Fu *et al. Virology Journal* (2025) 22:29
https://doi.org/10.1186/s12985-025-02644-7

Virology Journal

RESEARCH Open Access

Association between cadherin-related family member 3 rs6967330-A and human rhinovirus-C induced wheezing in children

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Abstract

Background The heterogeneity of childhood wheezing illnesses is associated with viral and host factors. Human rhinoviruses (HRV) are the major pathogens in severe wheezing in young children. The single nucleotide polymorphism (SNP) rs6967330 G > A proved to heighten the risk of wheezing. However, the relation between rs6967330 variants of cadherin-related family member 3 (CDHR3) and wheezing induced by human rhinovirus (HRV)-C has not been determined.

Methods A total of 11,756 respiratory specimens collected from hospitalized children with acute respiratory infections (ARIs) between September 2017 and March 2023 were screened for enterovirus (EV)/HRVs by the capillary electrophoresis-based multiplex PCR (CEMP) assay, and those positive only for HRVs were amplified and sequenced for HRV and CDHR3 genotyping. The clinical data of the enrolled patients were obtained and analyzed.

Results EV/HRVs (15.2%; 1,616/10,608) were the more common viruses detected in inpatients with ARIs. Among the enrolled samples, 148 were positive for HRV-A (49.83%; 148/297), 129 for HRV-C (43.4%; 129/297), and 20 for HRV-B (6.7%; 20/297). More patients infected with HRV-C had history of allergy ($P=0.004$), family history of asthma ($P=0.001$), wheezing ($P=0.005$) and asthma ($P=0.001$) than those infected with HRV-A or HRV-B, while patients infected with HRV-C were less likely to have older siblings compared to those infected with HRV-A ($P=0.014$). The rs6967330-A variant was related to a high incidence of the three concave signs ($P=0.047$), asthma exacerbation ($P=0.025$), a higher risk of HRV-C infection determined by the dominant model (OR 1.91, 95% confidence interval 1.05–3.48; $P=0.033$), and a high proportion of wheezing (56.67%) in patients infected with HRV-C.

Conclusions HRV-C is the dominant species responsible for HRV-induced wheezing. The rs6967330-A variant is a risk factor for HRV-C infection, and was associated with the high rate of wheezing induced by HRV-C.

Keywords Children, Wheezing, Human rhinovirus species C, Cadherin-related family member 3, rs6967330 variant

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Introduction

Asthma is the most common chronic childhood disease worldwide. Approximately 14% of children are affected by asthma, and its prevalence is increasing globally [1]. Furthermore, asthma accounts for more than 14 million missed school days per year and costs billions of dollars in health care expenditures [2]. Diagnosis of asthma in older children and adults centers around identifying asthma symptoms and confirming variable airflow obstruction [3]. However, the diagnosis of asthma in children under five years has been controversial. One pragmatic approach to asthma diagnosis in this age group is based on the signs and symptoms of reversible airflow obstruction and patients' response to asthma medication [4]. Asthma exacerbation is results from complex interactions between environmental exposures and genetic predisposition [5]. Approximately 80% of asthma exacerbations are attributed to respiratory viral infections, with human rhinovirus (HRV) accounting for nearly two thirds of these cases [6].

HRV, a member of the *Enterovirus* genus within the *Picornaviridae* family, is a small, non-enveloped, positive-stranded RNA virus. To date approximately 165 genotypes of HRV have been identified based on the highly conserved nucleotide sequences of the 5' UTR and *VP4/VP2*, which have been classified into three species (A, B, and C) [7]. These include 80 genotypes of HRV-A, 30 of HRV-B and 55 of HRV-C. Genotypes in HRV-A and C are more likely to be associated with severe respiratory diseases than HRV-B [8]. HRVs are divided into three groups based on the receptors they use to bind and enter host epithelial cells. The major group, consisting of all genotypes of HRV-B and most genotypes of HRV-A, uses intercellular adhesion molecule 1 (ICAM-1) as a cellular receptor. The minor group, which include 12 genotypes of HRV-A, uses the low-density lipoprotein receptor (LDLR) family as the cellular receptor [9]. The third group, which includes all genotypes of HRV-C, uses cadherin-related family member 3 (CDHR3) as the cellular receptor [10].

