In accordance with our hypothesis regarding an association between MMPs and RV antigenemia, a positive correlation was identified between MMP-9 and RV antigenemia, while a reverse correlation was found between MMP-2 and RV antigenemia. These data suggest that these two MMPs have important roles in the pathogenesis of RV antigenemia. Since it has been suggested that the level of RV antigenemia is associated with disease severity [Ramani et al., 2010], an association between the serum MMPs concentrations and disease severity should be analyzed in a future study. Additionally, it is important to elucidate which cells are responsible for the local production of the MMPs in RV gastroenteritis, and the regulatory mechanisms for their production. Epithelial cells or infiltrating mucosal lymphocytes have been suggested to synthesize MMP-9 and MMP-2 in inflamed tissues [Castaneda et al., 2005]. As it is difficult to obtain biopsy specimen from RV gastroenteritis patients, pathological analysis of RV infected animals would be helpful to determine which cells in the intestinal tissue secret the MMPs. Since MMPs knockout mice have been useful to analyze role of MMPs in the pathogenesis of IBD [Garg et al., 2006; Munoz et al., 2009], we propose similar experiments to test our hypothesis.

The enzymatic activity of MMPs is controlled by TIMPs [Brew and Nagase, 2010]. Thus, the balance of MMPs and TIMPs within the tissue may be important for the regulation of local tissue damage. Two TIMPs (TIMP-1 and TIMP-2) were investigated in this study. TIMP-1 concentrations were found to be similar in patients with RV gastroenteritis and healthy controls. Meanwhile, TIMP-2 concentrations in RV gastroenteritis patients were significantly increased at the time of admission and discharge compared to healthy controls suggesting that TIMP-2 might be important for the pathogenesis of RV antigenemia. Although TIMP-1 concentration has been shown to increase in IBD patients [Louis et al., 2000; Arihiro et al., 2001; Wiercinska-Drapalo et al., 2003; Kapsoritakis et al., 2008], it has been demonstrated that TIPM-2 concentration remained normal in these patients [Kapsoritakis et al., 2008]. Thus, TIMPs expression may be differentially regulated in RV infection and IBD.

As several cytokines and chemokines have been suggested to be associated with upregulation of MMPs synthesis [Lotz and Guerne, 1991; Saren et al., 1996; Kusano et al., 1998], the correlation between serum cytokine concentrations and MMP-9 or MMP-2 was analyzed in this study. Although many reports have demonstrated a positive correlation between TNF-α and MMP synthesis [Pender et al., 1997; Pender et al., 1998; Louis et al., 2000], no remarkable association between these two biomarkers was found in the present study. It has been suggested that RV infection activates dendritic cells in Peyer's patches resulting in upregulation of TNF-α expression [Lopez-Guerrero et al., 2010]. Therefore, an association between locally produced MMP and TNF-α should be examined to elucidate the precise role of TNF-α in the regulation of MMPs in RV infection. Meanwhile, significant positive association between IL-6 and MMP-9 was identified. It has been suggested that IL-6 released from fibroblasts was responsible for secretion of MMP-9 from dendritic cells in in vitro dermal microenvironment model [Saalbach et al., 2010]. Similar pathophysiological mechanisms should be evaluated in the intestinal tissue of RV gastroenteritis patients.

In conclusion, these results suggest that MMP-9 and MMP-2 play important role in causing RV antigenemia as factors for attack and protection, respectively. Additionally, TIMP-2 might be important for the pathogenesis of RV antigenemia as a controller of MMPs. However, no other cohort such as Norovirus gastroenteritis patients was included in this study, further cohorts analysis is necessary to determine whether kinetics of MMPs and TIMPs were specific for RV gastroenteritis or not.

ACKNOWLEDGMENTS

We thank Mrs. Akiko Yoshikawa and Mrs. Chieko Mori for technical support.

REFERENCES

Arihiro S, Ohtani H, Hiwatashi N, Torii A, Sorsa T, Nagura H. 2001. Vascular smooth muscle cells and pericytes express MMP-1, MMP-9, TIMP-1 and type I procollagen in inflammatory bowel disease. Histopathology 39:50–59.

Bailey CJ, Hembry RM, Alexander A, Irving MH, Grant ME, Shuttleworth CA. 1994. Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collagenase in Crohn's disease and normal intestine. J Clin Pathol 47:113–116.

Bergin PJ, Anders E, Sicheng W, Erik J, Jennie A, Hans L, Pierre M, Qiang PH, Marianne QJ. 2004. Increased production of matrix metalloproteinases in Helicobacter pylori-associated human gastritis. Helicobacter 9:201–210.

Bergin PJ, Raghavan S, Svensson H, Starckx S, Van Aelst I, Gjertsson I, Opdenakker G, Quiding-Jarbrink M. 2008. Gastric gelatinase B/matrix metalloproteinase-9 is rapidly increased in Helicobacter felis-induced gastritis. FEMS Immunol Med Microbiol 52:88–98.

Blutt SE, Kirkwood CD, Parreno V, Warfield KL, Ciarlet M, Estes MK, Bok K, Bishop RF, Conner ME. 2003. Rotavirus antigenaemia and viraemia: A common event? Lancet 362:1445–1449.

Brew K, Nagase H. 2010. The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. Biochim Biophys Acta 1803:55–71.

Castaneda FE, Walia B, Vijay-Kumar M, Patel NR, Roser S, Kolachala VL, Rojas M, Wang L, Oprea G, Garg P, Gewirtz AT, Roman J, Merlin D, Sitaraman SV. 2005. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: Central role of epithelial-derived MMP. Gastroenterology 129: 1991–2008.

Dennehy PH. 2008. Rotavirus vaccines: An overview. Clin Microbiol Rev 21:198–208.

Fischer TK, Ashley D, Kerin T, Reynolds-Hedmann E, Gentsch J, Widdowson MA, Westerman L, Puhr N, Turcios RM, Glass RI. 2005. Rotavirus antigenemia in patients with acute gastroenteritis. J Infect Dis 192:913–919.

Gao Q, Meijer MJ, Kubben FJ, Sier CF, Kruidenier L, van Duijn W, van den Berg M, van Hogezand RA, Lamers CB, Verspaget HW. 2005. Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases. Dig Liver Dis 37:584–592.

Garg P, Rojas M, Ravi A, Bockbrader K, Epstein S, Vijay-Kumar M, Gewirtz AT, Merlin D, Sitaraman SV. 2006. Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis:

Contrasting role of gelatinases in the pathogenesis of colitis. J Immunol 177:4103–4112.

Garg P, Vijay-Kumar M, Wang L, Gewirtz AT, Merlin D, Sitaraman SV. 2009. Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis. Am J Physiol Gastrointest Liver Physiol 296: G175–G184.

Glass RI, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA, Clarke MJ, Ho MS, Gentsch JR. 1996. The epidemiology of rotavirus diarrhea in the United States: Surveillance and estimates of disease burden. J Infect Dis 174:S5–S11.

Mott JD, Werb Z. 2004. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 16:558–564.

Kapsoritakis AN, Kapsoritaki AI, Davidi IP, Lotis VD, Manolakis AC, Mylonis PI, Theodoridou AT, Germenis AE, Potamianos SP. 2008. Imbalance of tissue inhibitors of metalloproteinases (TIMP) - 1 and - 4 serum levels, in patients with inflammatory bowel disease. BMC Gastroenterol 8:55.

Koyama S. 2004. Significance of cell-surface expression of matrix metalloproteinases and their inhibitors on gastric epithelium and infiltrating mucosal lymphocytes in progression of Helicobacter pylori-associated gastritis. Scand J Gastroenterol 39:1046–1053.

Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, Kamoi K, Suda T. 1998. Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: Association of MMP induction with bone resorption. Endocrinology 139:1338–1345.

Lopez-Guerrero DV, Meza-Perez S, Ramirez-Pliego O, Santana-Calderon MA, Espino-Solis P, Gutierrez-Xicotencatl L, Flores-Romo L, Esquivel-Guadarrama FR. 2010. Rotavirus infection activates dendritic cells from Peyer's patches in adult mice. J Virol 84:1856–1866.

Lotz M, Guerne PA. 1991. Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem 266:2017–2020.

Louis E, Ribbens C, Godon A, Franchimont D, De Groote D, Hardy N, Boniver J, Belaiche J, Malaise M. 2000. Increased production of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 by inflamed mucosa in inflammatory bowel disease. Clin Exp Immunol 120:241–246.

Makitalo L, Kolho KL, Karikoski R, Anthoni H, Saarialho-Kere U. 2010. Expression profiles of matrix metalloproteinases and their inhibitors in colonic inflammation related to pediatric inflammatory bowel disease. Scand J Gastroenterol 45:862–871.

Medina C, Radomski MW. 2006. Role of matrix metalloproteinases in intestinal inflammation. J Pharmacol Exp Ther 318:933–938.

Meijer MJ, Mieremet-Ooms MA, van der Zon AM, van Duijn W, van Hogezand RA, Sier CF, Hommes DW, Lamers CB, Verspaget HW. 2007a. Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. Dig Liver Dis 39:733–739.

Meijer MJ, Mieremet-Ooms MA, van Duijn W, van der Zon AM, Hanemaaijer R, Verheijen JH, van Hogezand RA, Lamers CB, Verspaget HW. 2007b. Effect of the anti-tumor necrosis factor-alpha antibody infliximab on the ex vivo mucosal matrix metalloproteinase-proteolytic phenotype in inflammatory bowel disease. Inflamm Bowel Dis 13:200–210.

Meijer MJ, Mieremet-Ooms MA, van Hogezand RA, Lamers CB, Hommes DW, Verspaget HW. 2007c. Role of matrix metalloproteinase, tissue inhibitor of metalloproteinase and tumor necrosis factor-alpha single nucleotide gene polymorphisms in inflammatory bowel disease. World J Gastroenterol 13:2960–2966.

Munoz M, Heimesaat MM, Danker K, Struck D, Lohmann U, Plickert R, Bereswill S, Fischer A, Dunay IR, Wolk K, Loddenkemper C, Krell HW, Libert C, Lund LR, Frey O, Holscher C, Iwakura Y, Ghilardi N, Ouyang W, Kamradt T, Sabat R, Liesenfeld O. 2009. Interleukin (IL)-23 mediates Toxoplasma gondii-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. J Exp Med 206:3047–3059.

Nagase H, Visse R, Murphy G. 2006. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69:562–573.

Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. 2009. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 200:S9–S15.

Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. 2003. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 9:565–572.

Pedersen G, Saermark T, Kirkegaard T, Brynskov J. 2009. Spontaneous and cytokine induced expression and activity of matrix metalloproteinases in human colonic epithelium. Clin Exp Immunol 155:257–265.

Pender SL, Fell JM, Chamow SM, Ashkenazi A, MacDonald TT. 1998. A p55 TNF receptor immunoadhesin prevents T cell-mediated intestinal injury by inhibiting matrix metalloproteinase production. J Immunol 160:4098–4103.

Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT. 1997. A major role for matrix metalloproteinases in T cell injury in the gut. J Immunol 158:1582–1590.

Ramani S, Paul A, Saravanabavan A, Menon VK, Arumugam R, Sowmyanarayanan TV, Samuel P, Kang G. 2010. Rotavirus antigenemia in Indian children with rotavirus gastroenteritis and asymptomatic infections. Clin Infect Dis 51:1284–1289.

