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BRIEF REPORT

Re-emergence of genotype G9 during a five-and-a-half-year period in Turkish children with rotavirus diarrhea

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Abstract This study was done to understand the dynamics of rotavirus genotype distribution in Turkish children. Samples were collected from January 2006 through August 2011 from children at a hospital in Ankara. Rotavirus was detected in 28 % (241/889) of the samples. Genotype G9P[8] was predominant (28 %), followed by G1P[8] (16.3 %) and G2P[8] (15.9 %). G9 was absent in the samples from 2006 and 2007 and then re-emerged in 2008 and increased gradually. Phylogenetic analysis showed that Turkish G9 rotaviruses of the present study formed a sublineage with strains from Italy and Ethiopia, possibly indicating spread of a clone in these countries.

Keywords Rotavirus · Prevalence · Genotypes · Children · Turkey

In Turkey, diarrhea is an important cause of illness in children. Every year approximately 1–1.5 million diarrhea cases are identified in Turkey, of which 80 % are in children under two years old; however, the true prevalence of different viral agents is not well known [1]. With a large annual birth cohort (> 1.2 million), Turkey presumably experiences a high rotavirus disease burden, resulting in high healthcare costs and impaired quality of life [2].

Rotavirus infection is a major cause of infantile diarrhea worldwide, resulting in about 453,000 deaths yearly among children under 5 years of age [3]. The VP7 and VP4 proteins, which form the outer capsid, are most important for characterization of rotaviruses. Currently, 27 G and 38 P genotypes have been described in rotaviruses from humans and animal species [4, 5]. A few combinations (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) account for nearly 90 % of the human strains circulating worldwide. Globally, G1P[8] accounts for 60-80 % of all the strains. However, during the last decade, G9 has emerged as an important genotype [5, 6].

Rotavirus genotype diversity, particularly G9 and G12, is a great challenge for current vaccination programs [6]. Furthermore, an increased frequency of the G9P[4] genotype has been detected in Latin American countries during and after rotavirus vaccine implementation [5]. A national rotavirus vaccine program has not yet been implemented in Turkey, and data on the genotype distribution of rotaviruses is scarce in this country. Therefore, it is necessary to determine the rotavirus genotype distribution before the implementation of a national rotavirus vaccination program. Turkey's geographical location at the crossroads of Europe, Asia and Africa has epidemiological significance in the evolution and spread of pathogens. Therefore, molecular epidemiology of the circulating rotavirus strains may reveal unique information on genetic diversity and adaptation of viruses. We aimed in this study to determine the distribution of genotypes of rotavirus to understand their natural fluctuation before the introduction of rotavirus vaccines.

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This is part of a project to identify viral etiologies of diarrhea in Turkish children. Stool samples were collected prospectively from 889 children under 5 years old with watery diarrhea at the Ministry of Health Ankara Training and Education Hospital, Ankara from 1 January 2006 through 15 August 2011. The research was approved by the ethics committee of the Ankara Training and Education Hospital. Verbal consent was obtained from the child's guardian prior to sample collection.

Rotaviruses were identified by commercially available enzyme immunoassay (Rotaclone, Meridian Diagnostics Inc., Cincinnati, Ohio, U.S.A.) according to the manufacturer's instruction.

For VP7 and VP4 gene amplification, extracted RNA was transcribed to cDNA using AccessQuick RT-PCR (Promega Corporation, Madison, WI, USA). For G- and P-specific genotyping multiplex-PCR was done using PCR Master Mix (Promega) [7, 8]. Amplicons were analyzed in a 2 % agarose gel.

Full-length VP7 gene sequences were used for phylogenetic analysis. Partial sequencing of the VP7 and VP4 genes was performed with representative strains only to confirm that the PCR results were correct. The nucleotide sequences of the genes were determined using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the instruction of the manufacturer, and the product was analyzed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). A multiple sequence alignment was made using the ClustalW program, and phylogenetic analysis was done by neighbor-joining method [9].

There were year-to-year fluctuations in the percentage of rotavirus-positive samples from 22.2 % to 35.3 %, with a mean 28.0 % (95 % CI 22.2–33.9 %). The proportion of rotavirus-positive samples in males and females was 27.6 % and 26.3 %, respectively.

