A polymorphism in melanoma differentiation-associated gene 5 may be a risk factor for enterovirus 71 infection

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Abstract

Enterovirus 71 (EV71) infection has a wide variety of clinical manifestations, from no symptoms to fatal disease. Host immune response may be a determinant of disease severity. We investigated the association of polymorphisms in three pattern recognition receptor (PRR) genes —toll-like receptor 3 (*TLR3*) (rs3775291), retinoic acid-inducible gene I (*RIG-I*) (rs10813831) and melanoma differentiation-associated gene 5 (*MDA5*) (rs1990760)—with the severity of EV71 infection. Polymorphisms of candidate genes in 87 EV71-infected patients and 57 asymptomatic controls were detected. Binary logistic regression analysis revealed statistically significant differences in polymorphism of *MDA5* (rs1990760) between patients with severe EV71 infection and asymptomatic controls in an additive model (OR 0.424, 95% CI 0.213–0.845, p 0.015) and a dominant model (OR 0.256, 95% CI 0.103–0.635, p 0.003). Polymorphism of *MDA5* (rs1990760) (OR 0.399, 95% CI 0.199–0.798, p 0.009) was found to be associated with the severity of EV71 infection with the analysis of ordinal logistic regression. These results indicated the association between *MDA5* (rs1990760) polymorphism and an increased risk of a severe EV71 infection in Chinese children, which offers potential for investigating the innate immune mechanism of EV71 infection and identifying at-risk infants, for whom a preventive strategy may reduce the severity of EV71 infection.

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Introduction

Enterovirus 71 (EV71) belongs to human enterovirus species A in the genus *Enterovirus*, family *Picornaviridae*. EV71 was first isolated in the USA in 1969 and is the main pathogen in hand, foot and mouth disease (HFMD), a common illness that usually affects children under 10 years old [1]. Since then, EV71 epidemics and aperiodic global outbreaks have been reported (e.g. the 1975 epidemic in Bulgaria, the 1998 pandemic in

Taiwan and the 2008 outbreak in Singapore) [2–4]. A total of 489 073 HFMD cases, including 126 fatal cases, were reported in China in 2008, and EV71 was confirmed to be the major pathogen [5]. The number of reported cases has continued to increase. A total of 1 155 525 patients were reported in 2009; among these, 353 fatal cases were predominantly infections with EV71 in China [6]. In 2012, the Ministry of Health of the People's Republic of China announced a total of 2 198 442 HFMD cases, including 569 fatal cases [7].

The clinical spectrum of HFMD caused by EV71 in young children varies from asymptomatic (c.71%) to fatal disease (c.0.05%) [8]. The mechanisms of EV71-related disease are unclear. Recently, host genetic factors rather than EV71 itself have become a topic of interest in the study of pathogenesis of EV71-related disease. One study from Taiwan reported that children with meningoencephalitis had a higher frequency of the G/G genotype at position 49 in Exon 1 of the cytotoxic

T-lymphocyte-associated antigen 4 (*CTLA4*) gene compared with those without meningoencephalitis and control cases [8]. A subsequent study showed no association with meningoencephalitis, but another gene, *HLA-A33*, was correlated with increased susceptibility to EV71 infection [9]. A later study suggests that polymorphisms in interleukin-10 (*IL10*) and interferon-γ (*IFNG*) are associated with encephalitis during the progression of EV71 infection [10].

In terms of host factors related to virus infection, the innate immune system is essential for the initial recognition of invading viruses and subsequent activation of adaptive immunity. Viruses initially activate the innate immune system, which recognizes viral components through pattern-recognition receptors (PRRs). When PRRs are activated, interferons (IFNs) and cytokines are produced in response to viral infections. There are two main pathways for the recognition of RNA viruses. Toll-like receptor 3 (TLR3) in the endosome has been shown to recognize viral dsRNA [11]. A second pathway is mediated by cytosolic sensors of dsRNA. The cytoplasmic proteins retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) recognize viral RNA present in the cytoplasm. According to previous studies, TLR3, RIG-I and MDA5 are involved in picornavirus infection [12–14]. Lei *et al.* reported that EV71 inhibits type I IFN responses mediated by RIG-I and TLR3 [15,16]. Whereas recent study suggests that MDA5 plays a crucial role in the innate response to EV71 infection [17].

