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Updated classification of norovirus genogroups and genotypes

Preeti Chhabra[1,*], Miranda de Graaf[2], Gabriel I. Parra[3], Martin Chi-Wai Chan[4], Kim Green[5], Vito Martella[6], Qiuhong Wang[7], Peter A. White[8], Kazuhiko Katayama[9], Harry Vennema[10], Marion P. G. Koopmans[2] and Jan Vinjé[1]

Abstract

Noroviruses are genetically diverse RNA viruses associated with acute gastroenteritis in mammalian hosts. Phylogenetically, they can be segregated into different genogroups as well as P (polymerase)-groups and further into genotypes and P-types based on amino acid diversity of the complete VP1 gene and nucleotide diversity of the RNA-dependent RNA polymerase (RdRp) region of ORF1, respectively. In recent years, several new noroviruses have been reported that warrant an update of the existing classification scheme. Using previously described 2× standard deviation (SD) criteria to group sequences into separate clusters, we expanded the number of genogroups to 10 (GI-GX) and the number of genotypes to 49 (9 GI, 27 GII, 3 GIII, 2 GIV, 2 GV, 2 GVI and 1 genotype each for GVII, GVIII, GIX [formerly GII.15] and GX). Viruses for which currently only one sequence is available in public databases were classified into tentative new genogroups (GNA1 and GNA2) and genotypes (GII.NA1, GII.NA2 and GIV.NA1) with their definitive assignment awaiting additional related sequences. Based on nucleotide diversity in the RdRp region, noroviruses can be divided into 60 P-types (14 GI, 37 GII, 2 GIII, 1 GIV, 2 GV, 2 GVI, 1 GVII and 1 GX), 2 tentative P-groups and 14 tentative P-types. Future classification and nomenclature updates will be based on complete genome sequences and will be coordinated and disseminated by the international norovirus classification-working group.

INTRODUCTION

The genus *Norovirus* in the family *Caliciviridae* consists of a genetically diverse group of viruses infecting a wide range of mammalian host species that include humans, dogs, cats, pigs, mice, sheep and cattle [1–8]. To add to the diversity, several new unclassified noroviruses have recently been identified in bats, sea lions and harbour porpoise [9–12] and in non-human primates (unpublished GenBank submissions). Noroviruses found in humans are genetically and antigenically divergent and the leading cause of acute gastroenteritis in people of all ages worldwide associated with an estimated 70 000–200 000 deaths annually [13, 14].

Noroviruses are non-enveloped viruses with a single-stranded RNA genome approximately 7.5 kb in length. At the 5′-end, the RNA is covalently linked to a viral protein (VPg) and the 3′-end of the genome is polyadenylated. The genome of most noroviruses is organized into three ORFs, except for murine noroviruses, which contain a fourth ORF. ORF1 encodes a polyprotein that generates six non-structural proteins (NS1/2 to NS7) after post-translational cleavage by the viral protease [2, 15]. ORF2 encodes the major structural protein (VP1) that has shell (S) and protruding (P) domains. The S domain surrounds the viral RNA and the P domain, which consists of the P2 domain, is linked to the S domain through

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Author affiliations: [1]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA; [2]Department of Viroscience, Erasmus University Medical Center, Rotterdam, The Netherlands; [3]Division of Viral Products, Food and Drug Administration, Silver Spring, MD, USA; [4]Department of Microbiology, Stanley Ho Centre for Emerging Infectious Diseases and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, PR China; [5]Caliciviruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; [6]Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy; [7]Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [8]School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney 2052, Australia; [9]Laboratory of Viral infection I, Kitasato Institute for Life Sciences Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan; [10]Division for Virology, Centre for Infectious Diseases Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
***Correspondence:** Preeti Chhabra, PChhabra@cdc.gov
Keywords: Norovirus, acute gastroenteritis, classification, 2xSD criteria, genogroup, genotype, P-group, P-type.
Abbreviations: Bo, bovine; Ca, canine; DDBJ, DNA Data Bank of Japan; EMBL, European Molecular Biology Laboratory; G, genogroup; HBGA, histo-blood group antigen; Hu, human; Mu, murine; NA, not assigned; NCWG, Norovirus Classification Working Group; ORF, open reading frame; Ov, ovine; PCR, polymease chain reaction; P-groups, polymerase groups; Po, porcine; Pp, Phocoena phocoena; P-types, polymerase types; RdRp, RNA dependent RNA polymerase; Rn, Rattus norvegicus; RNA, ribonucleic acid; Rs, Rhinolophus sinicus; SD, standard deviation; VP, viral protein; Zc, Zalophus californianus.
Four supplementary tables and five supplementary figures are available with the online version of this article.

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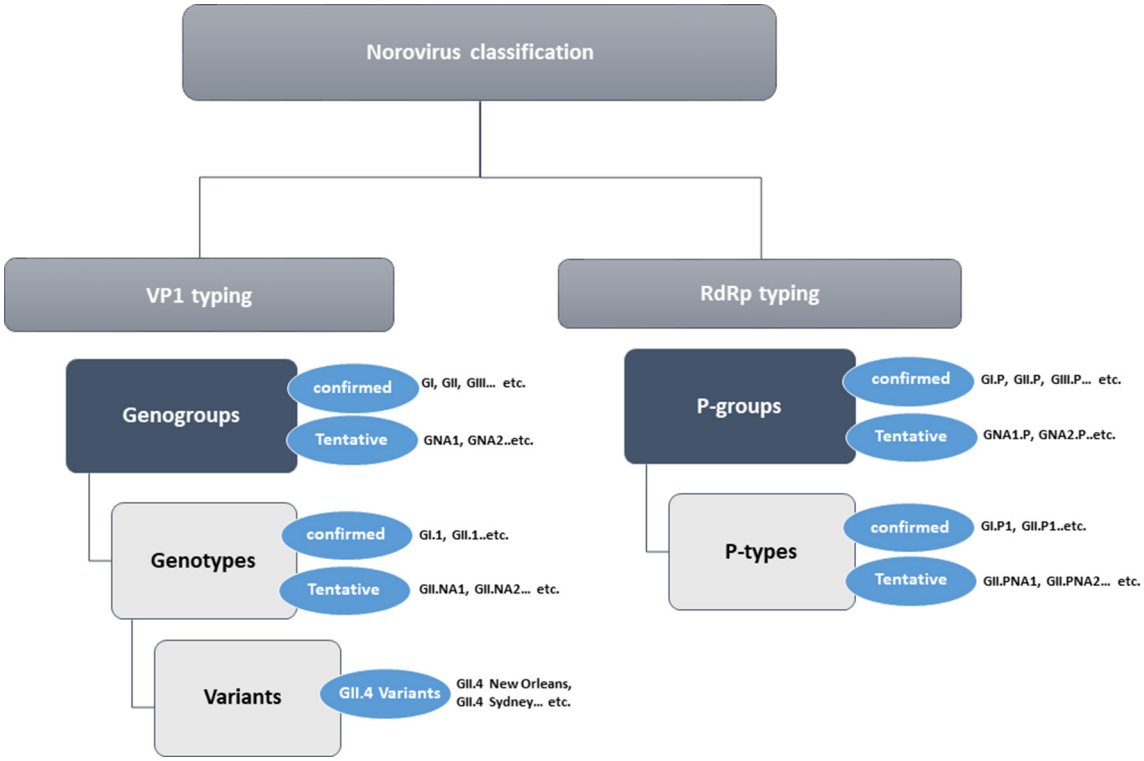

Fig. 1. Classification of noroviruses into genogroups, genotypes, variants, P-groups and P-types. Tentative genogroups, genotypes, P-groups and P-types are currently represented only by a single sequence or multiple non-identical sequences from a single geographic location and are therefore named as non-assigned (NA).

a flexible hinge [2, 14]. The P2 sub-domain is highly variable, harbours major neutralization epitopes and interacts with histo-blood group antigens (HBGAs) [16]. ORF3 encodes a minor structural protein (VP2), which is located inside the virus particle and has been proposed to be involved in capsid assembly and genome encapsidation [17] and, as described recently for feline calicivirus, entry of the virion into the host cell [18]. Complete VP1 amino acid sequences and the ORF1 NS7 region (which encodes the RNA-dependent RNA polymerase [RdRp]) nucleotide sequences are the basis of the current genetic classification of noroviruses [19].