Ravi A, Garg P, Sitaraman SV. 2007. Matrix metalloproteinases in inflammatory bowel disease: Boon or a bane? Inflamm Bowel Dis 13:97–107.

Ray P, Fenaux M, Sharma S, Malik J, Subodh S, Bhatnagar S, Greenberg H, Glass RI, Gentsch J, Bhan MK. 2006. Quantitative evaluation of rotaviral antigenemia in children with acute rotaviral diarrhea. J Infect Dis 194:588–593.

Saalbach A, Klein C, Schirmer C, Briest W, Anderegg U, Simon JC. 2010. Dermal fibroblasts promote the migration of dendritic cells. J Invest Dermatol 130:444–454.

Saren P, Welgus HG, Kovanen PT. 1996. TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages. J Immunol 157:4159–4165.

Stallmach A, Chan CC, Ecker KW, Feifel G, Herbst H, Schuppan D, Zeitz M. 2000. Comparable expression of matrix metalloproteinases 1 and 2 in pouchitis and ulcerative colitis. Gut 47:415–422.

Sugata K, Taniguchi K, Yui A, Miyake F, Suga S, Asano Y, Ohashi M, Suzuki K, Nishimura N, Ozaki T, Yoshikawa T. 2008. Analysis of rotavirus antigenemia and extraintestinal manifestations in children with rotavirus gastroenteritis. Pediatrics 122:392–397.

Widdowson MA, Steele D, Vojdani J, Wecker J, Parashar U. 2009. Global rotavirus surveillance: Determining the need and measuring the impact of rotavirus vaccines. J Infect Dis 200:S1–S8.

Wiercinska-Drapalo A, Jaroszewicz J, Flisiak R, Prokopowicz D. 2003. Plasma matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 as biomarkers of ulcerative colitis activity. World J Gastroenterol 9:2843–2845.

Short Communication

Whole-genomic analysis of a human G1P[9] rotavirus strain reveals intergenogroup-reassortment events

Souvik Ghosh,[1] Tsuzumi Shintani,[1] Noriko Urushibara,[1] Koki Taniguchi[2] and Nobumichi Kobayashi[1]

[1]Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan

[2]Department of Virology and Parasitology, School of Medicine, Fujita Health University, Toyoake, Japan

Correspondence
Souvik Ghosh
souvikrota@gmail.com *or*
souvik8@rediffmail.com

Received 21 March 2012
Accepted 14 May 2012

Group A rotavirus (RVA) strain K8 (RVA/Human-tc/JPN/K8/1977/G1P[9]) was found to have Wa-like VP7 and NSP1 genes and AU-1-like VP4 and NSP5 genes. To determine the exact origin and overall genetic makeup of this unusual RVA strain, the remaining genes (VP1–VP3, VP6 and NSP2–NSP4) of K8 were analysed in this study. Strain K8 exhibited a G1-P[9]-I1-R3-C3-M3-A1-N1-T3-E3-H3 genotype constellation, not reported previously. The VP6 and NSP2 genes of strain K8 were related closely to those of common human Wa-like G1P[8] and/or G3P[8] strains, whilst its VP1–VP3, NSP3 and NSP4 genes were related more closely to those of AU-1-like RVAs and/or AU-1-like genes of multi-reassortant strains than to those of other RVAs. Therefore, strain K8 might have originated from intergenogroup-reassortment events involving acquisition of four Wa-like genes, possibly from G1P[8] RVAs, by an AU-1-like P[9] strain. Whole-genomic analysis of strain K8 has provided important insights into the complex genetic diversity of RVAs.

Group A rotaviruses (RVAs) are a major cause of severe childhood diarrhoea (Cashman *et al.*, 2012; Estes & Kapikian, 2007). To date, RVAs are classified into at least 27 G and 35 P genotypes on the basis of differences in the nucleotide sequences of their outer-capsid VP7- and VP4-encoding genes, respectively (Matthijnssens *et al.*, 2011a). In humans, G1, G2, G3, G4 or G9 strains in conjunction with P[4], P[6] or P[8] have been reported widely, whilst G12 is emerging as an important VP7 genotype (Matthijnssens *et al.*, 2009, 2010a; Santos & Hoshino, 2005).

By RNA–RNA hybridization, human RVAs have previously been classified into at least two major genogroups, represented by reference strains RVA/Human-tc/USA/Wa/1974/G1P1A[8] and RVA/Human-tc/USA/DS-1/1976/G2P1B[4], and one minor genogroup, represented by strain RVA/Human-tc/JPN/AU-1/1982/G3P3[9] (Nakagomi *et al.*, 1989). Recently, a whole genome-based genotyping system has been accepted as the standard method for classification of RVAs by researchers worldwide (Matthijnssens *et al.*, 2008a, b, 2011a). Applying this classification system, the VP1–VP3, VP6 and NSP1–NSP5 genes of most human RVA strains with different G and P genotypes were found to exhibit an RVA strain Wa-like (designated genotypes R1, C1, M1, I1, A1, N1, T1, E1 and H1) or DS-1-like (designated genotypes R2, C2, M2, I2, A2, N2, T2, E2 and H2) genotype, whilst a limited number of strains possessed genes of the AU-1-like (designated genotypes R3, C3, M3, I3, A3, N3, T3, E3 and H3) genotype (Ghosh & Kobayashi, 2011; Heiman *et al.*, 2008; Matthijnssens, *et al.*, 2008a, b, 2011a). Results obtained using this genotyping system concurred with the previous classification of human RVA strains into the three RVA genogroups (Wa, DS-1 and AU-1) (Ghosh & Kobayashi, 2011; Matthijnssens *et al.*, 2008a, b). Human RVA strains possessing mixed genotype constellations have been also reported (Ghosh & Kobayashi, 2011).

RVA G1P[9] is an uncommon VP7–VP4 genotype combination, reported in RVA strains from humans and environmental samples (Matthijnssens *et al.*, 2009; Villena *et al.*, 2003). The first G1P[9] RVA strain, RVA/Human-tc/JPN/K8/1977/G1P[9], was detected in a diarrhoeal stool sample collected from a 14-year-old child in the city of Kitami, Hokkaido prefecture, Japan, in 1977 (Urasawa *et al.*, 1984). Since then, only a few human G1P[9] RVA strains have been reported, from Brazil, Burkina Faso, China, Italy, South Korea and Spain (Bonkoungou *et al.*, 2011; Fang *et al.*, 2002; Grassi *et al.*, 2012; Le *et al.*, 2008; Leite *et al.*, 1996; Santos *et al.*, 2003; Villena *et al.*, 2003).

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences of the VP1–VP3, VP6 and NSP2–NSP4 genes of rotavirus strain RVA/Human-tc/JPN/K8/1977/G1P[9] are JQ713645–JQ713651, respectively.

Two supplementary figures are available with the online version of this paper.

 043026 © 2012 SGM *Printed in Great Britain*

Whole-genomic analyses of atypical RVA strains are essential to obtain conclusive data on their true origin and evolution (Ghosh & Kobayashi, 2011; Matthijnssens *et al.*, 2008a, b). However, to date there are no reports on the whole-genomic analysis of the unusual G1P[9] RVA strains. RNA–RNA hybridization studies involving a single G1P[9] strain, K8, pointed towards possible intergenogroup-reassortment events (Nakagomi *et al.*, 1992). By partial genomic analysis, strain K8 was found to possess Wa-like VP7 and NSP1 genes and AU-1-like VP4 and NSP5 genes (Kojima *et al.*, 1996; Matthijnssens *et al.*, 2008b; Taniguchi *et al.*, 1989; Wu *et al.*, 1998). Therefore, to gain insights into the exact origin and overall genetic makeup of a G1P[9] RVA strain, the remaining seven genes (VP1–VP3, VP6 and NSP2–NSP4) of strain K8 were analysed in the present study. Moreover, only a few RVA gene sequences were available during analyses of the NSP1 and NSP5 genes of strain K8 in previous studies (Kojima *et al.*, 1996; Wu *et al.*, 1998), prompting us to repeat phylogenetic analyses of these genes with a larger number of RVA strains.

Human G1P[9] strain K8 was isolated successfully by tissue culture in MA-104 cells in our laboratory (Urasawa *et al.*, 1984) and stored at −80 °C until further analysis. Primers used for the amplification of the VP1–3, VP6 and NSP2–4 genes of strain K8 have been described previously (Ghosh *et al.*, 2010a, b, 2011; Wang *et al.*, 2010). RT-PCR, nucleotide sequencing and sequence analysis were carried out as described previously (Ghosh *et al.*, 2011). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) using MEGA (v5.01) software (Tamura *et al.*, 2011). The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were measured by the Kimura two-parameter model.

The VP4, VP7, NSP1 and NSP5 genes of RVA strain K8 were shown previously to belong to the P[9], G1, A1 and H3 genotypes, respectively (Matthijnssens *et al.*, 2008b). In the present study, based on nucleotide sequence identities and phylogenetic analyses of the nearly full-length nucleotide sequences (minus the 5′- and 3′-end primer sequences), the VP1–VP3, VP6 and NSP2–NSP4 genes of strain K8 were assigned to the R3, C3, M3, I1, N1, T3 and E3 genotypes, respectively (Table 1; Fig. 1). Therefore, strain K8 exhibited a G1-P[9]-I1-R3-C3-M3-A1-N1-T3-E3-H3 genotype constellation, not reported previously. Four of the 11 genotypes (G1, I1, A1 and N1) of K8 were closely related genomically to those of the Wa-like RVAs, whilst its remaining seven genotypes were AU-1-like, revealing a mixed genotype constellation (Table 1). The Wa-, DS-1- or AU-1-like genogroup is assigned to a human RVA strain if at least seven gene segments belong to the respective Wa-, DS-1-, or AU-1-like genotype (Matthijnssens *et al.*, 2008a). Therefore, strain K8 was assigned to the AU-1 genogroup.

The VP1 gene of strain K8 shared low nucleotide sequence identities (maximum nucleotide sequence identity of 89.9 % with strain RVA/Human-tc/THA/T152/1998/G12P[9], followed by 89.5 % with strain AU-1) with those of other RVAs, and phylogenetically, it clustered separately, near strain AU-1, AU-1-like G12 strain T152 (Matthijnssens *et al.*, 2008a, b; Rahman *et al.*, 2007) and strain RVA/

Table 1. Genotype nature of the 11 gene segments of RVA strain K8 compared with those of selected RVA strains with known genomic constellations

Bold type indicates gene segments with a genotype identical to that of strain K8; – indicates that no sequence data were available in GenBank. Strains K8, Wa and AU-1 are underlined.