Polymorphisms in the genes encoding PRRs are reportedly associated with many diseases. Polymorphism of rs3775291 in *TLR3* have been suggested to have associations with increased risk of tick-borne encephalitis virus infection of which the severe symptoms are in the central nervous system, as in EV71 infection [18]. A common non-synonymous single-nucleotide polymorphism (SNP) in *RIG-I*, rs10813831, was reported that modified the innate immune response of human dendritic cells [19]. A genome-wide association study identified that non-synonymous SNP rs1990760 in *MDA5* increased type 1 diabetes, which involved the enteroviral infection [20]. In this study, we investigated the relationships between these three polymorphisms in PRR genes (*TLR3*, *RIG-I* and *MDA5*) and the severity of EV71 infection to identify potential at-risk infants, for whom a preventive strategy could reduce the severity of EV71 infection.

Materials and Methods

Ethics statement

The use of patient specimens in this study was approved by the Ethics Committee of the National Institute for Viral Disease Prevention and Control, China CDC and Nanjing Children's Hospital. Informed consents were obtained from the parents of each of the enrolled children.

Study cohort

Peripheral EDTA-blood samples were collected from 87 inpatients between August 2010 and August 2011 at Nanjing Children's Hospital (Jiangsu Province, China). EV71 infection in these patients was confirmed by EV71-specific IgM ELISAs. According to the handbook of treatment for HFMD (2010) from the Ministry of Health of the People's Republic of China all of the cases were diagnosed as severe cases of HFMD.

The control group was composed of healthy children who came to Nanjing Children's Hospital for routine health examinations between November 2010 and August 2011. Because the children who do not have EV71-IgG antibodies might not contact the virus, to reduce bias we excluded children who were tested negative for EV71-IgG antibodies. We collected 391 samples from healthy children who were EV71-IgM negative. After excluding 230 children who were EV71-IgG negative, 65 children who failed to contact, nine children who had a history of HFMD or herpangina and 30 children who were more than 6 years old, 57 children who were EV71-IgG positive and who had no history of HFMD were enrolled as asymptomatic controls.

DNA extraction and SNP genotyping

DNA was extracted from peripheral EDTA-blood samples using a QIAamp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Following the amplification of genomic DNA by PCR, genotypes were determined by sequencing. PCR primers were designed separately for each gene using PRIMER PREMIER 5 software or as previously described (Table 1) [21]. The reactions were performed at 94°C for 2 min followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and finally one cycle of 5 min at 72°C.

Statistical analysis

Chi-squared tests and Student's *t*-test were used to analyse the demographic data and polymorphism frequencies between groups. Hardy–Weinberg equilibrium was checked for each gene. Associations between the alleles, genotypes and severity of EV71 infection were estimated by calculating OR and 95% CI using multiple logistic regression analyses. Age is an important factor in EV71 infection and disease, and we used age as a factor in the multivariate analysis. First, the association between candidate polymorphisms and the EV71 infection was assessed by binary logistical regression with additive and dominant models. For each model the dependent variable was