The *CDHR3* gene, located on chromosome 7q22.3, encodes CDHR3 [10]. A genome-wide scan aimed at identifying severe childhood asthma exacerbation revealed a related single-nucleotide polymorphism (SNP), rs6967330, which leads to an amino acid substitution from cysteine (T*G*T) to tyrosine (T*A*T) at position 529 of the CDHR3 amino acid sequence. The variant encoding Cys529 in two alleles, named the GG genotype, was observed in 69% of participants and had little clinical correlation with early childhood asthma. By contrast, the variant encoding Tyr529 in two alleles, named the AA genotype, showed A/A homozygosity in 3% of participants, whereas another 28% were A/G heterozygous, named the GA genotype. The rs6967330 risk allele (A) was associated with a greater risk of asthma hospitalizations (hazards ratio = 1.7; 95% confidence interval (CI) = 1.2–2.4; $P = 0.002$) and severe exacerbations (hazards ratio = 1.4; 95% CI = 1.1–1.9; $P = 0.007$) in combined analyses [11]. The rs6967330 variant has been associated with increased surface expression of the CDHR3 protein, and the use of CDHR3 by HRV-C creates a direct phenotypic link between human genetics (G versus A alleles) and the efficiency with which HRV-C infects cells. Therefore, rs6967330 confers a greater risk of severe childhood asthma exacerbation, likely through increased HRV-C infection levels and greater surface localization of the protein [12].

Wheezing is a common symptom in preschool children with acute respiratory tract infections (ARIs), with up to half experiencing at least one wheezing episode within the first 6 years of life [13]. Compared to HRV-A and HRV-B, HRV-C is associated with an increased risk of respiratory hospital admission and recurrent severe wheezing illness in children [14]. Therefore, wheezing in early life may be a predictor of childhood asthma [15]. The key role of environmental factors, such as HRV infection, in determining the progression from preschool wheezing to childhood asthma has been reported [13].

HRV-C infection is associated with asthma exacerbation via the rs6967330 variant of CDHR3, and HRV-C infection has been identified as a risk factor for severe wheezing. However, the relationship between the variation of rs6967330 of cadherin-related family member 3 (CDHR3) and wheezing induced by human rhinovirus (HRV) -C has not been fully clarified. In this study, respiratory specimens were retrospectively collected from children with ARI who were hospitalized at the Children's Hospital Affiliated with the Capital Institute of Pediatrics in Beijing from September 2017 to March 2023 for HRV screening and typing. The CDHR3 fragment containing the rs6967330 SNP was amplified and sequenced from HRV-positive specimens, which were then genotyped as GG, GA, or AA. Based on the clinical data of children positive for HRV, the association between the rs6967330 variant of CDHR3 and wheezing induced by different groups of HRVs was evaluated.

Materials and methods

Clinical specimens

Respiratory specimens were collected from pediatric patients under 14 years of age based on the following inclusion criteria: (1) diagnosed with ARIs; (2) hospitalized in the Affiliated Children's Hospital, Capital Institute of Pediatrics (Beijing, China) from September 2017 to March 2023; and (3) positive only for EV/HRVs in multiple respiratory pathogen screening using a capillary electrophoresis-based multiplex PCR (CEMP)-compatible assay-Respiratory Pathogen Multiplex Detection

Kit (Ningbo HEALTH Gene Technologies Ltd., Ningbo, China) [16]. The exclusion criteria were as follows: samples collected from the same patient during a single hospitalization; only the first HRV-positive sample was included.

Upon arrival at the laboratory, each clinical specimen was handled in a Class II biosafety cabinet and processed immediately using 2.5 mL of viral transport medium (Yocon Biotechnology Co., Ltd., Beijing, China), followed by centrifugation (500×g, 10 min). A portion of the supernatant was used for viral nucleic acid extraction, and the remainder was stored at -80℃ for future use.

Nucleic acid extraction and multiple respiratory pathogen screening

Total nucleic acid (DNA and RNA) was extracted from 140 µL of supernatant from each collected specimen using the QIAamp MinElute Virus Spin Kit (Qiagen GmbH, Germany) for multiple respiratory pathogens screening CEMP assay. According to the manufacturer's instructions, 15 pairs of primers for detecting 13 pathogens, deoxy-nucleotide triphosphates (dNTPs), MgCl2, and buffer were included in the kit for multiplex PCRs. Then, the amplification products were subjected to capillary electrophoresis on a GeXP capillary electrophoresis system (Sciex, Concord, ON, Canada) to detect the signals of the 15 labeled PCR products measured by fluorescence and separated by size: influenza virus (Flu) A 105 nt (2009H1N1 163.3 nt, H3N2 244.9 nt), Flu B 212.7 nt, adenovirus virus (ADV) 110.2/113.9 nt (representing different subtypes), human bocavirus (HBoV) 121.6 nt, human rhinovirus (HRV) 129.6 nt, human parainfluenza virus (PIV) 181.6 nt, chlamydia (Ch) 190.5 nt, human metapneumovirus (HMPV) 202.8 nt, *Mycoplasma pneumoniae* (Mp) 217 nt, human coronavirus (HCoV) 265.1 nt, and respiratory syncytial virus (RSV) 280.3 nt [16].