The need to organize norovirus strains into different genetic groups or clusters was recognized in the mid-1990s when noroviruses were primarily divided into genogroups and genotypes based on partial RdRp sequences [20–23]. When more sequences became available, classification shifted to designate genogroups and genotypes based on the complete VP1 amino acid sequence with 20% sequence difference used as a cut-off threshold for new genotypes, which was later adjusted to 15% [24, 25]. Due to the lack of internationally accepted standards for norovirus classification several research groups used a small nucleotide region of the 5′-end of VP1 (termed region C) to classify genotypes, which although appropriate for typing noroviruses, led to inconsistent classification of some strains [25, 26].

To allow efficient communication of epidemiologically important norovirus lineages, in 2013 researchers from the Norovirus Classification Working Group (NCWG) proposed a universal standardized nomenclature and typing system for genotyping of GI and GII noroviruses using phylogenetic clustering of the complete VP1 amino acid sequences [19]. Based on an analysis of the then available sequence data, noroviruses were classified into six genogroups (GI to GVI) [19, 27], with a proposed seventh genogroup (GVII) [27]. These genogroups were further divided into more than 40 genotypes [28]. Viruses of GI, GII and GIV infect humans however GII also includes three genotypes (GII.11, GII.18 and GII.19) detected in faecal specimens from swine and GIV viruses include a genotype (GIV.2) that has only been detected in carnivore species (cats and dogs) [2, 27, 29]. Since the nomenclature criteria could not be met for distinguishing GII.4 variants, it was decided that the subtyping of GII.4 strains into variants will be based on phylogenetic clustering and that new GII.4 variants will only be recognized after they become epidemic in at least two geographically diverse locations [19]. Over the past two decades, eight GII.4 variants have been circulating each replacing a previous dominant variant and since 2012 GII.4 Sydney is the most contemporary GII.4 variant [19].

In addition, as norovirus diversity may be further increased through recombination, and circulating recombinant strains appear as distinct epidemiological entities, dual typing was proposed to include diversity at the level of the partial RdRp sequences in strain designations [19]. Although several

Table 1. Classification of norovirus prototype strains in current and new genotype assignment as determined by (a) complete VP1 amino acid and (b) partial (762 nt) RdRp relatedness

Strain designation	GenBank accession no.	Current norovirus genotype	New norovirus genotype
(a) VP1			
GI/Hu/US/1968/GI.1/Norwalk	M87661	GI.1	GI.1
GI/Hu/GB/1991/GI.2/Southampton	L07418	GI.2	GI.2
GI/Hu/SA/1990/GI.3/DesertShield395	U04469	GI.3	GI.3
GI/Hu/JP/1987/GI.4/Chiba407	AB042808	GI.4	GI.4
GI/Hu/GB/1989/GI.5/Musgrove	AJ277614	GI.5	GI.5
GI/Hu/DE/1997/GI.6/BS5(Hesse)	AF093797	GI.6	GI.6
GI/Hu/GB/1994/GI.7/Winchester	AJ277609	GI.7	GI.7
GI/Hu/US/2001/GI.8/Boxer	AF538679	GI.8	GI.8
GI/Hu/CA/2004/GI.9/Vancouver730	HQ637267	GI.9	GI.9
GII/Hu/US/1971/GII.1/Hawaii	U07611	GII.1	GII.1
GII/Hu/GB/1994/GII.2/Melksham	X81879	GII.2	GII.2
GII/Hu/CA/1991/GII.3/TV24	U02030	GII.3	GII.3
GII/Hu/GB/1993/GII.4/Bristol	X76716	GII.4	GII.4
GII/Hu/GB/1990/GII.5/Hillingdon	AJ277607	GII.5	GII.5
GII/Hu/GB/1990/GII.6/Seacroft	AJ277620	GII.6	GII.6
GII/Hu/GB/1990/GII.7/Leeds	AJ277608	GII.7	GII.7
GII/Hu/NL/1998/GII.8/Amsterdam	AF195848	GII.8	GII.8
GII/Hu/US/1997/GII.9/VA97207	AY038599	GII.9	GII.9
GII/Hu/DE/2000/GII.10/Erfurt546	AF427118	GII.10	GII.10
GII/Po/JP/1997/GII.11/Sw918	AB074893	GII.11	GII.11
GII/Hu/GB/1990/GII.12/Wortley	AJ277618	GII.12	GII.12
GII/Hu/US/1998/GII.13/Fayetteville	AY113106	GII.13	GII.13
GII/Hu/US/1999/GII.14/M7	AY130761	GII.14	GII.14
GII/Hu/US/1999/GII.16/Tiffin	AY502010	GII.16	GII.16
GII/Hu/US/2002/GII.17/CS-E1	AY502009	GII.17	GII.17
GII/Po/US/2003/GII.18/OH-QW101	AY823304	GII.18	GII.18
GII/Po/US/2003/GII.19/OH-QW170	AY823306	GII.19	GII.19
GII/Hu/DE/2002/GII.20/Luckenwalde591	EU373815	GII.20	GII.20
GII/Hu/IQ/2002/GII.21/IF1998	AY675554	GII.21	GII.21
GII/Hu/JP/2003/GII.22/Yuri	AB083780	GII.22	GII.22
GII/Hu/PE/2010/GII.23/Loreto1847	KT290889	–	GII.23
GII/Hu/PE/2013/GII.24/Loreto1972	KY225989	–	GII.24
GII/Hu/CN/2007/GII.25/Beijing53931	GQ856469	GII.22	GII.25
GII/Hu/NI/2005/GII.26/Leon4509	KU306738	–	GII.26
GII/Hu/PE/2012/GII.27/Loreto0959	MG495077	–	GII.27
GII/Hu/PE/2013/GII.NA1/Loreto1257	MG495079	–	GII.NA1