TABLE 1. PCR primer sequences used to analyse candidate gene polymorphisms

Gene	Polymorphic site	GenBank Access. no.	Primer sequence (5′→3′)
TLR3	+1234A/G	rs3775291	Forward: GTCAGCTGCAGGTACTTGTTG Reverse: TGGAGCACCTTAACATGGAAGA [21]
RIG-I	Cys7Arg	rs10813831	Forward: GTAGGTAGGGTCCAGGGTCTTCCG Reverse: GTGCGGAGGGAAACGAAACTA
MDA5	Ala946Thr	rs1990760	Forward: CTGAATAGTCAAGATTGGGAAATG Reverse: GAAGGAAGGAATGCCGTGTA

case–control. The additive model and dominant model were fitted for polymorphisms of *TLR3* (rs3775291), *RIG-I* (rs10813831) and *MDA5* (rs1990760) separately. Second, ordinal logistic regression was used to evaluate the independent effect of polymorphisms on the severity of EV71 infection. There were three levels of severity status, such as asymptomatic controls, encephalitis patients and encephalitis patients with other complications. Third, due to the small number of samples, exact logistic regression analysis was used to analyse the association of candidate polymorphisms with the risk of developing severe brainstem encephalitis (BE) using the patients with encephalitis only as control. In all groups, p-values <0.05 were considered to be statistically significant. The above analyses were performed using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA) or SAS software (version 9.3; SAS Institute, Cary, NC, USA).

Results

Demographic data, clinical characteristics and Hardy–Weinberg equilibrium analysis

Based on the clinical manifestation, 87 patients were divided into two groups: 39 patients with encephalitis only (44.8%) and 48 encephalitis patients with other complications (55.2%) including five patients with encephalomyelitis, nine patients with myocardial damage, 20 patients with severe brainstem encephalitis (BE), four patients with pulmonary oedema, one patient with encephalomyelitis and myocardial damage, two patients with encephalomyelitis and BE, one patient with myocardial damage and BE, three patients with encephalomyelitis and pulmonary oedema, two patients with BE and pulmonary oedema and one patient complicated with encephalomyelitis, BE and pulmonary oedema (see Supporting information, Table S1).

Among the 87 EV71-infected cases, 65 (74.7%) were male and 22 (25.3%) were female. Similarly, there were 35 (61.4%) males and 22 (38.6%) females in the asymptomatic control group. There were no significant sex differences between the case and control subjects. The median age of the patients was 1.7 years (range 0.1–4.6 years), while that of the asymptom-

atic control group was 3.8 years (range 0.3–5.8 years) (p <0.05) (see Supporting information, Table S2).

The genotypic distributions of the candidate genes between the patients and controls did not differ significantly from the results of Hardy–Weinberg equilibrium (data not shown).

Distribution of allelic variants and genotypes of the candidate genes

The genotypes of three candidate SNPs in cases and controls are shown in Tables S1 and S2. After adjusting for age, a binary logistic regression analysis showed that there were differences in *MDA5* (rs1990760) between the patients with severe EV71 infection and asymptomatic controls, under an additive model for the A allele (OR 0.424, 95% CI 0.213–0.845, p 0.015). When comparing the GG and GA/AA genotypes for rs1990760 in patients with EV71 infection and asymptomatic controls, the GG genotype was significantly more frequent in patients with severe EV71 infection than controls (OR 0.256, 95% CI 0.103–0.635, p 0.003) in a dominant model. No significant differences in allele frequencies were observed in other genes between the two groups (Table 2).

The associations between polymorphisms of candidate genes and the severity of EV71 infection

We used ordinal logistic regression models to analyse the association between SNPs of candidate genes and severity of EV71 infection (asymptomatic controls, encephalitis patients and encephalitis patients with other complications). The severity of EV71 infection was going to be treated as ordinal under the assumption that the levels of severity status have a natural ordering (low to high), but the distances between adjacent levels are unknown. Associations were found between polymorphism of *MDA5* (rs1990760) (OR 0.399, 95% CI 0.199–0.798, p 0.009) and the severity of EV71 infection after adjusting for age (Table 3).

