genogroups (GI-GV) based on their capsid and/or polymerase genes: about14 genotypes in group I (GI); 17 in groupII (GII); two in groupIII(GIII); one in group4 (GIV), and one in group 5(GV) [5]. According to the capsid gene diversity, viruses own > 14.3% differences are classified as the same strain, those have 14.3 - 43.8% differences are classified as the same genotype, and viruses with 45 - 61.4% difference are classified as the same genogroup [6] .

The virus has three open reading frames (ORFs): ORF 1 encodes for the large polyprotein that breaks down into helicase, protease, and RNA polymerase after proteolytic cleavage. The ORF 2 which encodes for the capsid protein (VP3),and the ORF 3 encodes for a predicted 22.5 kD protein of unknown function. the capsid monomer is divided into the three domains: The shell domain (S) forms the core of the particle and the protruding domain (P) extends away from the core, the P domain is further subdivided into the P1 subdomain (residues 226–278 and 406–520) ,and the P2 subdomain (residues 279–405) [7]. The P2 subdomain is consider as the exposed region is interact with potential neutralizing antibodies and histo-blood group antigen (HBGA) ligands [8]. The P2 subdomain of VGII.4 strains is evolving rapidly, resulting in new epidemic strains with altered carbohydrate ligand binding properties and antigenicity [9]. The NoV genome undergoes frequent

change [10] by mechanisms including mutation [11] and recombination [12] .The estimated mutation rate (1.21 x 10−2 to 1.41 x 10−2 substitutions per site per year) in this virus is high even compared with other RNA viruses.[13]. Breakpoint analysis of recombinant NoV showed that the recombination site was at the open reading frame ORF1/ORF2 overlap [14;15]. Evidence of recombination in the NoV capsid gene, RNA-dependent RNA polymerase (RdRp) gene and ORF2/ORF3 overlap is also reported [16] .

Materials and Methods

Viral Isolation and Extraction

Stool samples were collected from 252 patients suspected with AGE[17] children under 5 through year 2013 fromEbn –Albalady Hospital and Child Central Hospital in Baghdad province. Viral genome was extracted from 30% (w/v) stool suspensions in phosphate-buffered saline (pH =7.2) using QIAamp1Viral RNA Mini kit (Qiaen, Germany) according to the manufacturer's instructions. [18] .The purity and concentration of extracts were determined using Nanodrope technique. The extracted RNA was stored at -70-C until used.

Genotyping

Viral detection by using reverse transcription real-time-PCR (RT-qPCR) [19] using reaction mix "Go Taq 1 Step RT-QPCR" supplied from Promega company (USA)81 samples tested positive for Norovirus by RT-qPCR were submitted for viral genotyping to amplify the 5' end of the capsid gene (region C in ORF2) for GI and GII, respectively, and 3' end of the RdRp gene (region A in ORF1) . DNA was generated by QIAGEN One Step RT PCR Kit (Qiagen, Germany) according to the manufacturer's instructions. The PCR products were visualized by gel electrophoresis [20]. Sequencing of PCR product was carried out by Microgen company (Korea) in two direction forward and reverse.

Sequencing analysis

Bands size 318 bp of A1 product, 326 bp ORF1 A2 product , 329 bp partial capsid gene C1 product, and 343 bp C2 Junction ORF1-ORF2 overlap product were Sequenced. Data was analyzed by sequence analyzer program from Applied Biosystem. Alignments and sequence blasting was conducted using BLAST program available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) , BioEdit program , and Evolutionary analysis conducted by the UPGMA method in MEGA6 software [21] using 16 nucleotide sequences of 288 nucleotide (nt) positions in the final dataset of RbRd partial gene [22]. The evolutionary distances were computed using the Maximum Composite Likelihood method [23], with branch length = 0.04690812 [21]. Figure 3 showed the analysis of 34 nucleotide sequences with branch length sum = 2.03539311 and are in the units of the number of base substitutions per site. The gaps and missing data were excluded. There were a total of 185 positions (nt) in the final dataset.