Reverse-transcription PCR for HRV typing

A semi-nested reverse-transcription polymerase chain reaction (PCR) targeting the *VP4*/*VP2* gene region was used to amplify a 539 bp fragment from clinical specimens positive only for EVs/HRVs, as determined by the capillary electrophoresis-based multiplex polymerase chain reaction (CEMP) assay. All PCR products were sequenced by Sino Geno Max Co., Ltd. (Beijing, China). Sequences were verified using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/). HRV and EV genotypes were identified via phylogenetic analyses of sequences using the maximum likelihood method in MEGA version 6.0. Phylogenetic trees of the HRV sequences from this study and GenBank were constructed to assess the reliability of each node [17].

CDHR3 rs6967330 genotyping

Clinical specimens positive for HRVs were subjected to nucleic acid extraction and CDHR3 genotyping by PCR using the primer pair 6967330-F (5'-AGGTACTTTATT CCTCCAG-3') and 6,967,330-R (5'-TTACTTGTTTCTC ACCACAT-3'), designed according to the CDHR3 coding sequence (GenBank No. NM_152750.5). All PCR products, with an expected size of 382 bp, were sequenced by Sino Geno Max Co., Ltd. (Beijing, China). The rs6967330 SNP is located at nucleotide position 179 of the 382 bp amplified fragment (5'-3'). There were three genotypes of the rs6967330 variant, named the GG genotype with G/G, the GA genotype with G/A, and the AA genotype with A/A, on two alleles.

Clinical data collection

The clinical data of the enrolled patients, including age, sex, date of admission, date of sample collection, symptoms or signs, diagnosis, laboratory results, and duration of hospitalization, were obtained from their electronic medical records. Especially, the special symptom of severe asthma, three concave signs, was evaluated, which was defined as three types of concave deformities observed in the chest, including suprasternal, supraclavicular and intercostal retraction. Suprasternal retraction is usually due to the increased negative pressure in the chest during inhalation, while supraclavicular retraction indicates increased airway resistance, and intercostal retraction suggests that the respiratory muscles are working harder to overcome airway resistance.

The severity of ARIs was assessed according to the following criteria [18]: (1) very mild: upper respiratory tract symptoms or signs only; (2) mild: lower respiratory tract symptoms or signs +/−, upper respiratory tract symptoms/signs +, without hospital admission; (3) moderate: lower respiratory tract symptoms or signs +/−, upper respiratory tract symptoms or signs +, hospital admission, and oxygen saturations in air > 93% on pulse oximetry; (4) severe: lower respiratory tract symptoms or signs +/−, upper respiratory tract symptoms or signs +, hospital admission, and oxygen saturations in air < 93%.

Patients who were positive only for HRVs and had symptoms of wheezing or three concave signs, or diagnosed with ARIs, asthma/asthma exacerbation, or acute bronchitis were identified as having HRV-induced wheezing. Patients diagnosed with other diseases that cause wheezing, such as chronic obstructive pulmonary disease, bacterial pneumonia, or left heart failure, were excluded from the study.

Statistical analysis

Statistical analysis was performed using SPSS Statistics (version 22.0; IBM, Armonk, NY, USA). In the descriptive analysis, data following a normal distribution were

presented as mean ± standard deviation ($\bar{x}\pm$ s), whereas data following a skewed distribution were presented as the median and interquartile range. Demographic and clinical characteristics were compared using the KruskalWallis H-test or analysis of variance for continuous variables and Pearson's χ^2 test or Fisher's exact test for categorical variables. HardyWeinberg equilibrium for CDHR3 rs6967330 was evaluated using the χ^2 exact test. Two-tailed *P*-values of < 0.05 were considered statistically significant.

Results

Multiple respiratory pathogenic screening

From September 2017 to March 2023, 11,756 clinical specimens were collected from children with ARI hospitalized in the Children's Hospital Affiliated with the Capital Institute of Pediatrics for respiratory pathogen screening using a CEMP assay. After removing outpatient specimens and duplicates from one hospitalization, there were 10,608 specimens male to female ratio: 1.39:1; average age: 3.05 ± 3.42 years). EV/HRVs were the most prevalent pathogen (15.23%; 1,616/10,608), followed by MP (11.80%; 1,252/10,608) and RSV (10.91%; 1,157/10,608). Among the 1,616 cases positive for HRV, 934 were positive only for EV/HRVs (57.80%; 934/1,616) (male tofemale ratio: 1.63:1; average age: 3.04 ± 2.91 years), and 682 (42.20%; 682/1,616) were positive for other pathogens as well. Among the co-infections, 150 were also positive for MP (21.99%; 150/682), 138 for HPIV (18.77%; 138/682), 119 for HBoV (16.57%; 119/682), 110 for RSV (14.96%; 110/682), 41 for HMPV (5.72%; 41/682), 38 for ADV (4.84%; 38/682), 14 for Flu (2.05%; 14/682), 8 for HCoVs (1.17%; 8/682), and 5 for Cp (0.73%; 5/682). Additionally, 47 were co-infected with two pathogens, and 12 were co-infected with three pathogens.