The associations between polymorphisms of candidate genes and the risk of developing BE

Brainstem encephalitis associated with cardiopulmonary dysfunction is a notable feature in EV71 epidemics in Asia, and is the primary cause of death. Brainstem encephalitis leads to

TABLE 2. **Differential distribution of candidate gene alleles and genotypes among cases with severe enterovirus 71 disease and asymptomatic control subjects**

Candidate genes	Genotypes	Case (%) $n = 87$	Control (%) $n = 57$	OR (95% CI)*	p-value*
TLR3 + 1234A/G	Additive model				
	G	128 (73.6)	78 (68.4)	1.000 (ref.)	0.318
	A	46 (26.4)	36 (31.6)	0.733 (0.398–1.348)	
	Dominant model				
	GG	47 (54.0)	28 (49.1)		0.389
	GA/AA	40 (46.0)	29 (50.9)	0.706 (0.319–1.560)	
RIG-I rs10813831	Additive model				
	G	159 (91.4)	106 (93.0)	1.000 (ref.)	0.531
	A	15 (8.6)	8 (7.0)	1.435 (0.464–4.443)	
	Dominant model				
	GG	73 (83.9)	49 (86.0)	1.000 (ref.)	0.551
	GA/AA	14 (16.1)	8 (14.0)	1.433 (0.439–4.677)	
MDA5 rs1990760	Additive model				
	G	141 (81.0)	86 (75.4)	1.000 (ref.)	**0.015**
	A	33 (19.0)	28 (24.6)	**0.424 (0.213–0.845)**	
	Dominant model				
	GG	57 (65.5)	30 (52.6)	1.000 (ref.)	**0.003**
	GA/AA	30 (34.5)	27 (47.4)	**0.256 (0.103–0.635)**	

For results shown in bold, p <0.05.
For additive model, reference group was G, and GG in dominant model. Additive model and dominant model were fitted for each candidate polymorphism separately.
*p-values and ORs are adjusted for age by binary logistic regression analysis.

TABLE 3. **Association between single nucleotide polymorphisms of candidate genes and severity of enterovirus 71 infection**

Candidate genes	Genotypes	Cases		Control ($n = 57$)	OR (95% CI)*	p-value*
		Complication patients ($n = 48$)	Encephalitis patients ($n = 39$)			
TLR3 + 1234A/G	GG	26 (54.2)	21 (53.8)	28 (49.1)	1.000 (ref.)	0.582
	GA + AA	22 (45.8)	18 (46.2)	29 (50.9)	0.834 (0.438–1.589)	
RIG-I rs10813831	GG	37 (77.1)	36 (92.3)	49 (86.0)	1.000 (ref.)	0.142
	GA + AA	11 (22.9)	3 (7.7)	8 (14.0)	1.999 (0.794–5.035)	
MDA5 rs1990760	GG	32 (66.7)	25 (64.1)	30 (52.6)	1.000 (ref.)	**0.009**
	GA + AA	16 (33.3)	14 (35.9)	27 (47.4)	**0.399 (0.199–0.798)**	

For results shown in bold, p <0.05.
Dependent variables were categorized as encephalitis patients with complications, encephalitis patients and asymptomatic controls. Three models were fitted for the single nucleotide polymorphisms of candidate genes as independent variables separately.
*p-values and ORs are adjusted for age by ordinal logistic regression analysis.

acute heart failure and this complication has a high mortality rate. The mechanism of BE caused by EV71 remains unclear. We explored the association between the three candidate polymorphisms of *TLR3*, *RIG-I* and *MDA5* and BE. Exact logistic regression was used to value the differences in genotype distributions of candidate SNPs between BE patients and patients with encephalitis only. There was statistical difference observed in polymorphism of *RIG-I* (rs10813831) (OR 5.278, 95% CI 1.097–34.963, p 0.036) with adjusting for age (Table 4).

Discussion

Although most HFMD cases are self-limiting, EV71 is neurotropic and can directly cause central neurological impairment. The case-fatality rate for HFMD ranges from 0.06 to 0.11%.