Results and Discussion

In this study the prevalence of NoVs was 32.1%.5 including four different genotypes: GGII.4 (61.6%), GGII.2 (5%), GGII.17 (5%), GGI.3 (28.39%), and the genotype GGII.4 was the most predominant strains in Baghdad province among 17 genotypes known of NoV, which is consistent with (15)in Bangladish who found that GII.4 was the predominant genotype during 2010-2013; Beginning from 1995, the emergence of novel NVGII.4 variants caused six pandemics. After the first detection of the Sydney_2012 NVGII.4 variant in March 2012 in Australia, many countries, including Iraq, reported increased levels of NoV activity associated with this novel variant during winter 2012–2013 [24; 25; 26; 27] ,also [28] demonstrated that from 2012 to 2016 the GII.4 variants prevailing in Canada [29] closed to our finding that the prevalence of NoV GI and GII in Seoul was 4.43% and 93.36%, respectively, and the genotypes GII.4 (50.92%), GII.17(18.08%), and GII.3 (9.96%) were found to be the dominant types.

The result revealed a higher incidence of NoV infection in the males 54% in compared with the 46% of females patients. The infection with genogroup NVGI 28.39% was affecting 56.52% of males and 43.48% of females, and the occurrence of infection with genogroup NVGII was 71.60% effecting 53.44% of males of AGE in comparison to 46.55% of females.

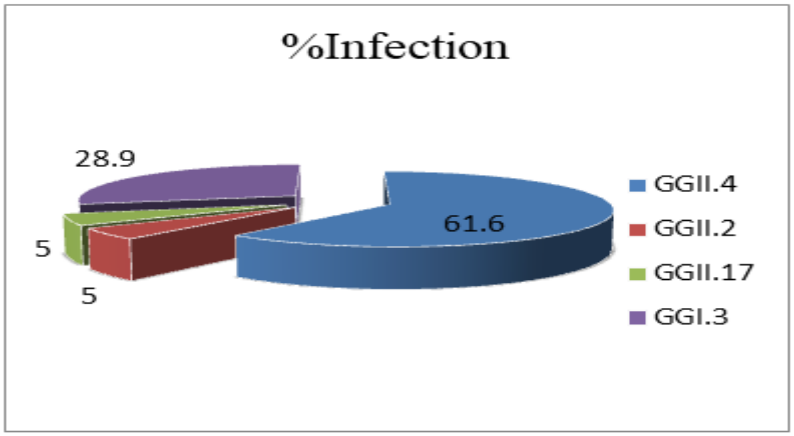

Figure1. Norovirus genotypes infection percentage among AGE patients

Sequence data was submitted to the NCBI and published under the Accession numbers (KU291998.1, KU292999.1, KU292001.1, KU292002.1, KU292003.1, KU292004.1, KU292005.1, and KU744839.1). The evolutionary trees of RpRd gene ,and RpRd &Vp1 Junction partial gene were conducted by MEGA6 software using the UPGMA method according [21] to the number of base substitutions per site in total of 288 , 201 positions, involving16, and 34 nucleotide sequences respectively figure 2,and 3 [22]. The sum of branch length = 0.04690812 for the RpRd gene phylogeny and 37.28372967 for the RpRd &Vp1 Junction gene phylogeny. The evolutionary distances were computed using the Maximum Composite Likelihood method [23].