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Human-tc/JPN/K8/1977/G1P[9]	**G1**	**P[9]**	**I1**	**R3**	**C3**	**M3**	**A1**	**N1**	**T3**	**E3**	**H3**
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	**G1**	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-wt/BEL/BE00097/2009/G1P[8]	**G1**	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-wt/USA/DC1505/1976/G3P[8]	G3	P[8]	**I1**	R1	C1	M1	**A1**	**N1**	T1	E1	H1
RVA/Human-tc/JPN/AU-1/1982/G3P3[9]	G3	**P[9]**	I3	**R3**	**C3**	**M3**	A3	N3	**T3**	**E3**	**H3**
RVA/Cat-wt/JPN/FRV1/1985/G3P[9]	G3	**P[9]**	–	–	–	–	–	–	–	**E3**	–
RVA/Human-tc/ITA/PA260-97/1997/G3P[3]	G3	P[3]	I3	**R3**	**C3**	**M3**	A15	N2	**T3**	**E3**	H6
RVA/Human-tc/USA/HCR3A/1984/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Cat-tc/AUS/Cat97/1984/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Dog-tc/AUS/K9/1981/G3P[3]	G3	P[3]	I3	**R3**	C2	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Simian-tc/USA/RRV/1975/G3P[3]	G3	P[3]	I2	R2	**C3**	**M3**	A9	N2	**T3**	**E3**	H6
RVA/Cat-wt/ITA/BA222/2005/G3P[9]	G3	**P[9]**	I2	R2	C2	M2	A3	**N1**	**T3**	E2	**H3**
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	G3	**P[9]**	I3	**R3**	C2	**M3**	A3	**N1**	T6	**E3**	**H3**
RVA/Human-wt/THA/CMH120/2004/G3P[9]	G3	**P[9]**	I3	–	–	–	–	–	–	**E3**	–
RVA/Human-wt/THA/CMH134/2004/G3P[9]	G3	**P[9]**	I3	–	–	–	–	–	–	**E3**	–
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	G3	P[24]	I9	**R3**	**C3**	**M3**	A9	**N1**	**T3**	**E3**	H6
RVA/Human-wt/JPN/KF17/2010/G6P[9]	G6	**P[9]**	I2	R2	C2	M2	A3	N2	**T3**	**E3**	**H3**
RVA/Human-tc/THA/T152/1998/G12P[9]	G12	**P[9]**	I3	**R3**	**C3**	**M3**	A12	N3	**T3**	**E3**	**H3**

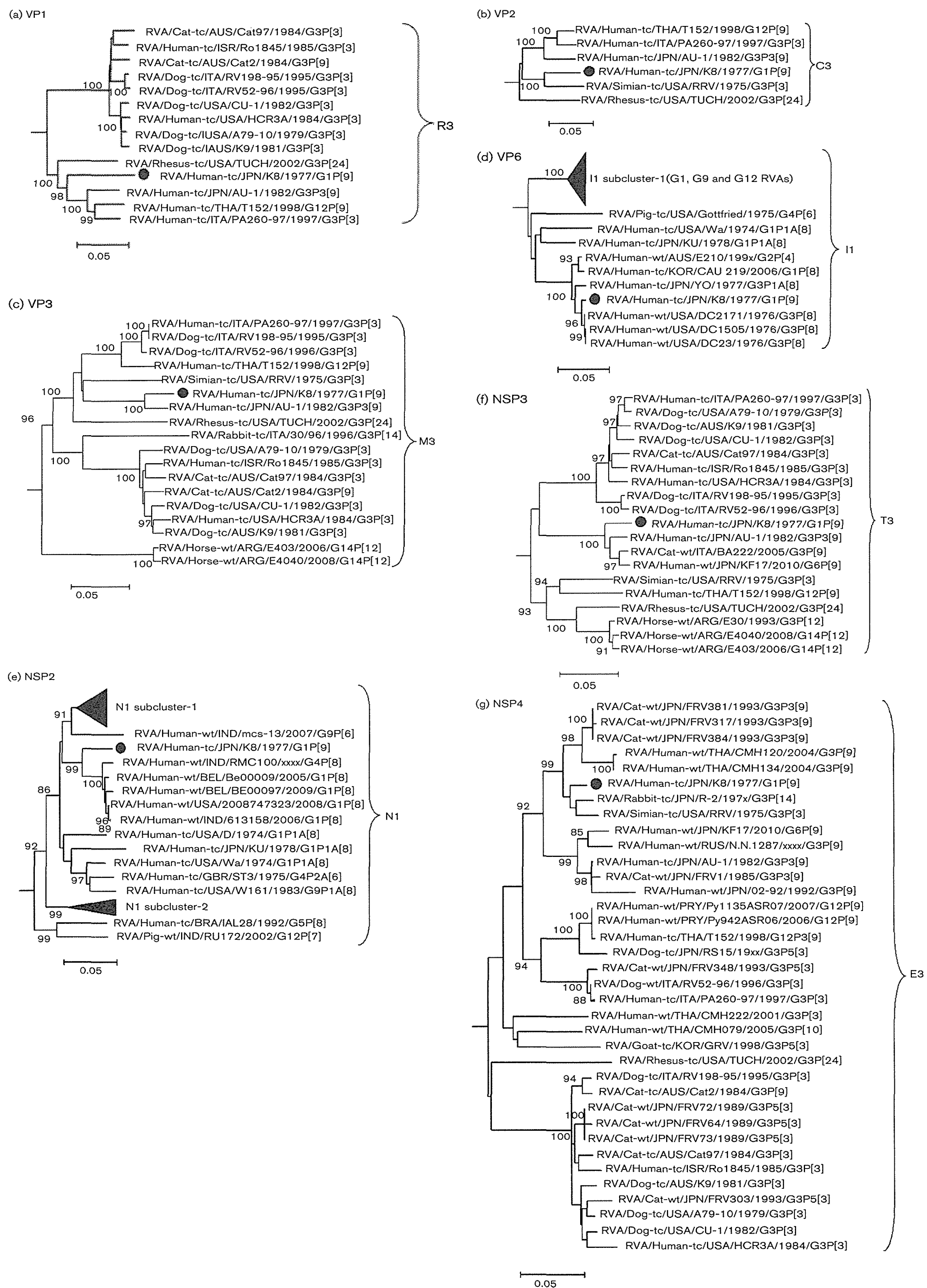

Fig. 1. Phylogenetic analyses of the VP1–VP3, VP6 and NSP2–NSP4 genes (a–g, respectively) of rotavirus strain RVA/Human-tc/JPN/K8/1977/G1P[9]. Although strains representing all RVA genotypes were included in the phylogenetic analyses, only those relevant to the present study are shown. Within the I1 and N1 genotypes, clade(s) consisting of strains that are not directly related to the present study, but were included for unbiased analysis, have been compressed and labelled as subcluster(s). In all trees, the position of strain K8 is highlighted by ●. Bootstrap values >85 % are shown. Bar, 0.05 substitutions per nucleotide.

Human-tc/ITA/PA260-97/1997/G3P[3] [a reassortant between RVAs of the AU-1-like and Cat97-like genogroups (Matthijnssens *et al.*, 2011b)] (Fig. 1a). Strain K8 exhibited maximum nucleotide sequence identity of 92.1 % to the AU-1-like VP2 gene of simian strain RVA/Simian-tc/USA/RRV/1975/G3P[3] (Matthijnssens *et al.*, 2010b), followed by identities of 89.0 and 88.9 % to those of strains AU-1 and T152, respectively. Phylogenetically, strain K8 clustered near strain RRV within the VP2-C3 genotype (Fig. 1b). The VP3 gene of strain K8 was related more closely to that of strain AU-1 (nucleotide sequence identity of 95.8 %) than to those of other RVAs (nucleotide sequence identities of <88 %) (Fig. 1c). The VP6 and NSP1 genes of K8 were related closely (nucleotide sequence identities of 99 %) to those of the common human Wa-like G3P[8] RVA strains detected in the USA in 1976 (Fig. 1d; Fig. S1, available in JGV Online). The NSP2 gene of K8 shared nucleotide sequence identities of 94–95 % and clustered phylogenetically with those of the common human Wa-like G1P[8] RVA strains (Fig. 1e).

The NSP3 gene of strain K8 was related more closely (nucleotide sequence identities of 96.0, 95.9 and 95.6 %, respectively) to those of strains AU-1, RVA/Cat-wt/ITA/BA222/2005/G3P[9] (a multi-reassortant strain derived from human, canine/feline, and bovine or bovine-like human RVAs) (Martella *et al.*, 2011) and RVA/Human-wt/JPN/KF17/2010/G6P[9] (a reassortant between bovine-like human and AU-1-like RVAs) (Yamamoto *et al.*, 2011) than those of other RVA strains (nucleotide sequence identities of <88 %) (Fig. 1f). The NSP4 gene of strain K8 exhibited high nucleotide sequence identities of 97.9, 97.5, 96.7, 96.7 and 96.5 % to those of lapine strain RVA/Rabbit-tc/JPN/R-2/197x/G3P[14], strain RRV, feline strains RVA/Cat-wt/JPN/FRV384/1993/G3P3[9], RVA/Cat-wt/JPN/FRV381/1993/G3P3[9] and RVA/Cat-wt/JPN/FRV317/1993/G3P3[9], respectively, and clustered phylogenetically with strains R-2 and RRV, close to strains FRV384, FRV381 and FRV317, within the NSP4-E3 genotype (Fig. 1g). The NSP5 gene of strain K8 formed a separate cluster with strain AU-1 and two other human P[9] RVA strains from Japan within the NSP5-H3 genotype (Fig. S2).

Taken together, the VP6, VP7, NSP1 and NSP2 genes of strain K8 were related closely to those of common human Wa-like G1P[8] and/or G3P[8] strains, whilst its VP1–VP4 and NSP3–NSP5 genes were related more closely to those of AU-1-like RVAs and/or AU-1-like genes of multi-reassortant RVA strains than those of other RVAs. Therefore, human G1P[9] RVA strain K8 might have originated from intergenogroup-reassortment events involving acquisition of four Wa-like gene segments, possibly from G1P[8] RVAs, by an AU-1-like P[9] strain.

Human AU-1-like strains are believed to be derived from feline/canine RVAs, as revealed by RNA–RNA hybridization studies (Nakagomi & Nakagomi, 1989). Among the AU-1-like genes of strain K8, the NSP4 gene was possibly derived from co-circulating feline RVAs (Fig. 1g). Phylogenetically, the VP1, VP3 and NSP3 genes appeared to share a common ancestry with those of typical feline/canine RVAs (Fig. 1a, c, f). The VP4 gene belonged to the same genotype as those of the feline G3P[9] RVAs, such as strains RVA/Cat-wt/JPN/FRV1/1985/G3P3[9], FRV-317, FRV381 and FRV384 from Japan. On the other hand, the VP2 and NSP5 genes appeared to be genetically distinct from those of the typical canine/feline RVAs (Table 1; Fig. 1b; Fig. S2). Moreover, phylogenetically, the NSP5 gene of strain K8 (and AU-1) appeared to share a common ancestry with those of artiodactyl and artiodactyl-like human strains (Fig. S2). Therefore, whole-genomic analyses of more AU-1-like human and typical canine/feline RVAs may be required to obtain conclusive data on the overall genetic relatedness between these RVAs, and with RVAs from other host species.

In conclusion, whole-genomic analysis of human RVA G1P[9] strain K8 provided important insights into the complex genetic diversity and evolutionary patterns of human RVAs. RVAs arising from intergenogroup-reassortment events, such as strain K8, are believed to be selected against in nature (McDonald *et al.*, 2009), as evident from the detection of only a few G1P[9] RVA strains in the last three and a half decades since the isolation of strain K8. However, compared with the high rates of detection of RVAs in humans, to date only a limited number of human RVA strains have been analysed for their whole genomes. Therefore, large-scale whole genome-based surveillance studies may be required to elucidate the actual frequency of RVA intergenogroup-reassortment events occurring under natural conditions, and to monitor the stability of RVA strains arising from such events. To our knowledge, the present study is the first report on the whole-genomic analysis of an intergenogroup-reassortant G1 RVA strain.