Recent studies suggested that host immune responses were related to serious complications in EV71 patients. Clinical studies have also provided clues about the relationship between EV71 pathogenesis and immune responses in patients [22,23]. The innate immune system is the first line of defence against invading organisms. Sensing of RNA virus infections is carried out by membrane-bound TLRs or the cytoplasmic proteins RIG-I and MDA5. EV71 is a single-stranded RNA virus. Studies suggest that EV71-encoded 3C protease inhibits the RIG-I-mediated IFN regulatory factor 3 activation and cleaves the adaptor protein of TLR3 to inhibit type I IFN production [15,16]. Recent study reveals MDA5 is an important factor for EV71 RNA-activated type I IFN expression [17]. Which PRR plays the pivotal factor in the EV71 infection is still unclear. Detection of viral components by PRRs activates signalling cascades, leading to the secretion of type I IFNs, proinflammatory cytokines and chemokines. A cytokine storm

TABLE 4. Association of candidate genes' genotypes with the risk of developing brainstem encephalitis (BE)

Candidate genes	Genotypes	BE (%) n = 26	Encephalitis patients (%) n = 39	OR (95% CI)*	p-value*
TLR3 + 1234A/G	GG	15 (57.7)	21 (53.8)	1.000 (ref.)	0.932
	GA + AA	11 (42.3)	18 (46.2)	0.843 (0.274–2.542)	
RIG-I rs10813831	GG	18 (69.2)	36 (92.3)	1.000 (ref.)	**0.036**
	GA + AA	8 (30.8)	3 (7.7)	**5.278 (1.097–34.963)**	
MDA5 rs1990760	GG	17 (65.4)	25 (64.1)	1.000 (ref.)	1.000
	GA + AA	9 (34.6)	14 (35.9)	1.032 (0.300–3.485)	

For results shown in bold, p <0.05.
*p-values and ORs are adjusted for age by exact logistic regression analysis.

has been found in EV71-infected patients with serious complications [22,24].

In our study, we found a significant difference in *MDA5* (rs1990760) polymorphism between patients with severe EV71 infection and asymptomatic controls. MDA5 is a DEx (D/H) box helicase that can detect intracellular viral products and recognizes *Picornaviridae* family members [25]. Upon activation, MDA5 triggers nuclear factor-κB and/or IFN regulatory factor pathways and induces an antiviral IFN-β response. MDA5 is encoded by the gene IFN-induced with helicase C domain 1 (*IFIH1*) on chromosome 2q24.3. The A allele of *MDA5* (rs1990760) encodes an alanine to threonine amino acid substitution at codon 946 in a region that is important for the dimerization of RIG-I-like proteins and may influence its binding to dsRNA. Shigemoto *et al.* constructed the A946T mutation of MDA5, but found that it affected neither dsRNA binding nor the IFN gene activation [26]. Although a previous study did not find differences in the expression of the protein between its two alleles, the authors proposed that it might act through changes in the tertiary structure of the protein [27]. The mechanism through which the mutation affects the diseases remains unclear. Liu *et al.* reported that expression of MDA5 was up-regulated in peripheral blood mononuclear cells from carriers of *IFIH1* variants [28]. Significant association has been reported between IFIH1 and many autoimmune diseases. A genome-wide association study has identified that *MDA5* rs1990760 is associated with susceptibility to type 1 diabetes, which is associated with enteroviral infection [20]. Infection with enteroviruses is more common among newly diagnosed type 1 diabetes patients. Another study also reported that genotypes of *MDA5* rs1990760 might modify the frequency of enterovirus RNA in blood [29]. These studies suggest that *MDA5* rs1990760 might correlate with enterovirus infection. Our study showed that children with severe EV71 infection had a higher frequency of the GG genotype of *MDA5* compared with asymptomatic control subjects. This result indicates that *MDA5* rs1990760 may play an important role in the pathogenesis of EV71 infection, which provides new insights into the relationship between MDA5 and EV71 infection.