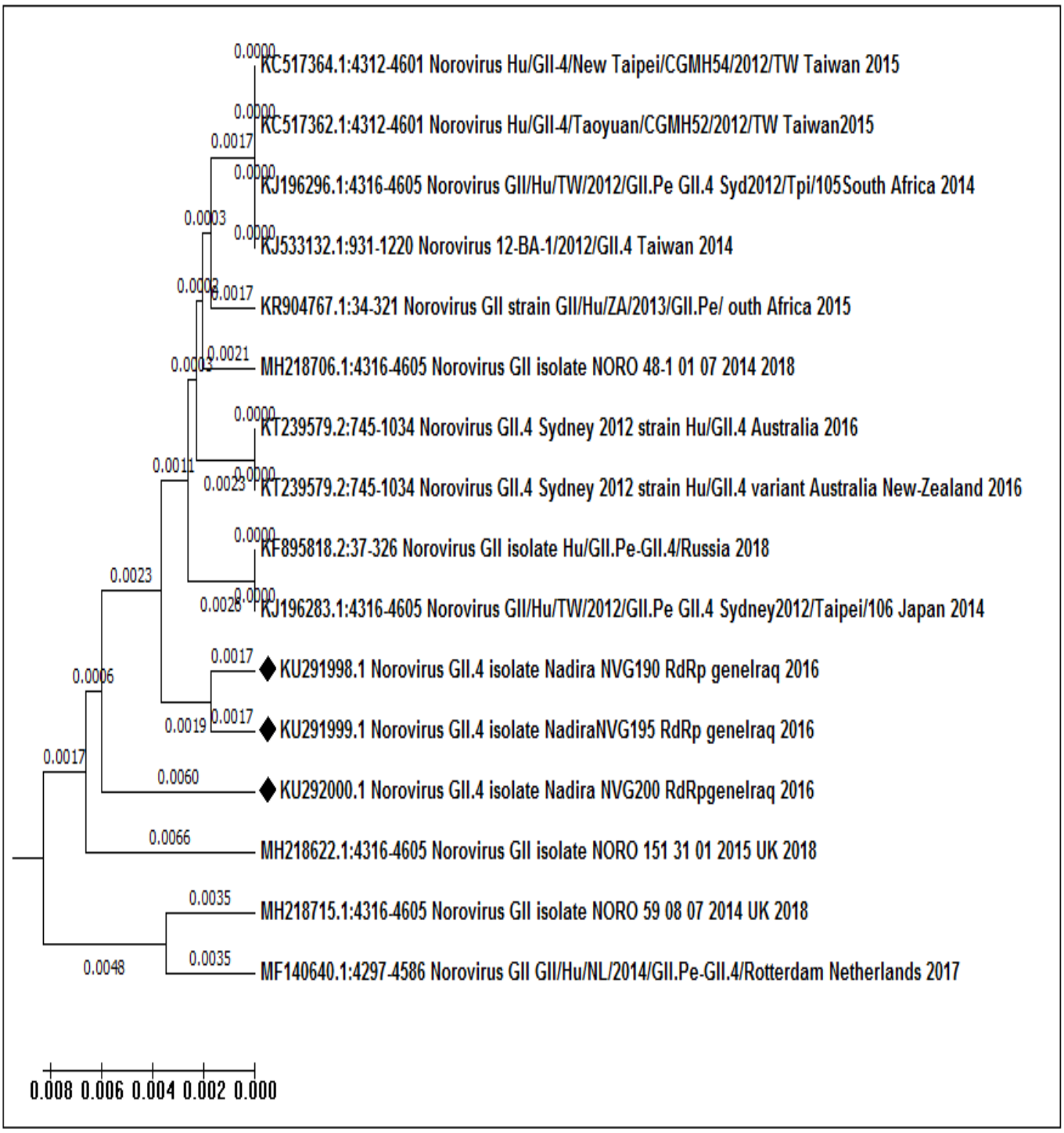

Figure2. The Evolutionary tree of Iraqi Norovirus genotypes depending on RpRd gene marked with dark rhombus in compared with NCBI sequenced data.