HRV typing

Among the 934 patients positive only for EV/HRVs in the CEMP assay, 569 patients were excluded from the study, including 464 co-infected with bacteria, 57 diagnosed with chronic obstructive pulmonary disease, 48 suffering from cardiovascular diseases that cause wheezing symptoms, and 365 were included for HRV typing. Of these, 41 patients had low RNA loads for sequencing, 27 were identified as EV, and 297 were determined to be HRV and were finally enrolled in the study.

According to the phylogenetic analysis of the 539 bp fragment of the *VP4*/*VP2* genes amplified from 297 clinical specimens, 148 were clustered into 47 genotypes of HRV-A (49.83%, 148/297), 129 into 32 genotypes of HRV-C (43.4%, 129/297), and 20 into 12 genotypes of HRV-B (6.7%, 20/297). The dominant genotypes of HRV-A were HRV-A49 (8.11%, 12/148) and HRV-A78 (7.43%, 11/148), and the most common genotypes of HRV-B were HRV-B52 (15.00%, 3/20) and HRV-B86 (15.00%, 3/20), whereas HRV-C53 (9.30%, 12/129), HRV-C15 (7.75%, 10/129), and HRV-C6 (6.98%, 9/129) were the most frequent genotypes of HRV-C (Fig. 1).

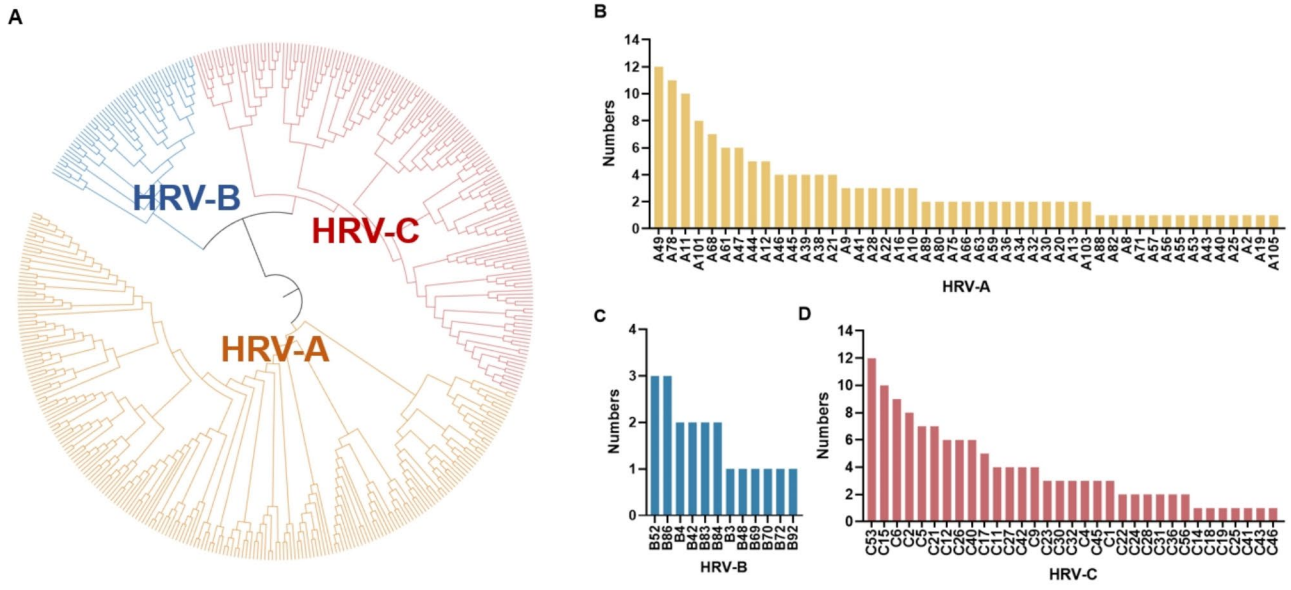


Fig. 1 Human rhinovirus (HRV) typing by semi-nested reverse-transcription polymerase chain reaction and sequencing of the 539 bp fragment of the *VP4/VP2* genes amplified from 297 clinical specimens. (**A**) Phylogenetic tree constructed using the maximum likelihood method in MEGA version 6.0 software to identify the genotypes of HRV in the enrolled clinical specimens. The orange branches represent HRV-A, the blue branches HRV-B, and the red branches HRV-C. (**B**) Numbers of patients infected with each genotype of HRV-A among the 148 patients. C: Numbers of patients infected with each genotype of HRV-B among the 20 patients. D: Numbers of patients infected with each genotype of HRV-C among the 129 patients