Acknowledgements

The study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 22406017) and Health and Labour Sciences Research Grants in Research on Emerging and Re-emerging Infectious Disease from the Ministry of Health, Labour and Welfare of Japan (H23-shinkou-ippan-005).

References

Bonkoungou, I. J., Damanka, S., Sanou, I., Tiendrébéogo, F., Coulibaly, S. O., Bon, F., Haukka, K., Traoré, A. S., Barro, N. & Armah, G. E. (2011). Genotype diversity of group A rotavirus strains in children with acute diarrhea in urban Burkina Faso, 2008–2010. *J Med Virol* **83**, 1485–1490.

Cashman, O., Collins, P. J., Lennon, G., Cryan, B., Martella, V., Fanning, S., Staines, A. & O'Shea, H. (2012). Molecular characterization of group A rotaviruses detected in children with gastroenteritis in Ireland in 2006–2009. *Epidemiol Infect* **140**, 247–259.

Estes, M. K. & Kapikian, A. Z. (2007). Rotaviruses and their replication. In *Fields Virology*, 5th edn, pp. 1917–1974. Edited by B. N. Fields, D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb,

M. A. Martin, B. Roizman & S. E. Straus. Philadelphia, PA: Lippincott Williams & Wilkins.

Fang, Z. Y., Yang, H., Qi, J., Zhang, J., Sun, L. W., Tang, J. Y., Ma, L., Du, Z. Q., He, A. H. & other authors (2002). Diversity of rotavirus strains among children with acute diarrhea in China: 1998–2000 surveillance study. *J Clin Microbiol* **40**, 1875–1878.

Ghosh, S. & Kobayashi, N. (2011). Whole-genomic analysis of rotavirus strains: current status and future prospects. *Future Microbiol* **6**, 1049–1065.

Ghosh, S., Alam, M. M., Ahmed, M. U., Talukdar, R. I., Paul, S. K. & Kobayashi, N. (2010a). Complete genome constellation of a caprine group A rotavirus strain reveals common evolution with ruminant and human rotavirus strains. *J Gen Virol* **91**, 2367–2373.

Ghosh, S., Kobayashi, N., Nagashima, S., Chawla-Sarkar, M., Krishnan, T., Ganesh, B. & Naik, T. N. (2010b). Full genomic analysis and possible origin of a porcine G12 rotavirus strain RU172. *Virus Genes* **40**, 382–388.

Ghosh, S., Adachi, N., Gatheru, Z., Nyangao, J., Yamamoto, D., Ishino, M., Urushibara, N. & Kobayashi, N. (2011). Whole-genome analysis reveals the complex evolutionary dynamics of Kenyan G2P[4] human rotavirus strains. *J Gen Virol* **92**, 2201–2208.

Grassi, T., Bagordo, F., Cavallaro, A., Guido, M., Malaventura, C., Gabutti, G. & De Donno, A. (2012). Sequence analysis of human rotavirus strains: comparison of clinical isolates from Northern and Southern Italy. *Eur J Clin Microbiol Infect Dis* **31**, 575–582.

Heiman, E. M., McDonald, S. M., Barro, M., Taraporewala, Z. F., Bar-Magen, T. & Patton, J. T. (2008). Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. *J Virol* **82**, 11106–11116.

Kojima, K., Taniguchi, K. & Kobayashi, N. (1996). Species-specific and interspecies relatedness of NSP1 sequences in human, porcine, bovine, feline, and equine rotavirus strains. *Arch Virol* **141**, 1–12.

Le, V. P., Kim, J. Y., Cho, S. L., Nam, S. W., Lim, I., Lee, H. J., Kim, K., Chung, S. I., Song, W. & other authors (2008). Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. *J Med Virol* **80**, 175–182.

Leite, J. P., Alfieri, A. A., Woods, P. A., Glass, R. I. & Gentsch, J. R. (1996). Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization, and sequence analysis. *Arch Virol* **141**, 2365–2374.

Martella, V., Potgieter, A. C., Lorusso, E., De Grazia, S., Giammanco, G. M., Matthijnssens, J., Bányai, K., Ciarlet, M., Lavazza, A. & other authors (2011). A feline rotavirus G3P[9] carries traces of multiple reassortment events and resembles rare human G3P[9] rotaviruses. *J Gen Virol* **92**, 1214–1221.

Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M., Palombo, E. A., Iturriza-Gómara, M., Maes, P. & other authors (2008a). Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *J Virol* **82**, 3204–3219.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M. K., Gentsch, J. R., Iturriza-Gómara, M., Kirkwood, C. D. & other authors (2008b). Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch Virol* **153**, 1621–1629.

Matthijnssens, J., Bilcke, J., Ciarlet, M., Martella, V., Bányai, K., Rahman, M., Zeller, M., Beutels, P., Van Damme, P. & Van Ranst, M. (2009). Rotavirus disease and vaccination: impact on genotype diversity. *Future Microbiol* **4**, 1303–1316.

Matthijnssens, J., Heylen, E., Zeller, M., Rahman, M., Lemey, P. & Van Ranst, M. (2010a). Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. *Mol Biol Evol* **27**, 2431–2436.

Matthijnssens, J., Taraporewala, Z. F., Yang, H., Rao, S., Yuan, L., Cao, D., Hoshino, Y., Mertens, P. P., Carner, G. R. & other authors (2010b). Simian rotaviruses possess divergent gene constellations that originated from interspecies transmission and reassortment. *J Virol* **84**, 2013–2026.

Matthijnssens, J., Ciarlet, M., McDonald, S. M., Attoui, H., Bányai, K., Brister, J. R., Buesa, J., Esona, M. D., Estes, M. K. & other authors (2011a). Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol* **156**, 1397–1413.

Matthijnssens, J., De Grazia, S., Piessens, J., Heylen, E., Zeller, M., Giammanco, G. M., Bányai, K., Buonavoglia, C., Ciarlet, M. & other authors (2011b). Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. *Infect Genet Evol* **11**, 1396–1406.

McDonald, S. M., Matthijnssens, J., McAllen, J. K., Hine, E., Overton, L., Wang, S., Lemey, P., Zeller, M., Van Ranst, M. & other authors (2009). Evolutionary dynamics of human rotaviruses: balancing reassortment with preferred genome constellations. *PLoS Pathog* **5**, e1000634.

Nakagomi, T. & Nakagomi, O. (1989). RNA–RNA hybridization identifies a human rotavirus that is genetically related to feline rotavirus. *J Virol* **63**, 1431–1434.

Nakagomi, O., Nakagomi, T., Akatani, K. & Ikegami, N. (1989). Identification of rotavirus genogroups by RNA–RNA hybridization. *Mol Cell Probes* **3**, 251–261.

Nakagomi, O., Kaga, E. & Nakagomi, T. (1992). Human rotavirus strain with unique VP4 neutralization epitopes as a result of natural reassortment between members of the AU-1 and Wa genogroups. *Arch Virol* **127**, 365–371.

Rahman, M., Matthijnssens, J., Yang, X., Delbeke, T., Arijs, I., Taniguchi, K., Iturriza-Gómara, M., Iftekharuddin, N., Azim, T. & Van Ranst, M. (2007). Evolutionary history and global spread of the emerging g12 human rotaviruses. *J Virol* **81**, 2382–2390.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Santos, N. & Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* **15**, 29–56.

Santos, N., Soares, C. C., Volotão, E. M., Albuquerque, M. C. & Hoshino, Y. (2003). Surveillance of rotavirus strains in Rio de Janeiro, Brazil, from 1997 to 1999. *J Clin Microbiol* **41**, 3399–3402.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Taniguchi, K., Nishikawa, K., Urasawa, T., Urasawa, S., Midthun, K., Kapikian, A. Z. & Gorziglia, M. (1989). Complete nucleotide sequence of the gene encoding VP4 of a human rotavirus (strain K8) which has unique VP4 neutralization epitopes. *J Virol* **63**, 4101–4106.

Urasawa, S., Urasawa, T., Taniguchi, K. & Chiba, S. (1984). Serotype determination of human rotavirus isolates and antibody prevalence in pediatric population in Hokkaido, Japan. *Arch Virol* **81**, 1–12.

Villena, C., El-Senousy, W. M., Abad, F. X., Pintó, R. M. & Bosch, A. (2003). Group A rotavirus in sewage samples from Barcelona and Cairo:

emergence of unusual genotypes. *Appl Environ Microbiol* **69**, 3919–3923.

Wang, Y. H., Kobayashi, N., Nagashima, S., Zhou, X., Ghosh, S., Peng, J. S., Hu, Q., Zhou, D. J. & Yang, Z. Q. (2010). Full genomic analysis of a porcine-bovine reassortant G4P[6] rotavirus strain R479 isolated from an infant in China. *J Med Virol* **82**, 1094–1102.

Wu, H., Taniguchi, K., Urasawa, T. & Urasawa, S. (1998). Serological and genomic characterization of human rotaviruses detected in China. *J Med Virol* **55**, 168–176.

Yamamoto, D., Kawaguchiya, M., Ghosh, S., Ichikawa, M., Numazaki, K. & Kobayashi, N. (2011). Detection and full genomic analysis of G6P[9] human rotavirus in Japan. *Virus Genes* **43**, 215–223.

Key words
ロタウイルス
迅速診断キット
イムノクロマト法
ラテックス凝集法
ELISA

ロタウイルス

田中 孝明* 中野 貴司*

要旨

ロタウイルスは，乳幼児における急性胃腸炎の主な原因ウイルスである．その疾病負担は大きく，家族内や施設内感染も問題となっている．これまでに電顕法からイムノクロマト法まで種々の診断方法が開発され，近年は迅速診断キットも汎用されている．外来やベッドサイドにおいて短時間で容易にロタウイルス胃腸炎の診断が可能であり，検査・治療方針の決定や施設内感染対策に有用である．しかし，受診時に便検体が得られない場合や血清型判定ができないという，キットの限界もある．最近，わが国においてもロタウイルスワクチンが導入された．ワクチン導入前後の流行疫学の解析においては，キットに加え遺伝子検査も重要な位置付けとなる．

はじめに

ロタウイルスは，世界中の乳幼児における急性胃腸炎の主な原因となっている．外来受診，入院，合併症や重症例など疾病負担は大きく，家族内および施設内感染も問題となっている．種々の診断方法が開発され，近年は外来診療やベッドサイドでの迅速診断も可能となった．診断が治療方針に直接結びつかないことも多いため，やみくもに検査を行うことは推奨されない．しかし，実際の臨床現場では，患者への病状説明が容易となり，不要な検査や投薬を回避でき，さらに公衆衛生学的あるいは施設内感染対策の観点からも有用な情報が得られるため，わが国では迅速診断キットが汎用されている．また，近年ロタウイルスワクチンが開発され，わが国でもすでに導入されたが，有効性の評価のために導入前後のサーベイランスが各地で行われており，急性胃腸炎に対する病因診断の必要性も増している．本稿では，ロタウイルス感染症の疫学，臨床的特徴，各種診断方法およびイムノクロマト法を中心とした迅速診断キットの有用性や限界について概説する．