Genotype G9[P8] was predominantly detected in 28 % of samples, followed by G1P[8] (16.3 %) and G2P[8] (15.9 %). Genotypes G2P[4] and G1P[4] were found in 10.4 % and 9 % of the samples, respectively. Other genotypes, G3P[8] and G4P[8], were found in 4.5 % and 2.2 %, respectively.

In 2006, the most common genotype was G1P[8], which was detected in 68.7 % of the samples. In 2007, G1P[4] (38.5 %) and G2P[4] (38.5 %) were found in equal proportion. In 2008, G9[8] emerged and became co-dominant (21.2 %) with G2P[4] (21.2 %). In 2009, G9P[8] (34.8 %) became the dominant type, followed by G1P[8] (32.6 %). Then, in 2010, the proportion of G9P[8] increased (44.3 %), followed by G2P[8] (31.4 %). Finally, in 2011, the proportion of G9P[8] increased further (40 %), followed by G2P[8] (30 %).

P[6] was associated with seven strains, and of these, four were G2, one was G9, and for two, the G type was undetermined. P[9] was associated with only one G9 strain.

(a)

Strain	Residues																											
											7-1a	7-1a			7-1a		7-2			7-1b	7-2						7-1a	
	29	37	41	49	65	66	68	72	74	75	94	97	101	108	123	141	147	170	202	212	217	218	266	268	281	291	321
Rotarix	I	S	Y	R	T	V	A	Q	G	I	N	E	S	T	S	L	N	I	M	V	M	V	S	V	T	K	A
RotaTeq	I	F	T	R	A	V	T	Q	E	V	N	D	T	T	S	F	S	V	T	V	M	I	S	V	T	K	S
AHP 525	I	F	F	K	T	V	S	Q	G	I	S	E	S	I	N	L	N	I	T	V	T	V	A	I	I	R	A
AHP 550	I	F	F	K	T	V	S	R	E	V	S	E	S	T	N	L	N	I	T	T	T	V	A	I	I	R	A
AHP 767	M	S	C	R	A	A	A	Q	G	I	N	E	S	I	S	L	N	I	T	V	M	V	S	V	T	K	A

(b)

Strain	Residues																	
							7-1a	7-1a	7-2		7-1b	7-1b						
	35	40	42	44	55	75	87	96	147	178	213	242	281	287	307	319	326	
RotaTeq	Y	V	V	I	I	T	A	D	T	S	S	S	I	I	V	A	V	
AHP 229	F	L	A	M	M	S	T	N	A	N	D	N	V	V	I	T	I	
AHP 757	F	L	A	M	M	S	T	N	A	N	D	N	V	V	I	T	I	

Fig. 1 Comparison of the antigenic residues of VP7 present in Turkish G1 strains with those of RotaTeq and Rotarix (a), and in Turkish G2 strains with that of RotaTeq (b). The respective antigenic epitopes are shown above the residue numbers. The amino acid in the Turkish strains that differed from those in the vaccine strains are highlighted in blue. The amino acid residues highlighted in yellow are different from those in the other vaccine strain and the Turkish strains

Compared with the Rotarix vaccine strain RIX4414 and the RotaTeq vaccine strain W179-9, the Turkish G1 strains shared 93 %–98 % amino acid sequence identity. Compared with Rotarix, there were substitutions at 6–16 residues in all of the Turkish strains (Fig. 1a). Two of the three Turkish strains had substitutions at 4–5 residues belonging to the 7-1a, 7-1b and 7-2 antigenic regions [10]. The third Turkish strain had no substitutions in any of these antigenic regions. Compared with RotaTeq, there were substitutions at 15–21 residues in all of the Turkish strains. These Turkish strains had substitutions at 2–7 residues belonging to the 7-1a, 7-1b and 7-2 antigenic regions. When compared with RotaTeq vaccine strain W179-9, the Turkish G2 strains shared 95 % amino acid sequence identity (Fig. 1b). There were substitutions at 17 residues in all of the Turkish G2 strains. Two strains had substitutions at five residues belonging to the 7-1a, 7-1b and 7-2 antigenic regions.

The Turkish G1 rotaviruses belonged to lineages I and V and were closely related to strains from Belgium and India, and Germany, respectively (Fig. 2a). Turkish G2 rotaviruses belonged to lineage II and clustered with strains from Russia (Fig. 2b). The G1 and G2 strains were closely related to globally circulating strains of similar lineages. The Turkish G9 rotaviruses in the present study belonged to lineage III, and they formed a sub-lineage with a significant bootstrap value, indicating that very similar strains are circulating in our study population (Fig. 3). These strains were closely related to strains from Ethiopia, Germany and Italy. The Turkish G9 strains circulating in 2005 belonged outside this sub-lineage, and none of these strains are currently circulating in Turkey.