Continued

Table 1. Continued

Strain designation	GenBank accession no.	Current norovirus genotype	New norovirus genotype
GII/Hu/PE/2008/GII.NA2/PNV06929	MG706448	–	GII.NA2
GIII/Bo/DE/1980/GIII.1/Jena	AJ011099	GIII.1	GIII.1
GIII/Bo/GB/1976/GIII.2/Newbury2	AF097917	GIII.2	GIII.2
GIII/Ov/NZ/2007/GIII.3/Norsewood30	EU193658	GIII.3	GIII.3
GIV/Hu/NL/1998/GIV.1/Alphatron	AF195847	GIV.1	GIV.1
GIV/Ca/IT/2006/GIV.2/Pistoia-387	EF450827	GIV.2	GIV.2
GIV/Hu/US/2016/GIV.NA1/WI7002	KX907728	–	GIV.NA1
GV/Mu/US/2002/GV.1/MNV-1	AY228235	GV.1	GV.1
GV/Rn/HK/2011/GV.2/HKU-CT2	JX486101	GV.2	GV.2
GVI/Ca/IT/2007/GVI.1/Bari91	FJ875027	GVI.1	GVI.1
GVI/Ca/PT/2007/GVI.2/C33-Viseu	GQ443611	GVI.2	GVI.2
GVII/Hu/HK/2007/GVII.1/Ca026F	FJ692500	GVII.1	GVII.1
GVIII/Hu/JP/2004/GVIII.1/Chiba-040502	AJ844470	–	GVIII.1
GIX/Hu/US/1999/GIX.1/J23	AY130762	GII.15	GIX.1
GX/Rs/CN/2010/GX.1/YN2010	KJ790198	–	GX.1
GNA1/Pp/NL/2012/GNA1.1/120906	KP987888	–	GNA1.1
GNA2/Zc/HK/2008/GNA2.1/PF080916-2	MG572715	–	GNA2.1
Strain designation	**GenBank accession no.**	**Current norovirus P-type**	**New norovirus P-type**
(b) RdRp			
GI/Hu/US/1968/GI.P1/Norwalk	M87661	GI.P1	GI.P1
GI/Hu/GB/1991/GI.P2/Southampton	L07418	GI.P2	GI.P2
GI/Hu/US/1998/GI.P3/VA98115	AY038598	GI.P3	GI.P3
GI/Hu/JP/1987/GI.P4/Chiba 407	AB042808	GI.P4	GI.P4
GI/Hu//Hu/2013/GI.P5/Siklos-HUN5407	KJ402295	GI.P5	GI.P5
GI/Hu/DE/1997/GI.P6/BS5(Hesse)	AF093797	GI.P6	GI.P6
GI/Hu/SE/2008/GI.P7/LillaEdet|	JN603251	GI.P7	GI.P7
GI/Hu/JP/2007/GI.P8/Nagoya	KJ196298	GI.P8	GI.P8
GI/Hu/CH/2012/GI.P9/CAIQ12110628	KF586507	GI.P9	GI.P9
GI/Hu/SA/1990/GI.P10/DesertShield395	U04469	GI.Pa	GI.P10
GI/Hu/CH/2007 GI.P11/Beijing53997	GQ856463	GI.Pb	GI.P11
GI/Hu/JP/2000/GI.P12/SzUG1	AB039774	GI.Pc	GI.P12
GI/Hu/FR/2003/GI.P13/Vesoul576	EF529738	GI.Pd	GI.P13
GI/Hu/JP/1979/GI.P14/Otofuke	AB187514	GI.Pf	GI.P14
GI/Hu/JP/2002/GI.PNA1/WUG1	AB081723	GI.Pb	GI.PNA1
GI/Hu/BD/2011/GI.PNA2/Dhaka1882	MH130046	–	GI.PNA2
GI/Hu/IN/2007/GI.PNA3/V1707	AB447414	–	GI.PNA3
GI/Hu/JP/1998/GI.PNA4/No20-Saitama-98–17	LC342057	–	GI.PNA4

Continued

Table 1. Continued

Strain designation	GenBank accession no.	Current norovirus P-type	New norovirus P-type
GII/Hu/US/1971/GII.P1/Hawaii	U07611	GII.P1	GII.P1
GII/Hu/GB/1994/GII.P2/Melksham	X81879	GII.P2	GII.P2
GII/Hu/CA/1991/GII.P3/TV24	U02030	GII.P3	GII.P3
GII/Hu/GB/1993/GII.P4/Bristol	X76716	GII.P4	GII.P4
GII/Hu/HU/1999/GII.P5/MOH	AF397156	GII.P5	GII.P5
GII/Hu/JP/2002/GII.P6/SaitamaU16	AB039778	GII.P6	GII.P6
GII/Hu/JP/2002/GII.P7/SaitamaU4	AB039777	GII.P7	GII.P7
GII/Hu/JP/2002/GII.P8/SaitamaU25	AB039780	GII.P8	GII.P8
GII/Po/JP/2003/GII.P11/swine43	AB126320	GII.P11	GII.P11
GII/Hu/JP/2005/GII.P12/Sakai-04–179	AB220922	GII.P12	GII.P12
GII/Hu/FR/2004/GII.P13/Briancon870	EF529741	GII.P13	GII.P13
GII/Hu/US/1999/GII.P15/Sapporo/HK299	KJ196290	GII.P15	GII.P15
GII/Hu/DE/2000/GII.P16/Neustrelitz260	AY772730	GII.P16	GII.P16
GII/Hu/JP/2014/GII.P17/Kawasaki323	AB983218	GII.P17	GII.P17
GII/Po/US/2003/GII.P18/OH-QW101	AY823304	GII.P18	GII.P18
GII/Hu/GE/2005/GII.P20/Leverkusen267	EU424333	GII.P20	GII.P20
GII/Hu/FR/2004/GII.P21/Pont de Roide673	AY682549	GII.P21	GII.P21
GII/Hu/JP/2003/GII.22/YURI	AB083780	GII.P22	GII.P22
GII/Hu/PE/2010/GII.P23/Loreto1847	KT290889	–	GII.P23
GII/Hu/PE/2013/GII.P24/Loreto1972	KY225989	–	GII.P24
GII/Hu/BD/2012/GII.P25/Dhaka1928	MG495083	–	GII.P25
GII/Hu/NI/2005/GII.P26/Leon4509	KU306738	–	GII.P26
GII/Hu/PE/2012/GII.P27/Loreto0959	MG495077	–	GII.P27
GVIII/Hu/JP/2011/GII.P28/Gira2HS	KJ196291	–	GII.P28
GII/Hu/JP/2004/GII.P29/SN2000JA	AB190457	GII.Pa	GII.P29
GII/Hu/US/1976/GII.P30/SnowMountain	AY134748	GII.Pc	GII.P30
GII/Hu/JP/2007/GII.P31/OC07138	AB434770	GII.Pe	GII.P31
GII/Hu/FR/1999/GII.P32/S63	AY682550	GII.Pf	GII.P32
GII/Hu/AU/2008/GII.P33/NSW199U	GQ845370	GII.Pg	GII.P33
GII/Hu/JP/1997/GII.P34/OC97007	AB089882	GII.Ph	GII.P34
GII/Hu/GR/1997/GII.P35/E3	AY682552	GII.Pj	GII.P35
GII/Hu/JP/1996/GII.P36/OC96065	AF315813	GII.Pk	GII.P36
GII/Hu/IN/2006/GII.P37/PunePC24	EU921353	GII.Pm	GII.P37
GII/Hu/CN/2007/GII.P38/Beijing53931	GQ856469	GII.Pn	GII.P38
GII/Hu/US/1974/GII.P39/CHDC5191	FJ537134	GII.P1	GII.P39
GII/Hu/JP/2004/GII.P40/OsakaNI	DQ366347	GII.P22	GII.P40
GII/Hu/AUS/1983/GII.P41/GoulburnValleyG5175B	DQ379714	GII.Pg	GII.P41