Clinical characteristics of patients with different species of HRV

Of the 297 patients, clinical information revealed that their median age was 2.98 ± 2.83 years. The proportions of male children infected with HRV-A, HRV-B, and HRV-C were 59.46%, 60.00%, and 62.02%, respectively (Table 1). Asthma is a chronic (long-term) respiratory disease with many phenotypes, usually characterized by chronic airway inflammation with symptoms including wheezing, difficult breathing, chest tightness and cough. Asthma exacerbation is featured with acute or sub-acute (sudden or gradual) worsening in symptoms of shortness of breath, cough, wheezing, or chest tightness and lung function, compared with the person's usual condition. For the limited number of cases diagnosed with asthma or asthma exacerbation, all these cases were grouped into cases diagnosed with asthma. Significant differences were observed in the history of allergy ($P = 0.004$), family history of asthma ($P = 0.001$), presence of older siblings ($P = 0.046$), incidence of wheezing symptoms ($P = 0.005$), and asthma diagnosis ($P = 0.001$) in the pairwise comparison of the clinical characteristics of patients infected with HRV-A, HRV-B, or HRV-C.

Among the patients infected with HRV-C, compared with those infected with HRV-A, more had history of allergy (28.68%, 37/129 VS 12.84%, 19/148) ($P = 0.001$) and family history of asthma (23.26%, 30/129 VS 7.43%, 11/148) ($P = 0.001$), whereas fewer had older sibling(s) (37.98%, 49/129 VS 52.70%, 78/148) ($P = 0.014$). More patients (50.39%, 65/129) infected with HRV-C had wheezing than those infected with HRV-A (33.11%) ($P = 0.005$) or HRV-B (25.00%) ($P = 0.034$). Additionally, among those infected with HRV-C, more patients were diagnosed with asthma (28.68%) as compared to those infected with HRV-A (12.16%) ($P = 0.001$).

CDHR3 rs6967330 genotyping and associated clinical features

By comparing the 179 nucleotides of the 382 bp CDHR3 fragment with NM_152750.5, 244 cases were identified as the GG genotype (82.15%, 244/297), 49 as the GA genotype (16.50%, 49/297), and four as the AA genotype (1.35%, 4/297). The minor allele frequency for CDHR3 rs6967330 was A, with a minor allele frequency of 0.096.

As only four cases were defined as the AA genotype, they were classified into the rs6967330-A group, along with the 49 cases of the GA genotype, for clinical

Table 1 Comparison of clinical characteristics of patients infected with human rhinovirus (HRV)-A, HRV-B, or HRV-C

	HRV-A (*n* = 148)	HRV-B (*n* = 20)	HRV-C (*n* = 129)	*P*-value	*P*-value (HRV-A vs. HRV-C)	*P*-value (HRV-B vs. HRV-C)
Basic characteristics						
Male, *n* (%)	88 (59.46)	12 (60.00)	80 (62.02)	0.908	0.664	0.863
Median Age, years	1.83	0.42	2.92	0.333	0.319	0.955
History of allergy, *n*	19	3	37	*0.004**	*0.001**	0.199
Family history of asthma, *n*	11	3	30	*0.001**	*0.001 **	0.408
Older sibling(s), *n*	78	10	49	*0.046**	*0.014 **	0.307
Cesarean delivery, *n*	69	9	59	0.984	0.883	0.951
Premature (< 37 weeks), *n*	24	2	13	0.292	0.134	0.991
Breastfed until 6 months, *n*	89	9	71	0.374	0.392	0.402
Symptoms						
Wheezing, *n*	49	5	65	*0.005**	*0.005 **	*0.034 **
Three concave signs, *n*	14	3	16	0.627	0.432	0.746
ARI disease severity						
Moderate, *n*	133	20	120	0.245	0.351	0.223
Severe, *n*	15	0	9			
LRTI						
Bronchitis, *n*	36	6	31	0.842	0.955	0.565
Pneumonia, *n*	67	11	65	0.569	0.395	0.701
Asthma, *n*	18	2	37	*0.001**	*0.001 **	0.077
Median duration of staying in hospital, days, median (IQR)	8 (5–13)	6 (4.5–16.5)	6 (4–11)	0.127	0.058	0.237
Oxygen supplement, *n*	22	5	23	0.483	0.505	0.445
PICU entry, *n*	42	8	30	0.249	0.332	0.110