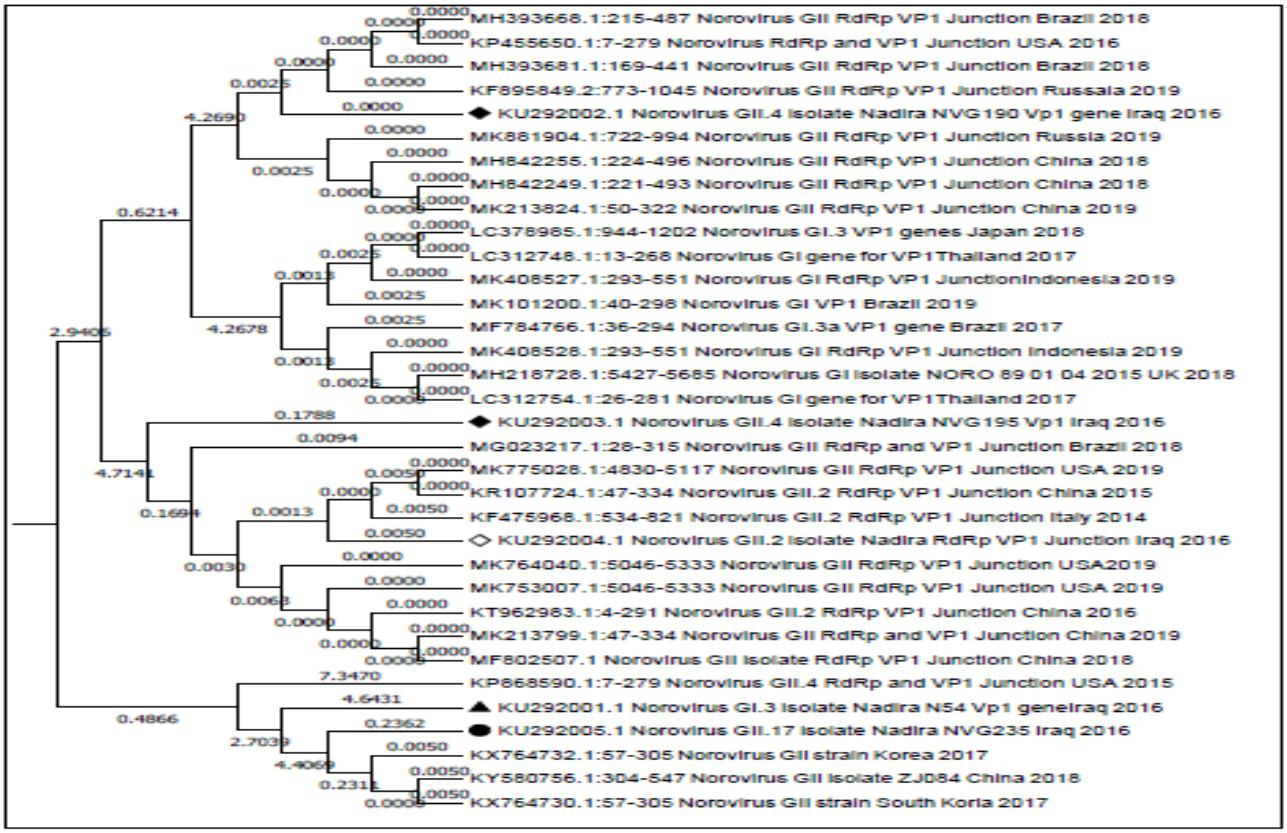

Figure3. The Evolutionary tree of Iraqi Norovirus genotypes depending on RpRd &Vp1 Junction partial gene marked with dark rhombus for NVGII.4, rhombus for NVGII.2, dark spot for NVGII.17 triangles for NVGI.3 genogroups respectively in compared with NCBI sequenced data.

Phylogenetic analysis demonstrated that the majority of these sequences belong to Sydney 2012 strains, and a recombinant sequence with Taipei is much closed to Iraqi isolates in figure 2 which explain that the capsid gene including hot spots lead to the highly diversity among them. On the other hand, the viral RNA polymerase, and protease genes still relatively more conserved region. Accurate detection for Norovirus need to update the markers design in the conserved region to cover all novel genotypes (Mohamed et al., 2019) Numerous amino acid substitutions were observed at each putative antigenic epitope of the VP1 protein, whereas few amino acid changes were identified in the polymerase protein.

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