Continued

Table 1. Continued

Strain designation	GenBank accession no.	Current norovirus P-type	New norovirus P-type
GII/Hu/PE/2013/GII.PNA1/Loreto1257	MG495079	–	GII.PNA1
GII/Hu/PE/2008/GII.PNA2/PNV06929	MG706448	–	GII.PNA2
GII/Hu/US/1974/GII.PNA3/CHDC2094	FJ537135	GII.P1	GII.PNA3
GII/Po/JP/1997/GII.PNA4/Sw918	AB074893	GII.P11	GII.PNA4
GII/Hu/BD/2012/GII.PNA5/Dhaka1940	MG495082	GII.P22	GII.PNA5
GII/Hu/AR/2015/GII.PNA6/Arg13099	KX061540	–	GII.PNA6
GII/Hu/JP/1999/GII.PNA7/No35-Saitama-99–1	LC342059	–	GII.PNA7
GII/Hu/ZA/2013/GII.PNA8/Empangeni_10403	KR904229	–	GII.PNA8
GII/Hu/AR/2019/GII.PNA9/Arg15813	MK733205	–	GII.PNA9
GIII/Bo/DE/1980/GIII.P1/Jena	AJ011099	GIII.P1	GIII.P1
GIII/Bo/UK/1976/GIII.P2/Newbury2	AF097917	GIII.P2	GIII.P2
GIV/Hu/US/1998/GIV.P1/FtLauderdale-560	AF414426	GIV.P1	GIV.P1
GIV/Hu/US/2016/GIV.PNA1/WI7002	KX907728	–	GIV.PNA1
GV/Mu/USA/2002/GV.P1/MNV1	AY228235	GV.P1	GV.P1
GV/Rn/HK/2011/GV.P2/HKU-CT2	JX486101	GV.P2	GV.P2
GVI/Ca/IT/2007/GVI.P1/Bari-91	FJ875027	GVI.P1	GVI.P1
GVI/Ca/PT/2007/GVI.P2/C33-Viseu	GQ443611	GVI.P2	GVI.P2
GVII/Ca/HK/2007/GVII.P1/HKU_Ca026F	FJ692500	GVII.P1	GVII.P1
GX/Rs/CN/2010/GX.P1/YN2010	KJ790198	–	GX.P1
GNA1/Pp/NL/2012/GNA1.P1/120906	KP987888	–	GNA1.P1
GNA2/Zc/HK/2008/GNA2.P1/PF080916-2	MG572715	–	GNA2.P1

recombination breakpoints have been identified across the norovirus genome, they are most frequently found in the ORF1-ORF2 junction region [29–36]. Dual typing (ORF1-RdRp=P type, ORF2=genotype) is now used routinely in many laboratories worldwide and at least 14 GI P-types and 27 GII P-types as well as 9 GI capsid genotypes and 22 GII capsid genotypes have been described [2, 19, 27]. Inclusion of norovirus diagnostic testing by real-time quantitative reverse transcription PCR into clinical and public health routine in recent years [27, 37–39] has confirmed the importance of GI and GII noroviruses globally [13, 31, 33, 35, 37, 40–44]. The increasing use of pathogen genome sequencing to unravel modes of transmission, sources of outbreaks, or to study the burden of infections has led to identification of several new candidate norovirus genogroups and genotypes since the last norovirus classification update in 2013 [9, 12, 33, 40, 41, 43, 45–49]. In this paper, we update the classification scheme for noroviruses by proposing new genogroups and genotypes based on the complete capsid amino acid sequences using the previously agreed criteria [19]. To provide a uniform basis to norovirus classification in the face of recombination and potential 'orphan' sequences, and to eliminate orphan ORF1 naming system (e.g. GI.Pa, GI.Pb, GII.Pa, GII.Pe, etc.), we grouped nucleotide sequences from the partial RdRp region into polymerase (P)-groups and P-types independently from the classification of their corresponding capsid genogroups and genotypes, respectively.

RESULTS

Nomenclature criteria for new genogroups and genotypes

VP1 designations

We followed the genotype designation and numbering as reported previously [19]. Sequences from strains that could not be typed, and for which at least two non-identical complete VP1 sequences from geographically diverse locations were available, were identified as candidate novel genogroups or genotypes (Fig. 1). After confirmation that they formed separate phylogenetic clusters using the 2×SD criteria [19], defined as the average distance between all sequences within a new genogroup or genotype and its nearest cluster(s), should not overlap within two standard deviations (2×SD) from each

Table 2. Examples of new proposed dual-typing designations of norovirus strains

Current designation	New designation
GI.P6-GI.6	GI.6[P6]
GI.Pd-GI.3	GI.3[P13]
GII.P1-GII.1	GII.1[P1]
GII.P12-GII.3	GII.3[P12]
GII.Pe-GII.4 Sydney	GII.4 Sydney[P31]
GII.P16-GII.4 Sydney	GII.4 Sydney[P16]
GII.P4 New Orleans-GII.4 Sydney	GII.4 Sydney[P4 New Orleans]
GII.Pe-GII.17	GII.17[P31]
GII.P15-GII.15	GIX.1[GII.P15]
GVI.P1-GIV.2	GIV.2[GVI.P1]

other, the next available number for new genogroups (e.g. GVIII) and genotypes (e.g. GII.23) was assigned. Genogroups or genotypes for which only a single complete VP1 sequence or only sequences from a single geographic location were available were referred to as not assigned (NA). For example, GNA1 and GNA2 for not yet assigned new genogroups and GII.NA1 and GII.NA2 for not yet assigned new genotypes [19] (Fig. 1). New GII.4 variants were named only when they became epidemic in at least two geographically diverse locations and were given the name of the city of the first full-length capsid sequence available in the public domain, e.g. GII.4 Sydney [19].

RdRp designations

At the genogroup level, RdRp clusters were referred to as P-groups and at the genotype level as P-types. To distinguish VP1 genogroups or genotypes from P-groups or P-types, a capital P was introduced after the Roman genogroup (e.g. GI.P, GII.P, GIII.P) or genotype (GI.P1, GII.P3) designation. To eliminate 'orphan' P-types from the norovirus classification scheme, all RdRp nucleotide sequences for which at least two sequences from two geographic locations were available were assigned a P-type number independent of their corresponding VP1 genotype (Table 1b). When only one RdRp sequence was available, we assigned 'NA' to those P-types with a number after NA (e.g. GI.PNA1, GII.NA2) (Fig. 1).

For dual-typing nomenclature, we propose the following designations of norovirus strains: GI.genotype[P-type] or GII. genotype[P-type], first listing the capsid genotype followed by the P-type between brackets (Table 2). For those strains where VP1 and RdRp sequences segregate into different genogroups the proposed designations are Genogroup.genotype[P-group.P-type] (Table 2).

Strain designation for GenBank submission

With the species name (norovirus GI, norovirus GII) in the organism field, we propose a minor update of the current cryptogram [19] when submitting sequences to GenBank as follows: Organism/host/country code (two letter)/year of sample collection/genotype[P-type]/strain name. For example: Norovirus GII/Hu/US/2014/GII.4 Sydney[P4 New Orleans]/Pierce0249 or Norovirus GII/Hu/PE/2013/GII.24[P24]/Loreto1972.

Norovirus prototype strains

Prototype strains for each new genogroup or genotype include those strains for which the first full-length VP1 amino acid sequence and at least 762 nucleotides at the 3′-end of the RdRp region were available in public-domain databases (GenBank, EMBL, DDBJ). Prototype strains of novel genogroup/types are the first sequences of that lineage deposited into public-domain databases. The prototype strains for all genotypes and P-types are listed in Table 1.