In the present study, there was a yearly fluctuation in genotype distribution and a decreasing frequency of rotavirus-positive samples, which is the natural trend for rotaviruses. Genotype G9 strains were the most predominant, followed by G2 and G1 strains circulating in our study population. Usually, G1 and G2 are in combination with P[8] and P[4], respectively. However a considerable number of strains were G1P[4] and G2P[8], indicating inter-genogroup reassortment events. More studies are needed on the complete genomic characteristics of these strains to determine how such reassortment occurred.

The VP7 sequences of Turkish G1 strains had less amino acid sequence similarity to that of the RotaTeq strain W179-9 than to that of Rotarix RIX4414. There were more amino acid differences compared with RotaTeq than with the Rotarix strain. With respect to the amino acid differences that are known to be responsible for generating neutralization escape strains [6], the Turkish G1 strains contained more amino acid differences when compared to RotaTeq than when compared to Rotarix. The VP7 sequences of the Turkish G2 strains also had low amino acid sequence similarity to those of RotaTeq strain W179-9. Compared with RotaTeq strain W179-9, Turkish G2 showed differences at several amino acid residues, including all of the antigenic epitopes present on VP7. These results suggest that Rotarix may be a better choice, although the mechanism responsible for vaccine-induced protection is not clearly understood. It is known that serotype-specific neutralizing antibodies directed against VP7 and VP4, as well as virus-specific cytotoxic T lymphocyte induction are responsible for protection; however, other proteins may be involved in immune protection against rotaviruses [11, 12].

Phylogenetically, the G1 and G2 strains detected in this study were similar to the strains found in other parts of the world. The share of G3 and G4 remained insignificant in Turkey. A study done on samples collected during 2012–2014 from all over Turkey showed similar results [13]. Studies conducted during approximately the same time period as the present study showed that G9 was a predominant strain in Kenya [14], Zimbabwe, Zambia [15] and India [16].

Genotype G9 strains have spread globally since 1995 and have been established in many countries, and they are therefore regarded as the fifth common human strain. During 2000–2002, G9 was present sporadically in only 3.4 % of Turkish stool samples. In 2004, G9 emerged in Turkey and then increased substantially in 2005 to 17.2 % of the samples [17]. Now, 28 % of the samples are G9 after a disappearance for 2 years. A decrease in G9 has been noted in other countries as a natural fluctuation of genotype distribution [18] or after implementation of rotavirus vaccines in the USA, Australia and Brazil [6], but studies on the disappearance of G9 for two consecutive years and its re-emergence are absent. Phylogenetically, the G9 strains in the present study formed an independent sub-lineage, indicating the possible spread of a clone responsible for the current dominance of G9 in Turkish children. The present study highlights that genotype G9 can disappear completely from circulation but can re-emerge through the invasion of a new sub-lineage.

In Turkish samples, G9 was mainly found in combination with P[8], except in three samples where it was in combination with P[9], P[6], or P[4]. Rotavirus G9P[4] has increased in Mexico, Guatemala and Honduras during and after rotavirus vaccination, and recently in Bangladesh, where rotavirus vaccine has not yet been introduced in the government vaccination program [19, 20], but in Turkey, G9P[4] remains at low level, as found in this and the previous study.

Two live attenuated rotavirus vaccines are available at present commercially. Both vaccines have been used successfully in several countries [21]; however, there are concerns whether the vaccine is effective against other genotypes, particularly G9 and G12. A study in Brazil