I ロタウイルス胃腸炎の概要

ロタウイルスは乳幼児における急性胃腸炎の主要な原因ウイルスである．感染症法に基づく感染症発生動向調査において，全国約 3,000 の小児科定点から報告される 5 類感染症の感染性胃腸炎患者数は 11～12 月および 2～4 月に発生のピークを認める．地方衛生研究所では，定点の約 10% の胃腸炎患者において病原体検査が行われ，前半にノロウイルス，後半にロタウイルスが主に検出されている[1]．わが国の小児科外来において，ウイルス性胃腸炎と診断された便検体を用いてマルチプレックス・ポリメラーゼ連鎖反応（multiplex polymerase chain reac-

* 川崎医科大学附属川崎病院小児科
〒700-8505 岡山県岡山市北区中山下 2-1-80

表1 小児における急性胃腸炎の原因ウイルス

ウイルス名	核酸	頻度 (%)*	迅速診断キット	ワクチン
ロタウイルス	RNA	17〜20	あり	あり
ノロウイルス	RNA	16〜18	あり	なし
アデノウイルス	DNA	2〜5	あり	なし
サポウイルス	RNA	3〜4	なし	なし
アストロウイルス	RNA	0〜2	なし	なし
ヒトパレコウイルス	RNA	0〜5	なし	なし
ヒトボカウイルス	DNA	0〜1	なし	なし

*：わが国の小児科外来を受診した急性胃腸炎罹患児から検出されたウイルスの頻度
(牛島廣治, 2009[2])

tion) 法でウイルス検出率を調べた報告では, ロタウイルス, ノロウイルス, アデノウイルスの順に多くみられた. 三者のいずれもが迅速診断キットによる病因診断が可能である (ノロウイルスは健康保険適応外). 検出されたウイルスの頻度と特徴を表1に示す[2].

ロタウイルスは, レオウイルス科のロタウイルス属に分類されるRNAウイルスである. ウイルス粒子は, 外層, 内層および最内層の3層構造からなる. 外層に存在する感染防御抗原VP7 (viral protein 7) およびVP4は, それぞれG (glycoprotein) 血清型とP (protease) 遺伝子型を規定し, G1P [8] などのように表現する. 流行する血清型は, 年度・地域によってさまざまである. また内層に存在するVP6の抗原性の差異によりA〜G群に分類される. そのうちA, B, C群がヒトに感染するが, その大部分がA群であり, わが国ではまれにC群の散発例がみられる (2〜3%). B群はバングラデシュや中国で報告されているが, わが国の報告例はない[1].

感染者の便や吐物を介してヒト-ヒト感染する. 便1g中に10億個以上のウイルスが存在し, 数個のウイルスで感染が起こるとされている. 糞口感染によりウイルスが腸管内で増殖した後, 通常1〜3日の潜伏期間を経て発熱や嘔吐で発症し, 引き続き頻回の水様性下痢を生じる[3]. 便の性状に関する記述は少ないが, 臨床の現場では酸臭を伴う黄白色の特徴的な水様便にしばしば遭遇する. 外来受診, 入院, 高度の脱水やけいれん, 脳炎・脳症のような合併症や重症例など疾病負担は非常に大きく, また感染力も強いため家族や集団施設, 病院内での感染もしばしば問題となる. 特別な治療法はなく, 経口補液療法 (oral rehydration therapy：ORT) や輸液療法, 食事療法などの対症療法を行う. 諸外国ではRotarix®およびRotaTeq®の2種類の有効かつ安全なワクチンが開発され, 2004年以降世界中で普及しつつある. 前者は流行の多いG1P[8]の血清型を含む単価ヒトロタウイルスワクチン, 後者はG1P[5], G2P[5], G3P[5], G4P[5], G6P[8]を含む5価ウシ・ヒトロタウイルス組換え体ワクチンである. これらのワクチンは, ロタウイルス胃腸炎罹患の約80%, 重症胃腸炎の約90%を予防すると報告されている. わが国では2011年にロタリックス®内用液が承認・発売され, 2012年1月にはロタテック®内用液が承認された[4].

II 診断

ロタウイルス胃腸炎の診断は, 疫学・臨床的特徴, 便の性状などから容易に推測可能であるが, 確定するには検査診断が必要である. かつては電子顕微鏡によるロタウイルス粒子を検出する方法が主に使用されていたが, ELISA

(enzyme-linked immunosorbent assay) 法やラテックス凝集法 (latex agglutination assay : LA) を用いて便中のロタウイルス抗原を検出する迅速診断キットが広く利用されるようになった[5]. ELISA 法は 2〜3 時間の測定時間と機械が必要であるが, 集団発生などの多検体測定には有用である[6]. その後, より迅速で操作法も簡便なイムノクロマト (immunochromatographic assay : IC) 法を用いたキットも開発され, わが国の臨床現場では主流となっている. ただし, これらの免疫学的検査は A 群ロタウイルスを検出するものであり, B, C 群は検出し得ない. C 群ロタウイルスの検出には, 逆受身血球凝集反応 (reverse passive hemagglutination : RPHA) を用いたキットを使用する.

その他に, 逆転写ポリメラーゼ連鎖反応 (reverse transcription polymerase chain reaction : RT-PCR) 法や核酸ハイブリダイゼーション (nucleic acid hybridization) 法, ポリアクリルアミドゲル電気泳動 (polyacrylamide gel electrophores : PAGE) 法, ウイルス遺伝子のシーケンス解析 (sequence analysis), マルチプレックス PCR 法, 細胞培養法などの検査法がある. RT-PCR 法は, A, B, C 群の VP7 領域にプライマーを設定して PCR を行い, さらに nested PCR を行うことで G 血清型や P 遺伝子型の判別も可能であり, サーベイランスにおいて重要な役割を果たしている. マルチプレックス PCR 法は, 同時に複数のウイルスを検出でき, 重複感染も判定し得るため, スクリーニングとして適している. しかしこれらの検査法は費用や時間を要し, 実施可能な施設は限られているため, 主に研究ベースで行われている[7,8].

Ⅲ 迅速診断キットの適応と意義

迅速診断キットは, POCT (point of care testing ; 臨床現場即時検査) に含まれ, リアルタイムで短時間に検査を行うことで, 患者の QOL (quality of life) や満足度を向上させることを目的としている. 多くの場合, 特別な機器を使用せず, 簡単なトレーニングで操作が可能であり, 安価であることが求められる[9].

急性ウイルス性胃腸炎の診療において, 多くの場合迅速診断キットの結果は治療方針に影響を及ぼさないため, ルーチンでの使用は推奨されていない. しかし実際の臨床現場では, 病因診断がつくことで臨床経過が予測でき, 早期から ORT または輸液療法, 食事療法などによる適切な治療を開始したり, 不要な検査や投薬を回避できるメリットもある. さらに家族や集団施設, 病院内における感染対策として重要な疫学情報となり得る. とくに入院施設では, 少しでも院内感染の機会を減らすために感染性胃腸炎の病原微生物により入院する病室を分けることも行われている.

わが国でも, ロタウイルスワクチン導入前後の血清型分布を把握することは, ワクチン効果を評価する上できわめて重要である. 迅速診断の結果を元にその便検体から解析を行うことが多く, 臨床疫学研究の視点からもキットは有用性がある.

Ⅳ 迅速診断キットの実際

ロタウイルス迅速診断キットはウイルス抗原を迅速に検出する検査法である. 2012 年 2 月現在, 国内で市販されている主なキットを**表2**に示す[10)〜12)]. 対象となるウイルスは A 群ロタウイルスであり, 腸管アデノウイルスを同時に検出できるキットも開発されている. 感度・特異度は主に電顕法や RT-PCR 法との比較で評価したものを示したが, 検査の精度は優れているものが多く, 臨床現場での使用に値する. 一般的に, イムノクロマト法はラテックス凝集よりも感度がよく, ELISA 法とほぼ同等といわれている. 医療機関ではイムノクロマト法が大

表2 国内で市販されている主なロタウイルス迅速診断キット

測定原理	イムノクロマト法				ラテックス凝集法	ELISA
製品名	イムノカードSTロタウイルス®	ラピッドテスタ®ロタ・アデノ	BD Rota/Adeno エグザマンスティック®	ディップスティック'栄研'ロタ®	ロタ-アデノドライ®	ロタクロン®
メーカー	テイエフビー	積水メディカル	日本BD	栄研化学	積水メディカル	テイエフビー
検体量	糞便25 μL	糞便0.1 g	水様便100 μL 固形便30～50 mg	糞便12.5 mg	糞便0.5 g	糞便約0.1～0.2 g
抗体	抗ロタウイルス抗体（マウス）	抗ロタウイルス抗体（ウサギ）	抗ロタウイルス抗体（マウス）	抗ロタウイルス抗体（マウス）	抗ロタウイルス抗体（ウサギ）	抗ロタウイルス抗体（マウス）
反応時間	10分	10分	5～10分	15分	2分（前処置を除く）	40～70分
最小検出感度	1.9×10^{6}/テスト	10^{4}（TCID50/テスト）	31 ng/mL	100 ng/mL	10^{4}/mL	1.5×10^{6}/mL
感度	93.1*	92*	56.8**	94***	93.5*	92*
特異度	95.8*	100*	97.3**	100***	98.9*	89*
陽性尤度比	22.2	∞	21.04	∞	85	8.36
陰性尤度比	0.07	0.08	0.46	0.06	0.07	0.09

それぞれ，*電顕法，**PCR法，***ラテックス凝集法と比較　　（各種添付文書および文献10)～12) より作成）

部分を占めるが，ラテックス凝集法も使用されている．ELISA法は前二者と比較して，操作の煩雑さや時間を要することから主に委託検査施設や衛生研究所などで使用される．

イムノクロマト法には，ディップスティック'栄研'ロタ®，イムノカードSTロタウイルス®，BD Rota/Adenoエグザマンスティック®，ラピッドテスタ®ロタ・アデノ，ラピッドエスピー®《ロタ》などがあり，シェアの多くを占める．ラテックス凝集法はロタスクリーン®，ロタ-アデノドライ®，ロタレックスドライ®，ELISAはロタクロン®が市販されている．

本稿ではイムノクロマト法を使用したイムノカードSTロタウイルス®を例として解説する[11)12)]．

1. 原理

本法は金コロイドを結合したマウスの抗ロタウイルスモノクローナル抗体（検出抗体）と便検体中のウイルス抗原を反応させた後，キットのメンブレン（濾紙）上に流し，特定の部位（テスト領域）にあらかじめ固定されたウサギの抗ロタウイルスポリクローナル抗体（捕捉抗体）と反応させることにより，10分後に目視でウイルスの有無を判定する方法である．検体中にロタウイルスが存在すると，〔検出抗体-検体中のロタウイルス抗原-捕捉抗体〕の複合体を形成し，集積した金コロイドによりテスト領域に赤～紫色のラインが現れる．

2. 便検体の採取方法

便検体は，症状出現後なるべく早く採取する．第3～5病日頃に便中ウイルス量が最高になるといわれており，8日以上経過した検体では陰性と判定されることがある．

各種キットの添付文書には，他の細菌やウイルスとの交差反応性は認めないと記載があるが，水洗便所に排泄した便を採取するのはなるべく避ける．その理由は，便器内の水には他の微生物や塩素などが含まれているため，検査結果に影響を及ぼす可能性があるからである．厚手の使い捨て紙製便器や差し込み便器，新聞紙の上にサランラップを敷いたものなどに排泄し，容器へ採取するとよい[7)]．