In our study, we did not find a difference in the distributions of alleles and genotypes between patients with severe EV71 infection and asymptomatic controls for the polymorphisms of *TLR3* (rs3775291) and *RIG-I* (rs10813831). Surprisingly, the statistical difference indicated a polymorphism of *RIG-I* (rs10813831) between BE patients and encephalitis patients. RIG-I is another cytoplasmic RNA sensor that senses 5′-triphosphate single-stranded RNA with poly(U/A) motifs and short dsRNA in cells infected with a variety of RNA viruses. The *RIG-I* rs10813831 encodes an Arg7Cys amino acid change in the caspase recruitment domain (CARD). CARD is a functional domain of RIG-I that can activate the signalling cascade leading to IFN-β expression. In our study, *RIG-I* rs10813831 was associated with patients suffering from BE caused by EV71 infection. Previous study suggested that RIG-I initiates inflammatory immune responses by human glial cells following exposure to neurotropic RNA virus and plays an important role in the neuronal cell death associated with acute viral central nervous system infections [30]. However, given the small number of non-brainstem cases and the rarity of the G allele the exact role of *RIG-I* (rs10813831) and the complication of BE needs to be addressed in a further study.

There were some limitations in our study. No mild cases without complications due to EV71 were enrolled, which is the major limitation of our study, and which is very important in exploring the role of host genetic factors in the severity of disease. In addition, the number of severe cases with different complications was limited, which might hide potentially significant factors and influence the results of the statistical analysis. Because children who do not have EV71-IgG antibodies might not contract the virus, to reduce bias we excluded children from the control group who tested negative for EV71-IgG antibodies, while decreasing the size of control samples.

In summary, we report an association between the rs1990760 polymorphism in *MDA5* and EV71 infection in

children in eastern China, suggesting that it may be a risk factor for sever symptoms following EV71 infection. Our findings may throw light on elucidating the innate immune mechanism of EV71 infection and identifying at-risk infants. Additional studies, especially with a greater number of cases and molecular biological experiments, should be carried out to elucidate the role of host genetic factors in the pathogenesis of EV71-related disease.

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Transparency Declaration

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The demographic, clinical characteristics and genotypes data of enterovirus 71-infected patients.

Table S2. The demographic and genotypes data of asymptomatic control subjects.