Classification of VP1 sequences

We applied 2×SD criteria to classify 305 complete VP1 amino acid sequences (Table S1, available in the online version of this article) into known and tentative new norovirus genogroups and genotypes. We identified and confirmed four new genogroups including GVII (dog), GVIII (human), GIX (human) (re-classified from GII.15) and GX (bat) and two tentative new genogroups: GNA1 (harbour porpoise) and GNA2 (sea lion) (Fig. 2a, Table S2). The 2×SD criteria could not be applied to GNA1 and GNA2 viruses (each representing a tentative new genogroup) as at least two sequences per cluster are required to calculate the standard deviation for within cluster distances. We confirmed five new genotypes: GII.23, GII.24, GII.25, GII.26 and GII.27 (Fig. 2b and c and Table S2) as well as two tentative new GII genotypes: GII.NA1 and GII.NA2 (Table S2 and Fig. 2b and c), along with one tentative new GIV genotype (GIV.NA1).

Classification of RdRp sequences

Phylogenetic analysis of 232 partial RdRp nucleotide sequences (Table S3) from all norovirus genogroups confirmed eight (GI.P, GII.P, GIII.P, GIV.P, GV.P, GVI.P, GVII.P and GX.P) P-groups and two tentative (GNA1.P and GNA2.P) P-groups (Fig. 3a, Table S4). The RdRp sequences of GIV viruses that infect humans clustered together and were classified as GIV.P groups whereas GIV and GVI viruses from cats, lions and dogs clustered together and are classified as GVI.P groups (Fig. S1).

The RdRp sequences of GI viruses could be divided into 14 P-types and 4 tentative P-types (GI.PNA1, GI.PNA2, GI.PNA3 and GI.PNA4) with one available sequence or multiple strains from the same geographic location (Tables 1b, S4, and Fig. 3b). Similarly, RdRp sequences of GII viruses could be separated into 37 P-types (GII.P1- GII.P8, GII.P11-GII.P13, GII.P15-GII.P18, GII.P20-GII.P41) and 9 tentative P-types (GII.PNA1-GII.PNA9) (Table S4, Fig. 3c). GIII viruses could be divided into two P-types (GIII.P1-P2); GIV into one confirmed (GIV.P1) and one tentative (GIV.PNA1); GV into two (GV.P1 and GV.P2); GVI into two (GVI.P1-P2); GVII

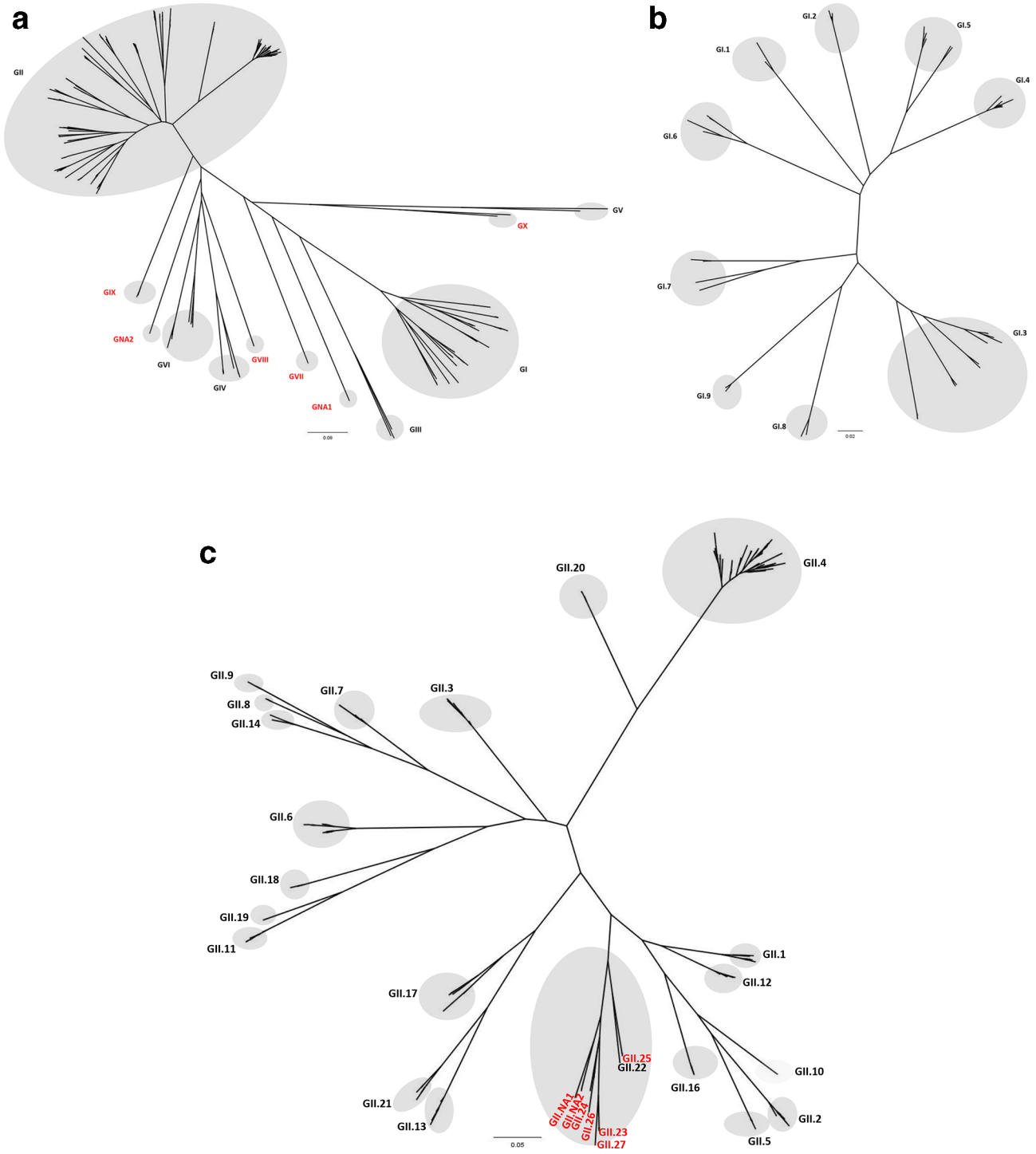

Fig. 2. Phylogenetic classification of noroviruses based on VP1 amino acid sequences into ten norovirus genogroups and two non-assigned (NA) genogroups (a), GI genotypes (b) and GII genotypes (c). Phylogenetic analysis was performed using maximum likelihood (PhyML). Resulting trees were plotted and edited in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Newly identified genogroups and genotypes are labelled in red.