表3 迅速診断キットの有用性と限界

有用性
・場所を問わず，簡単な操作で迅速に診断が可能である
・特別な機器を要さない
・感度・特異度が高い
・患者へ臨床経過の病状説明ができる
・早期の対症療法を開始し，不要な検査や投薬を回避できる
・家族内，施設内感染対策に対する情報が得られる
・診断することで医療者の知識と経験の蓄積となる
限界
・受診のタイミングや便性により検体が得られない場合がある
・直腸拭い液でも検査可能であるが，偽陰性もあり得る
・他のウイルスの検索や詳細な検査を行う場合は便検体を要する
・発症から一定期間経過すると感度が低下する
・検査結果が治療選択に影響しない場合がある
・A 群以外のロタウイルスは検出できない
・血清型の判定はできない

外来診療の場では，受診時に便検体を採取できない場合も多く，また水様便がおむつに染み込んで十分量の検体が得られないこともある．その際は，直腸拭い液を用いた検体でもロタウイルスの検出も可能である．電顕法でロタウイルスが検出された 20 例のうち，19 例が直腸拭い液を用いた本キットで陽性であり，検出されなかった 15 例では，キットによる陽性例はなかった（感度 95%，特異度 100%）[13]．

3．便検体の保存方法

採取後速やかに検査することが望ましいが，2～8℃で 72 時間の検体保存が可能であり，この時間を超える場合は－20℃以下で凍結保存する．過剰の便，おむつの切れ端，血液が混入した便を検体として用いると，正確な結果が得られない場合があるため注意する．

4．操作方法

① 希釈液（陰性コントロール）350 μL を検体調整用チューブに加える．

② 検体 25 μL を，ピペットを用いてチューブに加え，よく混和する．

③ 希釈検体 150 μL をテストカードの検体窓に加え，21～25℃で 10 分間反応させる．

④ テスト（TEST）領域とコントロール（CONTROL）領域の赤～紫色のラインを目視判定する．

5．感度・特異度

電顕法を基準とした場合の本キットの感度は 93.1%（121/130），特異度は 95.8%（114/119），有効度は 94.4%（235/249）であり，本キット陰性，電顕法陽性の 9 例中 8 例は，電顕法で認められたウイルスの粒子数が少量であった．また，ELISA 法（ロタクロン®）を基準とした場合の本キットの感度は 97.7%（126/129），特異度は 100%（121/121），有効度は 98.8%（247/250）であり，本キット陰性，ELISA 法陽性の 3 例は，電顕法で 2 例が陰性であった．

おわりに

ロタウイルス感染症の現状や各種診断方法，迅速診断キットの原理や測定意義について概説した．キットの登場により，急性胃腸炎の患者に対してリアルタイムで短時間の確定診断および除外診断が可能となった．必要な治療や検査に関して即時の判断を行うことができ，家族内および施設内感染対策としても有用な情報を得

られるため，POCTの目的である満足度および質の高い診療を達成できるようになった．また臨床医にとって病原微生物を追求することは基本姿勢であり，キットを用いた病因診断の経験を積むことで，曖昧であったロタウイルス感染症の臨床経過を確実なものとして理解することが可能となった．

諸外国では本症の疾病負担の大きさやワクチン費用対効果の検討から2種類のワクチンが導入され，有効性と安全性が示されつつある．わが国でも，それら2種類のワクチンが最近承認された．今後，ワクチン導入前後の流行疫学の解析においては，本症の診断のみならず血清型も測定する必要があるため，迅速診断キットに加え，遺伝子検査もこれまで以上に大きな役割を果たしていくと思われる．

迅速診断キットの有用性と限界について表3にまとめた．

文献

1) 国立感染症研究所感染症情報センター：ロタウイルス 2005～2010年．病原微生物検出情報 2011；32：61-62
2) 牛島廣治：ウイルス性胃腸炎の診断法と疫学の過去，現在と今後の展望．ウイルス 2009；59：75-90
3) American Academy of Pediatrics：Rotavirus. Pickering LK et al (eds)；Red Book. 28th ed, American Academy of Pediatrics, 2009：576-579
4) 田中孝明：ロタウイルス感染症とは．五十嵐隆，尾内一信（編）；保健指導者のための子どもの感染症と予防接種の手引き，初版，財団法人母子衛生研究会，2011：61-63
5) Bernstein DI：Rotavirus overview. Pediatr Infect Dis J 2009；28：S50-53
6) 田中智之：ウイルス性腸炎．Medical Technology 2008；36：1393-1399
7) 日本臨床微生物学会検査法マニュアル作成委員会・腸管感染症検査ガイドライン委員会：浅利誠志（編）；腸管感染症検査ガイドライン，初版，日本臨床微生物学会，2010：1-68
8) Treanor JJ：Clinical presentation and diagnosis of rotavirus infection. UpToDate19.3 [cited 2012 Jan. 16]
9) POC推進委員会：松尾収二（編）；POCTガイドライン，第2版，日本臨床検査自動化学会会誌 2009；33：1-123
10) 原三千丸：ロタウイルス迅速診断．日本小児科医会会報 2004；27：30-33
11) 田村真理子：イムノカードSTロタウイルス．Medical Technology 2008；36：1400
12) イムノカードSTロタウイルス添付文書．医薬品医療機器情報提供ホームページ，2011 http://www.info.pmda.go.jp/tgo/pack/21100AMY00013000_A_01_03/[cited 2012 Jan. 16]
13) 原三千丸：直腸拭い液によるロタウイルス胃腸炎の迅速診断．日本小児科学会雑誌 2002；106：766-767

○　　○　　○

J Infect Chemother
DOI 10.1007/s10156-012-0515-3

ORIGINAL ARTICLE

Mumps Hoshino and Torii vaccine strains were distinguished from circulating wild strains

Akihito Sawada · Yoshiaki Yamaji · Tetsuo Nakayama

Received: 4 September 2012 / Accepted: 22 October 2012
© Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases 2012

Abstract Aseptic meningitis and acute parotitis have been observed after mumps vaccination. Mumps outbreaks have been reported in Japan because of low vaccine coverage, and molecular differentiation is required to determine whether these cases are vaccine associated. RT-nested PCR was performed in the small hydrophobic gene region, and viruses were differentiated by restriction fragment length polymorphism assay. A total of 584 nucleotides were amplified. The PCR product of the Hoshino strain was cut into two fragments (313 and 271 nucleotides) by *Mfe*I; that of the Torii strain was digested with *Eco*T22I, resulting in 332- and 252-nucleotide fragments. Both strains were genotype B and had an *Xba*I site, resulting in two fragments: 299 and 285 nucleotides. Current circulating wild types were cut only by *Xba*I or *Mfe*I. However, the *Mfe*I site of the wild types was different from that of the Hoshino strain, resulting in 451- and 133-nucleotide fragments. Using three restriction enzymes, two mumps vaccine strains were distinguished from wild types, and this separation was applied to the identification of vaccine-related adverse events.

Keywords Mumps Hoshino strain · Mumps Torii strain · Molecular differentiation · Wild circulating genotypes

Introduction

In Japan, the MMR vaccine was introduced in 1989 but discontinued in 1993 because of an unexpectedly high incidence of aseptic meningitis caused by components of the mumps vaccine [1, 2]. The mechanisms responsible for the high incidence of aseptic meningitis with the MMR vaccine have not been elucidated in comparison to monovalent mumps vaccines used since 1993. Nagai et al. [3] investigated the incidence of aseptic meningitis after vaccination and identified 10 cases among 21,465 vaccine recipients. Moreover, 13 patients with aseptic meningitis were reported among 1,051 cases of naturally acquired mumps confirmed by viral isolation together with genome detection. The incidence of aseptic meningitis after vaccination was 1/27 of that observed for natural infections. However, in the post marketing study, the incidence of aseptic meningitis was approximately 0.01 % (1 case in 10,000 recipients) and that of acute parotitis, 2–3 %.

The mumps virus strains were divided into 12 genotypes based upon the sequence diversity of the small hydrophobic (SH) genome region [4, 5]. Parental strains of the Hoshino and Torii vaccine strains, isolated in the 1960s, are genotype B [6, 7]. Circulating wild-type strains were all genotype B in the 1970s and earlier and were genotypes J and B in the 1980s to 1990s. Genotype G appeared in the 2000s. Genotypes D, I, and L have been isolated sporadically [8–10], and recently genotype G was globally the major circulating genotype [11]. Large outbreaks have been observed every 3–5 years because of the low vaccine coverage, 30 % to 40 %. The mumps vaccine is voluntary (its cost is not covered by the government), and a guardian's decision usually depends on information on mumps outbreaks. Some recipients were immunized during the incubation period of natural infection, making it difficult to determine whether the mumps illness was caused by a natural infection or the vaccine.

In previous reports, the Hoshino vaccine strain was distinguished from circulating wild strains using the

A. Sawada · Y. Yamaji · T. Nakayama (✉)
Kitasato Institute for Life Sciences, Laboratory of Viral Infection I, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
e-mail: tetsuo-n@lisci.kitasato-u.ac.jp

Published online: 09 November 2012

reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) in the hemagglutinin-neuraminidase (HN) gene with *Sca*I and *Afl*II [12]. A simpler method was also reported through digestion with *Sca*I after DNA amplification by reverse transcription loop-mediated isothermal amplification (RT-LAMP) [13]. These methods are applied after immunization with the Hoshino vaccine. Now, two vaccine strains of the Torii and Hoshino are used, but no method of differentiation has been developed for the Torii strain. In this report, 584 nucleotides were amplified in the SH gene, and the two vaccine strains were distinguished from circulating wild types by unique restriction enzyme sites.

Materials and methods

Mumps virus and clinical samples

The Hoshino (Kitasato Institute, Tokyo, Japan) and Torii (Takeda Pharmaceutical, Osaka, Japan) vaccine strains were recovered from marketed vaccines. MuVi/Tokyo.JPN/77 (genotype B), MuVi/Akita.JPN/93-AK (genotype I), MuVi/Tokyo.JPN/94-H (genotype J), MuVi/Tokyo.JPN/94-0K (genotype B), and MuVi/Tokyo.JPN/01-III-10 (genotype L) were used as wild-type representatives for genotypes B, J, and L, which have already been reported [8–10]. A total of 47 clinical samples were examined: 20 cases of aseptic meningitis after immunization with the Torii strain, 25 cases after immunization with the Hoshino strain, and 2 cases of orchitis after immunization with the Hoshino strain. Two wild-type strains (MuVi/Tokyo.JPN/10-K and MuVi/Tokyo.JPN/10-F) were isolated and identified as genotype G. Cerebrospinal fluid (CSF) samples from the patients with aseptic meningitis and two salivary swab samples or nasopharyngeal swab (NPS) from the patients with orchitis were used.

RNA extraction

Total RNA was extracted from 200 μl CSF and salivary swabs or NPS using a magnetic bead RNA purification kit (MagExtractor-viral RNA; Toyobo, Osaka, Japan) and the RNA pellet was suspended in 30 μl distilled water.