References

1. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. *J Infect Dis* 1974; 129: 304–309.
2. Ho M, Chen ER, Hsu KH, *et al.* An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med* 1999; 341: 929–935.
3. Wu Y, Yeo A, Phoon MC, *et al.* The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. *Int J Infect Dis* 2010; 14: e1076–e1081.
4. Chumakov M, Voroshilova M, Shindarov L, *et al.* Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. *Arch Virol* 1979; 60: 329–340.
5. Yang F, Ren L, Xiong Z, *et al.* Enterovirus 71 outbreak in the People's Republic of China in 2008. *J Clin Microbiol* 2009; 47: 2351–2352.
6. Li R, Zou Q, Chen L, Zhang H, Wang Y. Molecular analysis of virulent determinants of enterovirus 71. *PLoS ONE* 2011; 6: e26237.
7. Mininstry of Health of the People's Republic of China. Available at: (http://www.nhfpc.gov.cn/zhuzhan/s3578/201304/b540269c8e5141e6b b2d00ca539bb9f7.shtml). Accessed 7 November 2013.
8. Yang KD, Yang MY, Li CC, *et al.* Altered cellular but not humoral reactions in children with complicated enterovirus 71 infections in Taiwan. *J Infect Dis* 2001; 183: 850–856.
9. Chang LY, Chang IS, Chen WJ, *et al.* HLA-A33 is associated with susceptibility to enterovirus 71 infection. *Pediatrics* 2008; 122: 1271–1276.
10. Yang J, Zhao N, Su NL, Sun JL, Lv TG, Chen ZB. Association of interleukin 10 and interferon γ gene polymorphisms with enterovirus 71 encephalitis in patients with hand, foot and mouth disease. *Scand J Infect Dis* 2012; 44: 465–469.
11. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 2001; 413: 732–738.
12. Barral PM, Sarkar D, Fisher PB, Racaniello VR. RIG-I is cleaved during picornavirus infection. *Virology* 2009; 391: 171–176.
13. Gitlin L, Barchet W, Gilfillan S, *et al.* Essential role of MDA-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. *Proc Natl Acad Sci USA* 2006; 103: 8459–8464.
14. Richer MJ, Lavallee DJ, Shanina I, Horwitz MS. Toll-like receptor 3 signaling on macrophages is required for survival following coxsackievirus B4 infection. *PLoS ONE* 2009; 4: e4127.
15. Lei X, Liu X, Ma Y, *et al.* The 3C protein of enterovirus 71 inhibits retinoid acid-inducible gene I-mediated interferon regulatory factor 3 activation and type I interferon responses. *J Virol* 2010; 84: 8051–8061.
16. Lei X, Sun Z, Liu X, Jin Q, He B, Wang J. Cleavage of the adaptor protein TRIF by enterovirus 71 3C inhibits antiviral responses mediated by Toll-like receptor 3. *J Virol* 2011; 85: 8811–8818.
17. Kuo RL, Kao LT, Lin SJ, Wang RY, Shih SR. MDA5 Plays a Crucial Role in Enterovirus 71 RNA-Mediated IRF3 Activation. *PLoS ONE* 2013; 8: e63431.
18. Kindberg E, Vene S, Mickiene A, Lundkvist A, Lindquist L, Svensson L. A functional Toll-like receptor 3 gene (TLR3) may be a risk factor for tick-borne encephalitis virus (TBEV) infection. *J Infect Dis* 2011; 203: 523–528.
19. Hu J, Nistal-Villan E, Voho A, *et al.* A common polymorphism in the caspase recruitment domain of RIG-I modifies the innate immune response of human dendritic cells. *J Immunol* 2010; 185: 424–432.
20. Smyth DJ, Cooper JD, Bailey R, *et al.* A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nat Genet* 2006; 38: 617–619.
21. Sironi M, Biasin M, Cagliani R, *et al.* A common polymorphism in TLR3 confers natural resistance to HIV-1 infection. *J Immunol* 2012; 188: 818–823.
22. Lin TY, Hsia SH, Huang YC, Wu CT, Chang LY. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. *Clin Infect Dis* 2003; 36: 269–274.
23. Chang LY, Hsiung CA, Lu CY, *et al.* Status of cellular rather than humoral immunity is correlated with clinical outcome of enterovirus 71. *Pediatr Res* 2006; 60: 466–471.
24. Wang SM, Lei HY, Huang KJ, *et al.* Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. *J Infect Dis* 2003; 188: 564–570.
25. Kato H, Takeuchi O, Sato S, *et al.* Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006; 441: 101–105.

26. Shigemoto T, Kageyama M, Hirai R, Zheng J, Yoneyama M, Fujita T. Identification of loss of function mutations in human genes encoding RIG-I and MDA5: implications for resistance to type 1 diabetes. *J Biol Chem* 2009; 284: 13348–13354.

27. Zouk H, Marchand L, Polychronakos C. Study of transcriptional effects in Cis at the IFIH1 locus. *PLoS ONE* 2010; 5: e11564.

28. Liu S, Wang H, Jin Y, *et al.* IFIH1 polymorphisms are significantly associated with type 1 diabetes and IFIH1 gene expression in peripheral blood mononuclear cells. *Hum Mol Genet* 2009; 18: 358–365.

29. Cinek O, Tapia G, Witso E, *et al.* Enterovirus RNA in peripheral blood may be associated with the variants of rs1990760, a common type 1 diabetes associated polymorphism in IFIH1. *PLoS ONE* 2012; 7: e48409.

30. Furr SR, Moerdyk-Schauwecker M, Grdzelishvili VZ, Marriott I. RIG-I mediates nonsegmented negative-sense RNA virus-induced inflammatory immune responses of primary human astrocytes. *Glia* 2010; 58: 1620–1629.