* Significant difference ($P < 0.05$). n: numbers. IQR: interquartile range. PICU: Pediatric Intensive Care Unit. ARI: acute respiratory infection. LRTI: lower respiratory tract infection

Table 2 Clinical features of the rs6967330-A (GA + AA cadherin-related family member 3 [CDHR3] genotype) and rs6967330-G (GG CDHR3 genotype) groups

	CDHR3 rs6967330 genotypes		
	GG (*n* = 244)	**GA + AA (*n* = 53)**	***P*-value**
Median duration of staying in hospital, days, median (IQR)	7 (5–13)	6 (4.5–10)	0.314
Symptoms			
Wheezing, *n*	97	22	0.813
Three concave sign, *n*	23	10	*0.047**
LRTI			
Bronchitis, *n*	63	10	0.287
Pneumonia, *n*	97	25	0.32
Asthma, *n*	41	16	*0.025**
ARI disease severity			0.69
Moderate, *n*	225	48	
Severe, *n*	19	5	
Oxygen supplement, *n*	60	10	0.374
PICU entry, *n*	67	13	0.663

* Significant difference ($P < 0.05$). n: numbers. IQR: interquartile range. PICU: Pediatric Intensive Care Unit. ARI: acute respiratory infection. LRTI: lower respiratory tract infection

characteristics analysis. These were compared with the rs6967330-G group, which contained 244 cases of the GG genotype (Table 2). The results indicated that the incidence of three concave signs in the rs6967330-A group (18.87%, 10/53) was higher than that in the rs6967330-G group (9.43%, 23/244) ($P = 0.047$), which may explain the higher diagnosis of asthma in the rs6967330-A group (30.19%, 10/53) compared with that (16.80%, 41/244) in the rs6967330-G group ($P = 0.025$). No significant differences in wheezing were observed between the rs6967330-A and rs6967330-G groups.

Associations between HRV-C infection and CDHR3 rs6967330 genotypes

Three models were used to evaluate the association between HRV-C infection and CDHR3 genotypes (Table 3), using HRV-A and HRV-B infections as controls. In the dominant model, the OR value for the GA + AA group compared with the GG genotype group was1.91, with a 95% CI of 1.05 to 3.48 ($P = 0.033$), confirming the association of the rs6967330-A variant with HRV-C infection. In the recessive model, the OR value between the GG + GA group and the AA group was 1.31, with a 95% CI of 0.18 to 9.41 ($P = 0.999$), indicating no significant association of the rs6967330-G variant with HRV-C infection. In the additive model, the GG genotype group was compared with the AA genotype group, and no significant difference was found ($P = 0.999$).

Association analyses between CDHR3 rs6967330 and HRV-induced wheezing

Among patients with HRV-A infection, the incidence of wheezing in the GA + AA genotype population was 21.05% (4/19), whereas in the GG genotype population, it was 34.88% (45/129) (Fig. 2), with a ratio of 0.60 (21.05% vs. 34.88%). For patients infected with HRV-B, the ratio between the GA + AA and GG genotypes, as shown in Fig. 2C, was 1.00 (25.00% vs. 25.00%), and for those infected with HRV-C, the ratio was 1.17 (56.67% vs. 48.48%).

Discussion

HRV is one of the most prevalent viruses that cause respiratory tract illnesses in children [19]. In this study, respiratory virus screening revealed that the prevalence of EV/HRVs was 15.2% (1,616/10,608) among patients with ARI hospitalized in the Children's Hospital Affiliated with the Capital Institute of Pediatrics from September 2017 to March 2023.Most of these patients were positive for HRV (297 HRV; 27 EV), which falls within the reported global range of 11.0 to 40.6% in prior studies [20–22] and is similar to the 15.4% (108/702) reported in Beijing in a previous study [23].

Phylogenetic analysis revealed 91 genotypes of HRVs, including 47 HRV-A, 12 HRV-B, and 32 HRV-C strains. The predominant genotypes were A49 and A78 of HRV-A; B52 and B86 of HRV-B; and C53, C15, and C6 of HRV-C. The diversity of predominant HRV genotypes has been widely reported. In another study conducted in Beijing in 2019, the predominant genotypes were A78, A12, B4, and C53 [17]. In a report from Shanghai, 77 HRV genotypes were detected in children with severe acute respiratory infections, with A78, B70, B86, C2, C6, and C24 being the predominant genotypes [24]. In Amsterdam, 129 genotypes of HRVs were detected in inpatients and outpatients, with A12, A78, and C2 being the predominant HRVs [25]. Therefore, the prevalence of HRV genotypes may vary according to time and region. Of the 297 samples enrolled in this study, HRV-A was the dominant species (49.8%, 148/297), followed by HRV-C (43.4%,