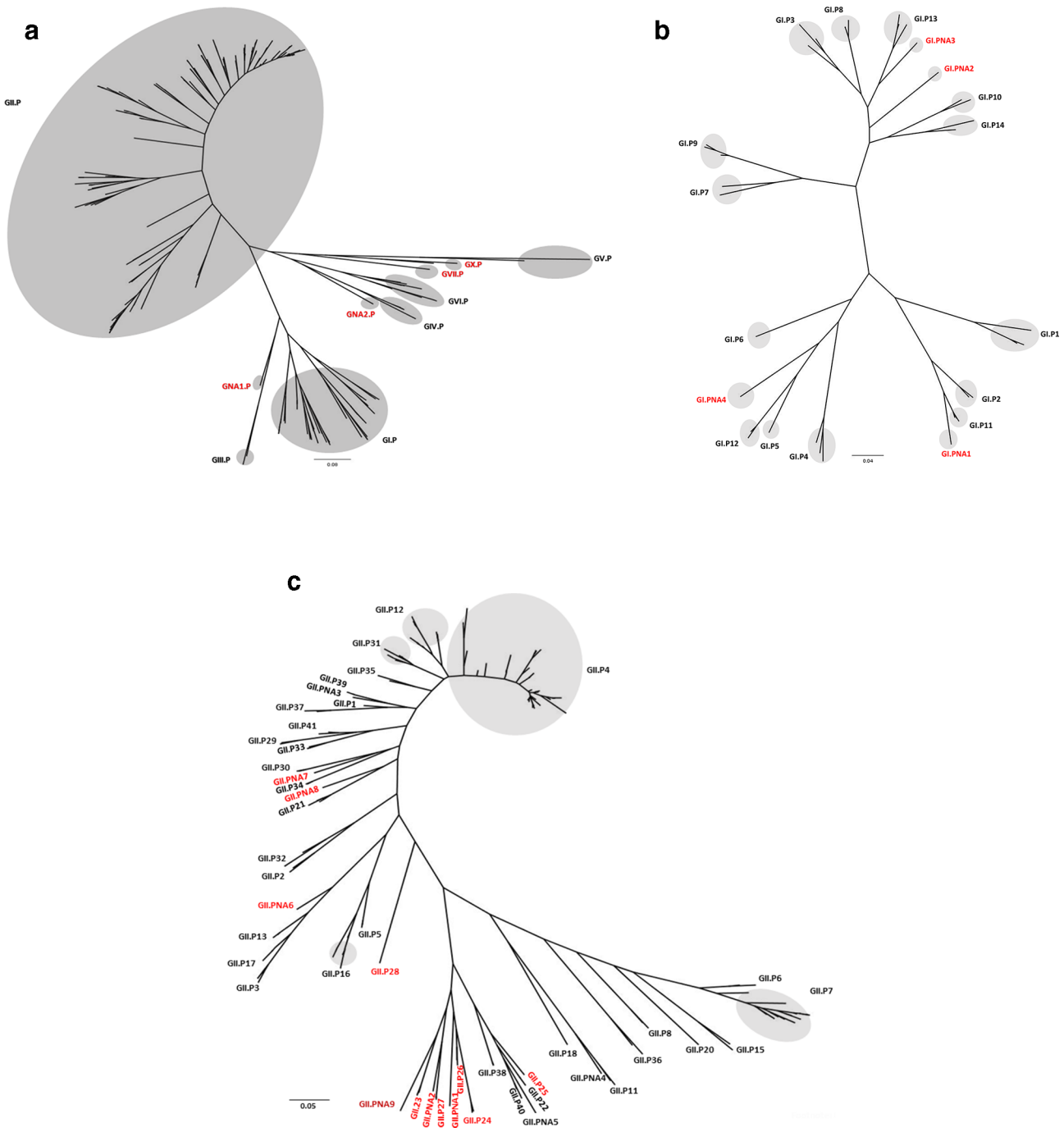

Fig. 3. Phylogenetic trees of partial (762 nucleotides at 3′-end of ORF1) RdRp sequences of norovirus genogroups (a), GI P-types (b) and GII P-types (c). Tentative P-groups and P-types with only a single sequence or multiple non-identical sequences from a single geographic location are referred to as non-assigned (NA). Phylogenetic analysis was carried out using maximum likelihood (ML) with PhyML. The resulting trees were plotted and edited in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Newly identified genogroups and genotypes are labelled in red. In Fig 3c, only GII P-types with highly divergent clusters are shown with a gray circle for clarity.

into one (GVII.P1) and GX into one (GX.P1) P-type(s) (Table S4). GNA1 and GNA2 P-groups both include a single strain and therefore are designated as GNA1.P1 and GNA2.P1. The RdRp sequences of GVIII and GIX viruses, which cluster with GII viruses, were assigned P-types GII.P28 and GII.P15, respectively (Table S4).

Viruses previously typed as GI.Pb, GII.P1, GII.P11, GII.P22 and GII.Pg did not meet the 2×SD criteria, either due to overlapping standard deviations (GI.Pb, GII.P22 and GII.Pg) (Fig. 4) or due to high intra-genotype diversity among the sequences (GII.P1 and GII.P11). To group these strains into P-types that meet the 2×SD criteria, viruses from