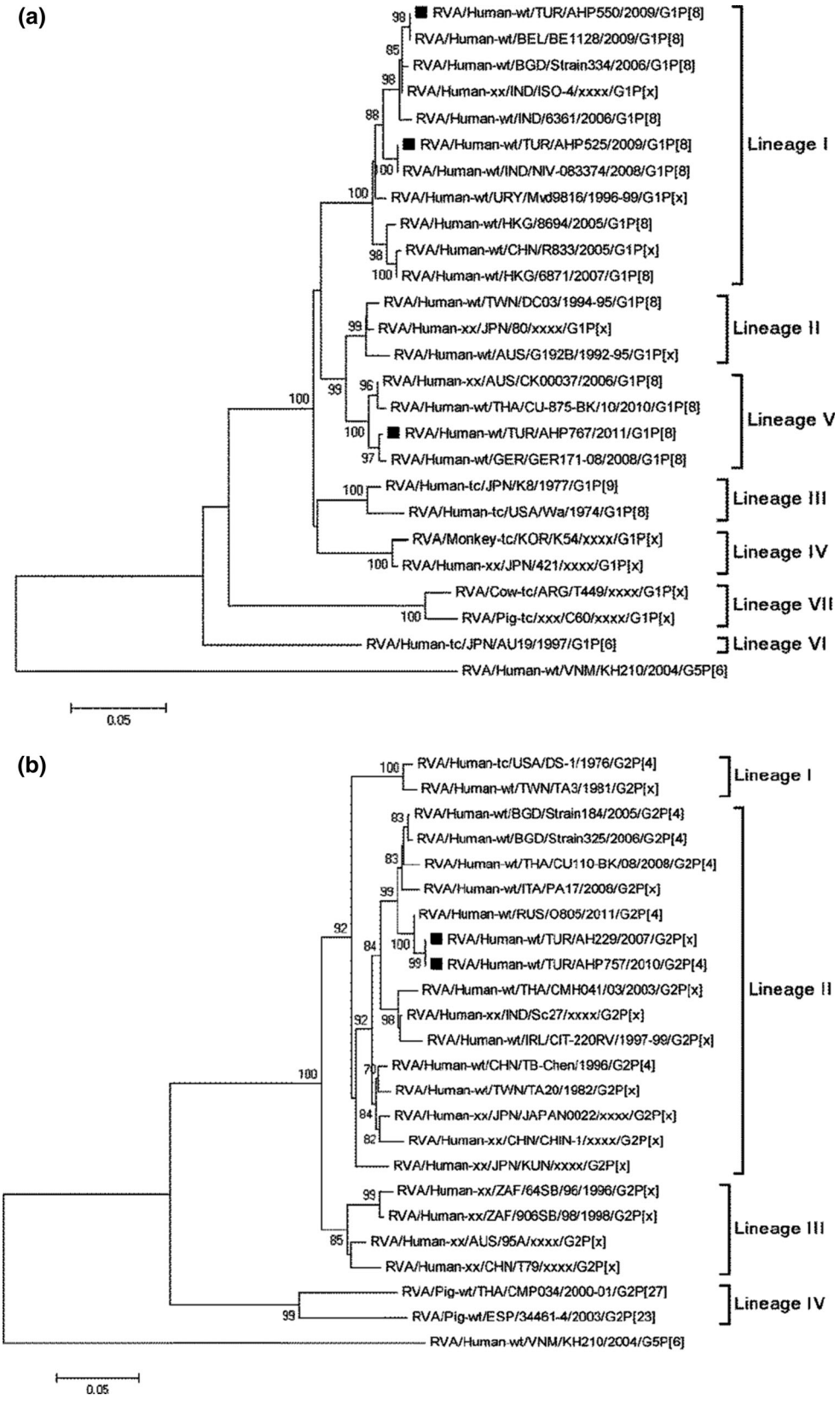

Fig. 2 Phylogenetic tree constructed using the nucleotide sequences of the VP7 gene of G1 (a) and G2 (b) strains. The strains from this study are marked with a filled square. A human G5 rotavirus strain was used as an outgroup. P[x] indicates that the P genotype is unknown. The number adjacent to the node represents the bootstrap value. Values lower than 70 % are not shown. The scale bar at the bottom shows the genetic distance expressed as nucleotide substitutions per site