RT-PCR and RLFP

RNA was transcribed to cDNA with a random hexamer using a PrimeScript RT reagent Kit (TaKaRa Bio, Japan) and amplified using Ex *Taq* DNA polymerase (TaKaRa Bio). The first PCR was done using MP F 921+ (5′TCTAT AATTCAATTGCCAGA) and MP HN241− (5′TGTCTGC AATTGAAGACAAC) and the nested PCR, using MpF0+ 5′GTCGATGATCTCATCAGGTAC) and Mp HN1− (5′C CAATATTCGGAAGCAGGTTCGGA), amplifying 584 nucleotides including the primer sequences from the genome positions 6139 to 6722 [10]. PCR products underwent electrophoresis after digestion with *Eco*T22I, *Mfe*I, and *Xba*I (New England BioLabs Japan).

Sequence analysis

PCR products were excised from low-melting gel electrophoresis and purified. DNA sequences were determined by the dye terminator method using an Applied Biosystems 3130 (Life Technologies Japan).

Results

Sequence analysis and restriction enzyme sites

The Hoshino and Torii strains were sequenced; alignments at the restriction enzyme sites are depicted in Fig. 1. The *Eco*T22I site (genome position 6386–6391) was unique to

	*Eco*T22 I	*Xba* I	*Mfe* I	*Mfe* I
	6386 6391	6437 6442	6451 6456	6589 6594
Torii [B]	ATGCAT	TCTAGA	CAGTTG	CAACTG
Hoshino [B]	-----G	------	--A---	T-----
JPN/77 [B]	-----G	------	---C--	----C-
JPN/93AK [I]	-----G	------	-----A	---T--
JPN/94H [J]	-----G	------	--T--A	----C-
JPN/94OK [B]	-----G	------	---C--	----C-
JPN/01-III-10 [L]	-----A	------	-----T	------
JPN/10K [G]	-----G	-----G	-----A	---T--
JPN/10F [G]	-----G	-----G	-----A	---T--

Fig. 1 Sequence alignment of the Torii and Hoshino vaccine strains and representative wild strains. MuVi/Tokyo.JPN/77 (genotype B), MuVi/Akita.JPN/93-AK (genotype I), MuVi/Tokyo.JPN/94-H (genotype J), MuVi/Tokyo.JPN/94-0K (genotype B), and MuVi/Tokyo.JPN/01-III-10 (genotype L) were used. MuVi/Tokyo.JPN/10-K and MuVi/Tokyo.JPN/10-F (genotype G) are isolated in this study. Nucleotide changes are depicted in comparison with the Torii strain, and restriction enzyme sequences are highlighted in *grey*

Table 1 DNA sizes of restriction fragments after treatment with *Eco*T22I, *Mfe*I, and *Xba*I

Mumps strains	*Eco*T22I	*Mfe*I	*Xba*I
Hoshino genotype B	–	+ (313/271)	+ (299/285)
Torii genotype B	+ (332/252)	–	+ (299/285)
Wild genotypes B, J, L	–	–	+ (299/285)
Wild genotype I	–	+ (451/133)	+ (299/285)
Wild genotype G	–	+ (451/133)	–

the Torii strain and *Mfe*I site (6451–6456) to the Hoshino strain. Genotypes B, I, J, and L had an *Xba*I (6437–6442) site, and old genotype I and the currently circulating genotype G had an *Mfe*I site (6589–6594) newly introduced by nucleotide change, not at position 6451–6456 of the Hoshino strain. Based on the results of the sequence analysis, RFLP and predicted fragment lengths are shown in Table 1. The PCR product of the Hoshino strain was cut into two fragments (313 and 271) by *Mfe*I and that of the Torii strain into two fragments (332 and 252) by *Eco*T22I. These two strains were also cut by *Xba*I into two fragments (299 and 285). RFLP of the circulating wild type had mainly two patterns: genotypes B, J, and L were cut by *Xba*I and genotype G by *Mfe*I but differently from the Hoshino strain.

The results of RFLP are shown in Fig. 2. The PCR product of the Hoshino vaccine strain was cut by *Mfe*I and *Xba*I, and that of the Torii strain by *Eco*T22I and *Xba*I. As for the RFLP of wild type, the PCR product of MuVi/Akita.JPN/93-AK (genotype I) was cut by both *Mfe*I and *Xba*I with different fragment sizes from the Hoshino strain. MuVi/Tokyo.JPN/94-OK (genotype B) was cut by *Xba*I, and the same RFLP pattern was noted for MuVi/Tokyo.JPN/94-H (genotype J) and MuVi/Tokyo.JPN/01-III-10 (genotype L). PCR products of MuVi/Tokyo.JPN/10-K and/10-F (genotype G) were cut by *Mfe*I. They showed different patterns from the vaccine strains, as predicted from the sequencing results.

Differentiation of vaccine strains from wild types

A total of 47 clinical samples were obtained: 20 cases of aseptic meningitis after immunization with the Torii strain, 25 cases after immunization with the Hoshino strain, and 2 cases of orchitis after immunization with the Hoshino strain. The results of RT-PCR and RFLP are shown in Table 2. RT-PCR was negative for two CSF samples from the recipients of the Torii strain, and among 18 RT-PCR positives, 16 were identified as the Torii vaccine strain. Among 25 CSF samples obtained from the recipients of the Hoshino strain, 3 were negative by RT-PCR, and 20 were considered positive for the vaccine strain. Two from each

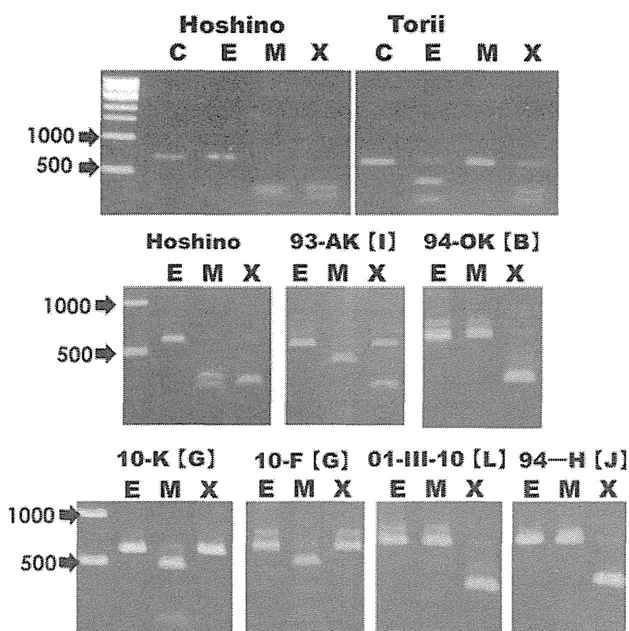

Fig. 2 Restriction fragment length polymorphism (RFLP) of the Hoshino and Torii vaccine strains and circulating wild strains. C, control; E, treatment with *Eco*T22I; M, treatment with *Mfe*I; X, treatment with *Xba*I. 93-AK, MuVi/Akita.JPN/93-AK [genotype I]; 94-H, MuVi/Tokyo.JPN/94-H [genotype J]; 94-OK, MuVi/Tokyo.JPN/94-0K [genotype B]; 01-III-10, MuVi/Tokyo.JPN/01-III-10 [genotype L]; 10-K, MuVi/Tokyo.JPN/10-K [genotype G]; 10-F, MuVi/Tokyo.JPN/10-F [genotype G]

were identified as wild strains. In 2 cases of orchitis after vaccination with the Hoshino strain, RT-PCR was positive in 1 case, identified as the wild type. Five of 45 patients with suspected adverse events were identified as having a concurrent wild-type genotype G.

Some strains identified as causing adverse events were sequenced; the phylogenetic analysis is shown in Fig. 3. Cases 1 and 2 were patients with aseptic meningitis after immunization with the Hoshino strain and cases A and B after that with the Torii strain. The sequencing results showed they were identical to the respective vaccine strains.

Table 2 Results of differentiation of mumps virus genome for clinical samples obtained from patients with aseptic meningitis and orchitis

	PCR negative	PCR positive	
		Vaccine strain	Wild strain
Aseptic meningitis after vaccination with			
Torii ($n = 20$)	2	16	2
Hoshino ($n = 25$)	3	20	2
Orchitis after vaccination with			
Hoshino ($n = 2$)	1		1

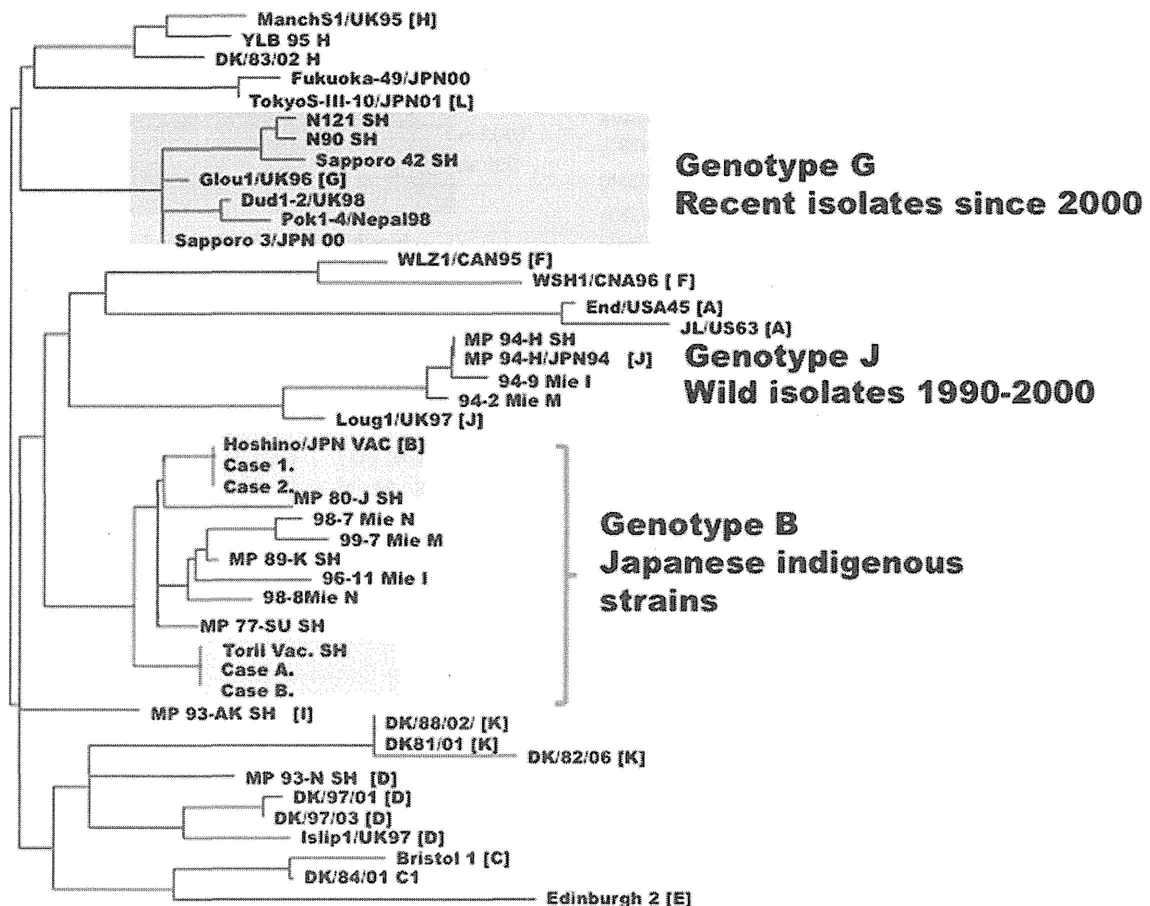

Fig. 3 Phylogenetic analysis of vaccine-associated cases in the small hydrophobic (SH) genome region. Cases 1 and 2 were patients with aseptic meningitis after immunization with the Hoshino strain and cases A and B after immunization with the Torii strain