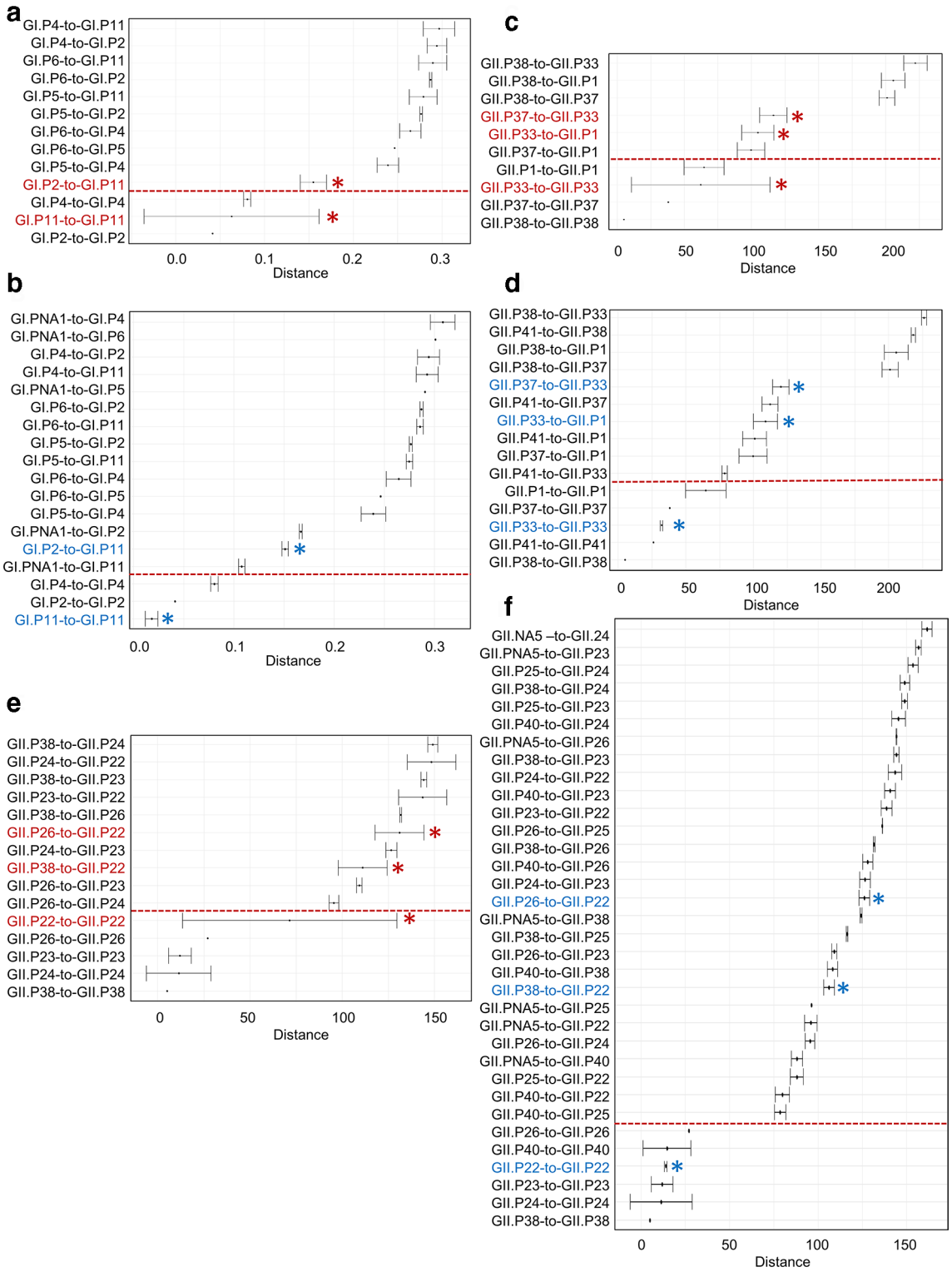

Fig. 4. Patristic and P-distance comparison of GI.P11 (previously GI.Pb) (a and b), GII.P33 viruses (c and d) and GII.P22 viruses (e and f) with their phylogenetically closest P-types. Error bars represent 2xSD for each P-type comparison. The red * and font indicate the overlap of 2×SD error bars for viruses within a cluster and its closest P-types (a, b and e). After re-assigning GI.P11 viruses into two clusters (GI.P11 and GI.PNA1) (c), GII.P33 into GII.P33 and GII.P41 (d) and GII.P22 into three clusters (GII.P22, GII.P40 and GII.PNA5) (f), no overlap of 2×SD error bars within or in-between P-types was present (blue * and font). Y-axis represents comparison of distances within and in-between P-types. Below the dotted line, distances within P-type are indicated and above the dotted line, distances between P-types are indicated. (2×SD criteria: phylogenetic distances of sequences within a P-type should not overlap with distances between different P-types.)

clusters GI.Pb, GII.P1, GII.P11 and GII.Pg were re-classified into eight P-types and four non-assigned P-types including (GI.P11 [previous GI.Pb], GII.P1, GII.P11, GII.P22, GII.P33 [previous P-type GII.Pg], GII.P39, GII.P40, GII.P41, GI.PNA1, GII.PNA3, GII.PNA4 and GII.PNA5) (Fig. 4, Table 1b and Table S4). Since segregation of these P-types was based on partial RdRp sequences, we also analysed available complete ORF1 sequences, which confirmed the need of re-classification into additional P-types (Figs S2 and S3). However, since no complete ORF1 sequences were available, we were unable to complete analyses for GII.P11 viruses.

DISCUSSION

We updated the number and classification of norovirus genogroups and genotypes statistically supported by 2×SD criteria (Fig. 5). Until recently, noroviruses were officially grouped into six genogroups (GI–GVI) [2]. Our updated analysis demonstrates that, based on VP1 amino acid sequence diversity, the number of genogroups in the genus *Norovirus* can be expanded to ten (GI–GX), as well as two tentative genogroups, which will need to be confirmed when additional full VP1 or partial RdRp sequence becomes available. Viruses in these ten genogroups can be further divided into 49 confirmed capsid genotypes based on amino acids of the complete VP1 and 60 confirmed P-types based on partial nucleotide sequences of RdRp regions.

Over the last decade, several new norovirus sequences have been detected from new hosts. These included canine norovirus sequences that were identified in faecal swab specimens of dogs in Hong Kong, China in 2007 [27, 49] as well as in sewage samples from Uruguay in 2012 [47]. Our analyses confirmed the classification of these viruses into a new genogroup, GVII. During routine surveillance of stool samples from children with acute gastroenteritis in Chiba, Japan in 2004, a tentative new GII genotype was reported [50] and a genetically similar strain from Yuzawa, Japan in 2011 was submitted to GenBank. We confirmed that the VP1 sequences of these strains represent a novel genogroup, GVIII.

Among GII viruses, GII.15 viruses have phylogenetically always been an outlier. With five VP1 sequences including one complete genome available in GenBank, we confirmed that based on 2×SD criteria, GII.15 viruses should be re-classified into a separate genogroup, designated as GIX. A multi-year virome analysis of pharyngeal and anal swab samples from a large collection of major bat species in China showed several novel norovirus sequences that could be classified into a separate genogroup (GX) [12]. Tentative new genogroups were also detected in harbour porpoises [9] (GNA1) and sea lions (GNA2). Applying the 2×SD criteria to the classification of phylogenetic clusters of GIV and GVI suggests that the three GIV genotypes and two GVI genotypes may each represent separate genogroups, however, we decided not to re-classify them but wait until additional GIV and GVI sequences become available.

In recent years, several new GII and GIV genotypes from different geographic locations around the world have been reported [41, 43, 45, 46, 48]. With the addition of five novel genotypes GII.23, GII.24, GII.25, GII.26 and GII.27, we now recognize 26 GII genotypes (GII.1–GII.27) (excluding GII.15, which now represents a novel genogroup) along with two tentative new GII genotypes (GII.NA1 and GII.NA2). The new GII genotypes were identified in samples collected from humans in Peru, Guatemala, Ecuador, Bangladesh, Germany, Argentina and the USA [43, 45, 48]. A tentative new GIV genotype was recently identified in wastewater (Brazil, Japan) and in stool samples from an acute gastroenteritis outbreak in a restaurant in the USA ([41, 46] J Vinjé, personal communication). However, since only one complete GIV genome sequence is currently available, classification into a new genotype is pending additional sequences and therefore this genotype is temporarily designated as not assigned (NA) (GIV.NA1[PNA1]).

Until now, classifying noroviruses into P-types was based on a naming system that used P-type designation linked to the corresponding VP1 genotype of the strain and when no VP1 sequence was known, orphan P-types and/or P types with alphabet letters were assigned [19]. Since an increasing number of recombinant strains have been recognized in recent years [33, 37, 40, 51], we propose an update for the norovirus classification system in which P-types and VP1 genotypes are assigned independently. We also propose designating dual types with the capsid genotype listed first followed by P-type (e.g. GI.6[P10], GII.4 Sydney[P16], GIX.1[GII.P15], GIV.2[GVI.P1]).

Five P-types, some of which include strains dating back to the 1970–1980s, were grouped into 12 P-types to meet 2×SD criteria. Of note, P-types (GII.P6 and GII.P7) have overlapping standard deviations and several GII.P4 types also did not satisfy the 2×SD criteria because of overlapping standard deviations with GII.P12, GII.P31 (former GII.Pe) and GII.P35 (former GII.Pj) viruses (Figs S4 and S5). Since all three P-types are historic types, especially GII.P4 strains, which are of epidemiological importance worldwide and the current P-typing classification is based on relatively short nucleotide sequence fragments (762 nucleotides), we decided that we will re-visit this issue in our next classification update, which is expected to be based on complete ORF1 sequences.