Table 3 Associations of human rhinovirus (HRV)-C infection with different cadherin-related family member 3 (CDHR3) rs6967330 genotypes

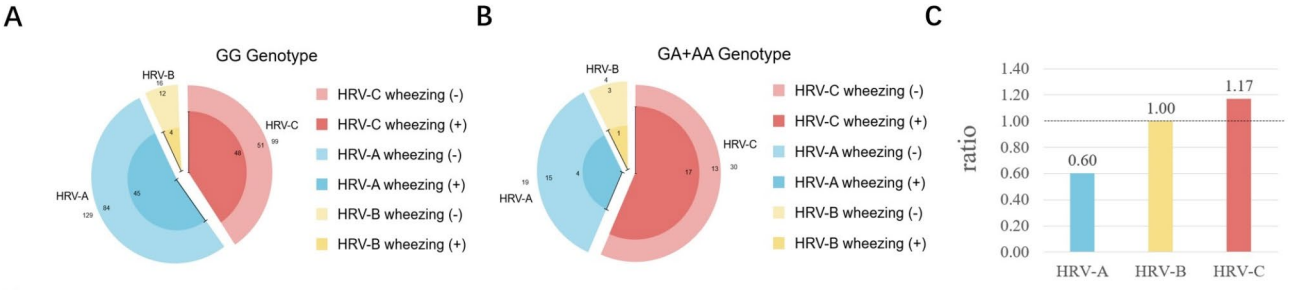
genotype	HRV-C		HRV-A + B		*P* value by Pearson χ^2	Dominant model		Recessive model		Additive model	
						(GG vs. GA + AA)		(GG + GA vs. AA)		(GG vs. AA)	
	n	%	*n*	%		OR (95%*CI*)	*P*-value	OR (95%*CI*)	*P*-value	OR (95%*CI*)	*P*-value
GG (*n* = 244)	99	40.6	145	59.4	0.101	***1.91 (1.05–3.48)***	***0.033****	1.31 (0.18–9.41)	0.999	1.47 (0.20-10.57)	0.999
GA (*n* = 49)	28	57.1	21	42.9							
AA (*n* = 4)	2	50	2	50							

* Significant difference (*P* < 0.05). *n*: numbers

129/297) and HRV-B (6.7%, 20/297). This suggests that HRV-A and HRV-C are more common in children than HRV-B.

HRV-induced wheezing is an important risk factor for asthma [10]. The relation between HRV species and wheezing was analyzed in this study, and more patients infected with HRV-C (50.39%, 65/129) experienced wheezing than those infected with HRV-A (33.11%, 49/148) ($P = 0.005$) or HRV-B (25.00%; 5/20) ($P = 0.034$). Of the 119 patients with wheezing, 54.62% (65/119) were infected with HRV-C, 41.18% (49/119) with HRV-A, and 4.20% (5/119) with HRV-B. It has been reported that the prevalence of HRV-C in children with acute wheezing, asthma, or lower respiratory infections is 26–68% [14, 26, 27], and HRV-C is the major cause of wheezing compared with HRV-A and HRV-B. Cox et al. also reported that HRV-C was the most common HRV species in children with wheezing [14], and Erkkola et al. found that HRV-C was more commonly associated with wheezing illnesses than HRV-A and HRV-B [19].

The CDHR3 rs6967330 SNP has been hypothesized to be correlated with HRV-induced wheezing [28]. The frequency of CDHR3 rs6967330-A in this study is 9.60%, which is consistent with the frequency of the A allele observed in Asian populations (7.37-11.53%) [28, 29]. A significant association ($P = 0.025$) was observed between asthma and the genetic variant CDHR3 rs6967330 in this study. In a genome-wide association study, Bonnelykke et al. reported that children with rs6967330-A showed an increased risk of an asthma phenotype characterized by recurrent respiratory infections with wheezing occurring in children between 2 and 6 years of age [11]. Moreover, rs6967330-A was also associated with a high risk of HRV-C infection, as evaluated by the dominant model (OR 1.91, 95% *CI* 1.05–3.48; $P = 0.033$) in this study. The allele rs6967330-A has been linked to increased binding and replication of HRV-C strains, as well as increased CDHR3 cell surface expression in HeLa cells. This indicates that rs6967330-A, and thereby surface overexpression of CDHR3, could be a specific risk factor for more severe HRV-C illnesses and, over time, an increased risk of asthma development [30]. These were consistent with observations in two birth cohorts that the CDHR3 rs6967330 variant modifies the frequency and severity of RV-C respiratory illness in early life [31]. Basnet et al. observed a tendency for increased secretion of CXCL10 and IFN-λ proteins in the asthma-risk genotype (G/A) nasal epithelial cells, and noted that rs6967330-A had greater effects on replication than binding, which indicates that the CDHR3-Y529 protein variant may promote both HRV-C binding and entry [32]. However, data on the varying effects of CDHR3 rs6967330 on wheezing induced by HRV-A, HRV-B, and HRV-C are limited. In this study, rs6967330-A was associated with a higher