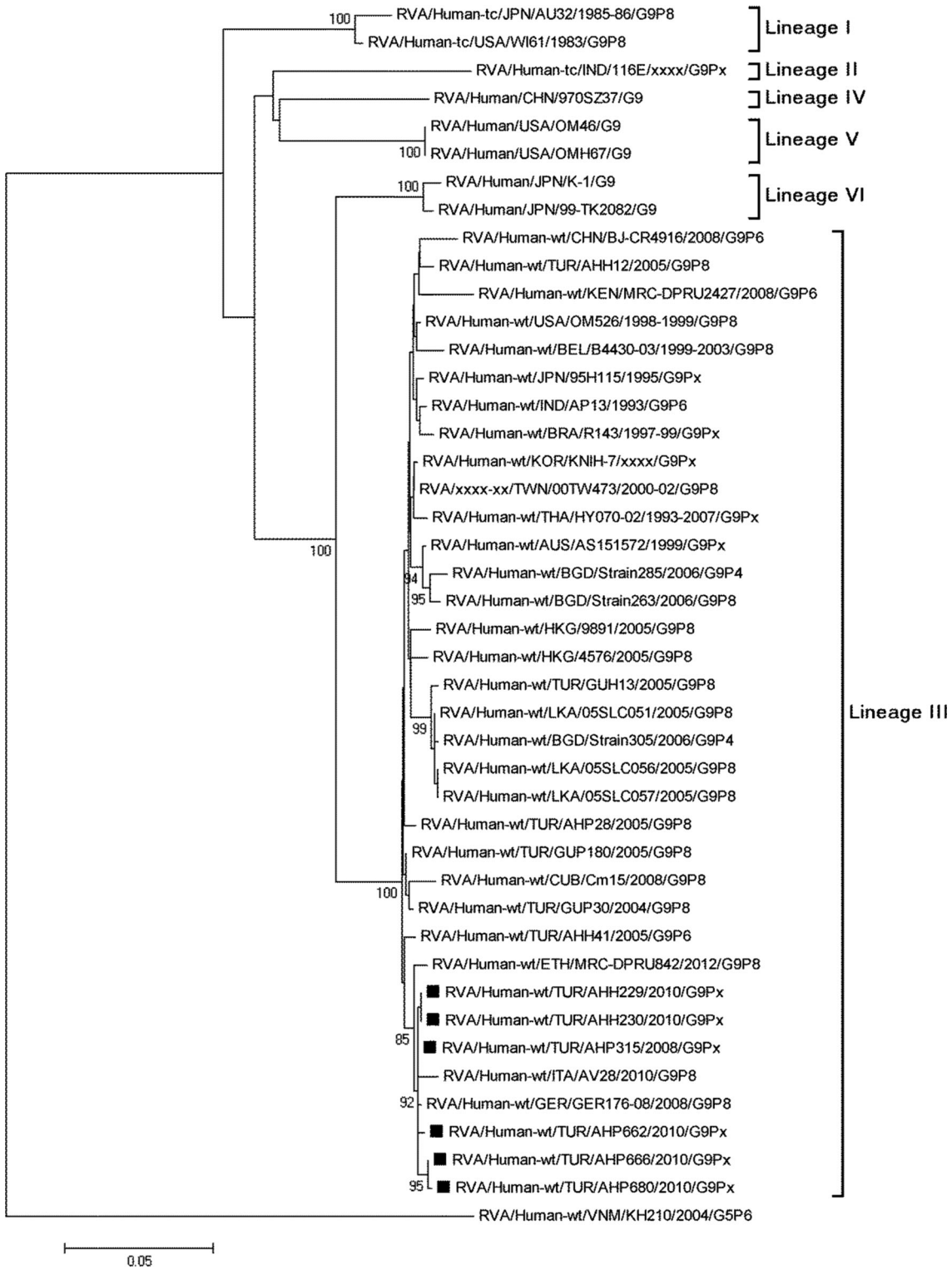

Fig. 3 Phylogenetic tree constructed using the nucleotide sequences of the VP7 gene of G9 strains. The strains form this study are indicated by a filled square. A human G5 rotavirus strain was used as an outgroup. [Px] indicates that the P genotype is unknown. The number adjacent to the node represents the bootstrap value. Values lower than 70 % are not shown. The scale bar at the bottom shows genetic distance expressed as nucleotide substitutions per site

found good efficacy of Rotarix against G9 rotaviruses [22], which provides support to a previous observation during vaccine trials that there is good efficacy against non-G1 types. Recently, a monovalent vaccine made from a G9P[11] strain has been shown to be effective in children in Indian [23, 24], where G9 is one of the predominant strains [25]. A trial of this vaccine might be advantageous to show whether the vaccine efficacy increases in Turkish children. In conclusion, continued surveillance of rotavirus is necessary in Turkey before and after the introduction of rotavirus vaccine in the national vaccination program in order to monitor changes and design a new course accordingly.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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