Discussion

The mumps virus is classified into 12 distinct genotypes, with genotype B indigenous to Japan [4, 5, 8]. Genotype J was a dominant circulating strain with some genotype B strains in 1990–2000, and genotype G appeared in 2000–2012 with sporadic outbreaks of genotype L [8–10]. Mumps has been circulating in Japan because of a low immunization rate, approximately 30–40 %, and mump outbreaks have also been reported in the EU and USA where high immunization coverage was achieved with two doses of MMR [14–16]. The outbreaks were caused by the accumulation of susceptible individuals with an insufficient two-dose MMR vaccination in childhood. Several vaccine strains have been developed, and the Jeryl Lynn strain, belonging to genotype A, has been widely used as a component of MMR. Neutralization test (NT) antibody titers in sera obtained after vaccination with Jeryl Lynn were lower against genotype G than those against the vaccine strain, but they completely neutralized the other genotypes [17, 18]. In contrast, the antigenicity of genotype A of the vaccine strain was quite different from the recent circulating wild types and considered one of the reasons for the recirculation of the mumps virus [19]. There would be some problem with immunogenicity and persistence of immunity after immunization with the Jeryl Lynn strain. In Japan, two vaccine strains, Hoshino and Torii, are used and they belong to genotype B. In our previous report, there was no antigenic difference among circulating wild types [10]. Immunogenicity paralleled the incidence of adverse reactions. The incidence of aseptic meningitis after immunization with the Jeryl Lynn strain was reported to be 1 case in 100,000, and that of Torii or Hoshi was higher. The mump vaccine is still a voluntary one and so the cost is not covered by regional governments. Thus, guardians consider a mumps vaccination only when an outbreak is coming according to surveillance data. Some recipients were vaccinated by chance during the incubation period, and infection with the wild type became mixed into the vaccine-adverse events [12, 13]. In this report, 4 cases were identified as wild types among 45 cases with aseptic meningitis and 1 of the 2 cases of orchitis after immunization. From the results of surveillance reports, mumps outbreaks were observed in moderate grade, and

approximately 10 % of the vaccine-associated cases were infected with the wild type around the immunization day. Most adverse events developed 2–3 weeks after vaccination, but wild-type-related illness developed a few days earlier. There was no difference in clinical symptoms and clinical laboratory findings between vaccine-related adverse events and wild-type-related illness [3]. Five samples in aseptic meningitis and 1 in orchitis showed negative for mumps RT-PCR. Enterovirus RT-PCR for the mump PCR-negative samples showed negative for 5 mumps PCR-negative clinical samples [20]; these were considered to be low virus doses or in inappropriate stocking or transporting conditions.

Vaccine safety is a major concern and depends on postmarketing surveillance. Postmarketing surveillance from 1994 to 2010 is summarized, adding new data to the previous report [21], compared with the incidence of natural infections, in Table 3. The incidence of aseptic meningitis was <1–15 % among mumps infections with different incidences [22], and enhanced surveillance data showed 2.9 % of mumps patients were hospitalized, 6.1 % had orchitis, 0.3 % had meningitis, and 0.25 % had pancreatitis in England in 2002–2006 [23]. For the other complications, permanent deafness was considered to occur in approximately 1 per 20,000 cases, but it would actually be higher, 1 per 1,000 cases [24]. The results of postmarketing studies are shown from 1994 to 2010. A total of 3.5 million doses of the Hoshino vaccine were shipped, and acute parotitis was observed in 2–3 % of recipients. Among them, 117 nasopharyngeal swabs were examined and 89 were positive for RT-PCR: 64 were identified as the Hoshino vaccine strain and 25 were wild type. Among CNS complications, 5 cases of encephalopathy, 223 cases of aseptic meningitis, and 3 cases of acute disseminated encephalomyelitis (ADEM) were reported. Two cases were identified as enterovirus infections by RT-PCR [20]. When 85 CSF samples were examined in 223 cases of aseptic meningitis, 58 were considered vaccine-associated illnesses among 66 PCR positives. In this study period from 2008 to 2012, approximately 10 % of the patients suspected of having a vaccine-associated illness were identified as having wild-type infections during the mump outbreaks. Therefore, a simple differentiation method would contribute to further understanding of the safety of mumps vaccines.

Table 3 Complications of mumps and vaccine adverse events after vaccination with the Hoshino strain reported from 1994 to 2010

Complications	Natural infection	Vaccination (3.5 million)
Acute parotitis	70 %	2–3 %[a]
CNS complications		
Encephalopathy	1/5,000–6,000	5 (1: enterovirus)
Aseptic meningitis	1–2 %	223[b]
ADEM		3 (1: enterovirus)
Deafness	1/15,000 (1/1,000)	4
Orchitis	25 % in adolescents	15[c]
Oophoritis	5 %	
Pancreatitis	4 %	2
Other		1: ITP
		1: allergic purpura

Incidence of complications during natural infection refers to Ref. [22]

CNS, central nervous system; ADEM, acute disseminated encephalomyelitis; ITP, idiopathic thrombocytopenic purpura

[a] Of 117 nasopharyngeal swab (NPS) samples examined from patients with acute parotitis after vaccination with the Hoshino strain, PCR was positive in 89; 64 were identified as the vaccine strain and 25 as the wild type

[b] Of 85 CSF samples examined, 66 were PCR positive; 58 were identified as the vaccine strain and 8 as the wild type

[c] Three NPS samples were examined; one was the vaccine strain and two were the wild type

Conflict of interest The author has a conflict of interest. T.N. has received a research fund for the development of a new concept of live recombinant vaccines (20 million yen a year) from Daiichi-Sankyo Pharmaceutical.

References

1. Kimura M, Kuno-Sakai H, Yamazaki S. Adverse events associated with MMR vaccines in Japan. Acta Paediatr Jpn. 1996;38: 205–11.
2. Ueda K, Miyazaki C, Hidaka Y, Okada K, Kusuhara K, Kadoya R. Aseptic meningitis caused by measles–mumps–rubella vaccine in Japan. Lancet. 1995;346:701–2.
3. Nagai T, Okafuji T, Miyazaki C, Ito Y, Kamada M, Kumagai T, et al. A comparative study of the incidence of aseptic meningitis in symptomatic natural mumps patients and monovalent mumps vaccine recipients in Japan. Vaccine. 2007;25:2742–7.
4. Jin L, Rima B, Brown D, Örvell C, Técle T, Afzal M, et al. Proposal for genetic characterization of wild-type mumps strains: preliminary standardisation of the nomenclature. Arch Virol. 2005;150:1903–9.
5. WHO. Mumps virus nomenclature update: 2012. A report based on the WHO mumps nomenclature update meeting, Geneva, 22 September 2011. Wkly Epidemiol Rec. 2012;87:217–24.
6. Sasaki K, Higashihara M, Inoue K, Igarashi Y. Studies on the development of a live attenuated mumps virus vaccine. Kitasato Arch Exp Med. 1976;49:43–52.
7. Hoshino M, Nishimitsu M, Ichimori Y, Oka Y, Kouno R, Yamashita K, et al. Development of live attenuated mumps Torii vaccine strain: development and biological characteristics. Clin Virol. 1981;9:323–30. (in Japanese).
8. Kashiwagi Y, Takami T, Mori T, Nakayama T. Sequence analysis of F, SH, and HN genes among mumps virus strains in Japan. Arch Virol. 1999;144:593–9.
9. Takahashi M, Nakayama T, Kashiwagi Y, Takami T, Sonoda S, Yamanaka T, et al. Single genotype of measles virus is dominant whereas several genotypes of mumps virus are co-circulating. J Med Virol. 1996;62:278–85.
10. Inou Y, Nakayama T, Yoshida N, Uejima H, Yuri K, Kamada M, et al. Molecular epidemiology of mumps virus in Japan and proposal of two new genotypes. J Med Virol. 2004;73:97–104.

11. Otto W, Mankertz A, Santibanez S, Saygilli H, Wenzel J, Jilg E, et al. Ongoing outbreak of mumps affecting adolescents and young adults in Bavaria, Germany, August to October 2010. Eur Surveill 2010;15(50):19748
12. Kashiwagi Y, Kawashima H, Takekuma K, Hoshika A, Mori T, Nakayama T. Detection of mumps virus genome directly from clinical samples and a simple method for genetic differentiation of the Hoshino vaccine strain from wild strains of mumps virus. J Med Virol. 1997;52:195–9.
13. Yoshida N, Fujino M, Ota Y, Notomi T, Nakayama T. Simple differentiation of mumps Hoshino vaccine strain from wild strains by reverse transcription loop-mediated isothermal amplification (RT-LAMP). Vaccine. 2007;25:1281–6.
14. CDC. Mumps epidemic—United Kingdom, 2004–2005. MMWR. 2006;55:173–5.
15. CDC. Mumps outbreak at a summer camp: New York, 2005. MMWR. 2006;55:175–7.
16. Barskey AE, Glasser J, LeBaron CW. Mumps resurgences in the United States: a historical perspective on unexpected elements. Vaccine. 2009;27:6186–95.
17. Rubin SA, Audet SA, Sullivan B, Carbone KM, Bellini WJ, Rota PA, et al. Antibody induced by immunization with Jeryl Lynn mumps vaccine strain effectively neutralizes a heterologous wild-type mumps virus associated with a large outbreak. J Infect Dis. 2008;198:508–15.
18. Rubin SA, Link MA, Sauder CJ, Zhang C, Ngo L, Rima BK, et al. Mumps outbreaks in vaccinated populations: no evidence of immune escape. J Virol. 2012;86:615–20.
19. Atrasheuskaya AV, Blatun EM, Kulak MV, Atrasheuskaya A, Karpov IA, Rubin S. Investigation of mumps vaccine failures in Minsk, Belarus, 2001–2003. Vaccine. 2007;25:4651–8.
20. Takami T, Kawashima H, Takei Y, Miyajima T, Mori T, Nakayama T, et al. Usefulness of nested PCR and sequence analysis in a nosocomial outbreak of neonatal enterovirus infection. J Clin Virol. 1998;11:67–75.
21. Nakayama T, Onoda K. Vaccine adverse events reported in post-marketing study of the Kitasato Institute from 1994–2004. Vaccine. 2007;25:570–6.
22. Plotkin SA, Rubin SA. Mumps vaccine. In: Plotkin SA, Orenstein W, Offit P, editors. Vaccines. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 435–65.
23. Yung CF, Andrews N, Bukasa A, Brown KE, Ramsay M. Mumps complications and effects of mumps vaccination, England and Wales, 2002–2006. Emerg Infect Dis. 2011;17:661–7.
24. Hashimoto H, Fujioka M, Kinumaki H, Kinki Ambulatory Pediatrics Study Group. An office-based prospective study of deafness in mumps. Pediatr Infect Dis J. 2009;28:173–5.