D

HRV species	GG			GA+AA			ratio
	Total	Wheezing	Percent (%)	Total	Wheezing	Percent (%)	
HRV-A	129	45	34.88	19	4	21.05	0.60
HRV-B	16	4	25.00	4	1	25.00	1.00
HRV-C	99	48	48.48	30	17	56.67	**1.17**

Fig. 2 Incidence of wheezing and their ratios induced by different species of human rhinovirus (HRV) in GG genotype and GA + AA genotype patients, respectively. (**A**) The incidence of wheezing in the GG genotype patients. (**B**) The incidence of wheezing in the GA + AA genotype patients. (**C**) The ratio of the incidence of wheezing in GA + AA genotype populations to that of GG genotype populations. (**D**) Detailed data of the comparison between GG and GA + AA genotype populations associated with wheezing induced by different species of HRV

incidence of wheezing (56.67%) in patients with HRV-C infection compared to those with HRV-A (21.05%) or HRV-B (25.00%) infections.

However, this study had several limitations. Although the wheezing rate in the AA group was 75%, higher than the 39.75% in the GG group and the 40.82% in the GA group, only four cases were identified as the CDHR3 rs6967330 AA genotype, which was not sufficient for statistical analysis and was grouped together with the GA genotype. Therefore, more data should be collected to accurately reveal the role of the AA genotype. Second, only hospitalized patients with detailed clinical information were enrolled, resulting in a lack of data from patients with mild or very mild ARIs. In addition, immunological data should be collected to further evaluate the relation between the CDHR3 rs6967330 variant and HRV-C-induced wheezing in children.

In conclusion, the results of this study suggest that a high prevalence of HRVs was observed in children in Beijing from September 2017 to March 2023, and that HRVC may be one of the key factors in determining the association between HRVs and wheezing. The rs6967330-A allele was related to a high risk of HRV-C infection, as confirmed by the dominant model, and cases with rs6967330-A were more likely to have HRV-induced wheezing. This helps in understanding the relation between the CDHR3 rs6967330 SNP and HRV-induced wheezing in Chinese children from the population of China.

Abbreviations

CDHR3	Cadherin-related family member 3
HRV	Human rhinovirus
EV	Enterovirus
ICAM-1	Intercellular adhesion molecule 1
LDLR	Low-density lipoprotein receptor
ARIs	Acute respiratory tract infections
Flu	Influenza virus
RSV	Respiratory syncytial virus
HPIV	Human Parainfluenza Virus
ADV	Adenovirus
HMPV	Human metapneumovirus
HCOVs	Human coronaviruses
HBoV	Human bocavirus
Mp	*Mycoplasma pneumoniae*
Cp	*Chlamydia pneumoniae*
PCR	Polymerase chain reaction
CEMP	Capillary electrophoresis-based multiplex polymerase chain reaction
IQR	Interquartile range
PICU	Pediatric Intensive Care Unit
LRTI	Lower respiratory tract infection

Acknowledgements
We would like to acknowledge the staff of Affiliated Children's Hospital, Capital Institute of Pediatrics for collecting the clinical samples, and the children and their parents who supported our research work. We would like to thank Editage (http://www.editage.cn) for language editing.

Author contributions
Conceptualization, ZLQ; methodology, FHHY, DR, SY, YY, CDM, ZYT, and GQ; software, FHHY and DR; validation, FHHY and ZLQ; formal analysis, FHHY; resources, SY, YY, CDM, ZYT, and GQ; data curation, FHHY, DR and ZRN; writing—original draft preparation, FHHY; writing—review and editing, DR and ZLQ; project administration, ZLQ; funding acquisition, ZLQ. All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by the High-Level Technical Talent Construction Project of the Beijing Municipal Health Commission (Discipline Leader – 02–20), Beijing Finance Bureau (CIP2024-0040), Clinical Testing Research Fund of the Capital Institute of Pediatrics (CTR-002), and Postdoctoral Research Fund of Chaoyang District, Beijing, China, in 2023.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The original study was approved by the Ethics Committee of the Capital Institute of Pediatrics (Approval number: SHERLLM2022027) and was a retrospective study. The ethical committee had voted that written informed consents were not required for EV/HRV positive specimens collected retrospectively.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 October 2024 / Accepted: 28 January 2025
Published online: 06 February 2025

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