RdRp sequences of GIV, GVI, GVIII, GIX viruses segregate independently of their corresponding capsid genogroups. For example, RdRp sequences of GVIII, GIX viruses cluster with GII viruses, and were designated as GII.P28 and GII.P15, respectively. Similarly, RdRp sequences of GIV and GVI viruses clustered into two distinct groups with sequences from GIV viruses that infect humans clustering together with other GIV strains, whereas sequences from GIV and GVI viruses that infect cats, lions and dogs clustered together as GVI strains. Thus, inter-genogroup recombination, which is considered a rare event among noroviruses [52, 53], may have occurred among viruses infecting humans (GVIII.1[GII.P28]; GIX.1[GII.P15]) and among viruses infecting canines

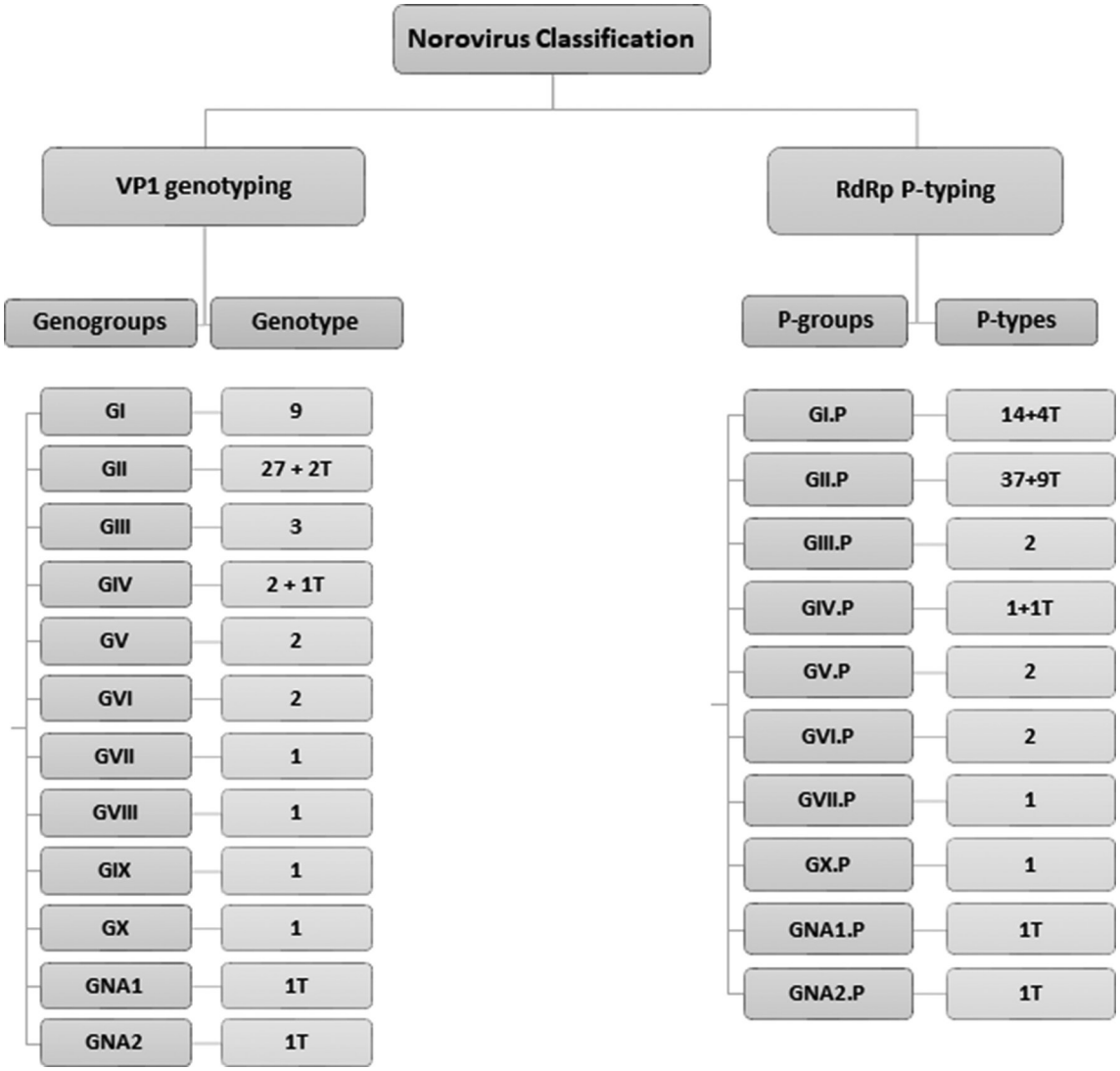

Fig. 5. Updated norovirus classification scheme with the number of genogroups and genotypes based on complete VP1 amino acids and the number of P-groups and P-types based on a partial region (762 nucleotides) of the RNA-dependent RNA polymerase (RdRp) at the 5′-end of ORF1. P=P-group, T=tentative genotype/P-type (when only a single sequence or multiple non-identical sequences from a single geographic location as available, they are non-assigned (NA).

(GIV.2[GVI.P1]). In addition, several recently reported RdRp sequences without an associated VP1 sequence were included in our updated classification scheme [33, 40].

One major limitation of the present classification is that current P-typing is based on a relatively short nucleotide sequence fragment (762 nucleotides) at the 3′-end of ORF1. Reassuringly, phylogenetic clustering of these shorter RdRp sequences was identical with segregation of P-types based on the complete RdRp sequences (~1500 nt), which were available for 85% of the sequences used in our study. Additional complete RdRp sequences or ideally complete genome sequences for all reference strains will help to improve the robustness of the present classification system for which several protocols have been described recently [54–56].

With consensus from the NCWG, we propose to base future nomenclature and classification updates on complete ORF1 and VP1 sequences of new genetic lineages of viruses in the genus *Norovirus*. The updated nomenclature, including new genogroup, genotypes and new P-type names are available via publicly accessible typing tools https://www.rivm.nl/mpf/norovirus/typingtool and https://norovirus.ng.philab.cdc.gov both of which use the same set of GenBank-available norovirus reference sequences. For high-quality sequences that cannot be typed using these typing tools, researchers have the option to contact members of the NCWG for additional analyses. Database administrators of both typing tools will coordinate among each other to periodically monitor public

databases for novel genotypes and update the reference sequences accordingly.

METHODS

GenBank sequences used for analysis

A total of 305 complete ORF2 sequences (ranging from 530 to 580 amino acids in length), representing the genetic diversity of all norovirus genogroups and genotypes including reference strains used by CaliciNet [37] and NoroNet [44] databases were downloaded from GenBank (last download date: March 2019) (Table S1). In addition, 232 partial nucleotide sequences (762 nucleotides) of the RdRp region at the 3′-end of ORF1 were also downloaded (Table S3).

Phylogenetic analyses

ORF2 nucleotide sequences of all 305 norovirus strains were translated into amino acids and aligned using ClustalW [57]. In addition, separate alignments were carried out for VP1 amino acid sequences of GI (n=50) and GII (n=218) noroviruses. Sequences of the remaining 37 strains belonged to GIII (n=3), GIV (n=11), GV (n=2), GVI (n=8), GVII (n=2) and unassigned genogroups (n=11). For the RdRp region, an alignment of all 232 norovirus sequences was generated, as well as separate alignments for GI (n=44) and GII (n=163) sequences. Phylogenetic analyses were performed as described previously [19]. Briefly, phylogenetic trees were computed with PhyML [58, 59] through the web server at the ATGC Montpellier Bioinformatics platform (http://www.atgc-montpellier.fr/phyml/) and the resulting trees were plotted and edited in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Known genogroups and genotypes were identified using the prototype sequences [2, 19, 27]. Phylogenetic distance matrices were computed using Patristic [60]. Mean distances and standard deviations (SD) values within and between phylogenetic clusters were computed in R [61]. According to 2×SD criteria, the average distance between all sequences within a new genogroup or genotype and its nearest cluster should not overlap within two standard deviations (2×SD) from each other [19]. New genogroups or genotypes were assigned by comparing the average distance between all sequences within and between phylogenetic cluster(s) (genogroup or genotype) (Fig. 1). Segregation of sequences into genogroups was based on 2×SD values followed by each individual genogroup, subdividing them further into genotypes.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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