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TURUN YLIOPISTO
Lääketieteellinen tiedekunta

HOMIL, KIARA: Rinovirus C:n neutraloivat vasta-ainereaktiot sekä infektioon liittyvät iästä riippuvaiset tekijät

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Rinovirukset (RV) ovat yleisiä hengitystieinfektioiden aiheuttajia. Nuorilla lapsilla rinovirusinfektiot voivat vaatia jopa tehohoitoa. Rinoviruslajeja on kolme (A, B ja C) ja näiden alatyyppejä on 160. Lasten rinovirusinfektioista yleisimmät ovat RV-A ja RV-C. Alttiuteen sairastaa RV-C infektio vaikuttavat useat tekijät, joista yksi esimerkki on CDHR3-geenin polymorfismi (rs6967330). Tutkimuksissa on todettu RV-C:n esiintyvyyden olevan lapsuudessa suurempaa kuin aikuisuudessa suhteessa RV-A:n esiintyvyyteen. Rinovirusinfektiot aiheuttavat spesifisiä vasta-ainereaktioita, joiden myötä uusintainfektion riski pienenee. RV-C:n suhteen neutraloivien vasta-ainereaktioiden muodostumista ei ole pystytty aiemmin tutkimaan puutteellisten in vitro -järjestelmien vuoksi. Tämän tutkimuksen tavoitteena on tunnistaa miten ikä ja muut yksilötason tekijät ovat yhteydessä RV-C-infektion sairastuvuuteen sekä taudin vaikeuteen. Lisäksi tutkitaan, liittyykö RV-C-infektioiden vähäisempi esiintyvyys iän myötä lisääntyneisiin neutraloiviin vasta-ainereaktioihin RV-C:tä vastaan.

Iän ja RV-C-infektion välistä yhteyttä tutkittiin COAST syntymäkohorttitutkimuksen pitkittäisdatasta. RV-A ja RV-C-spesifisiä neutraloivia vasta-aineita määrittämään käytettiin uuden laista PCR-pohjaista menetelmää. Lisäksi dataan on yhdistetty lisäksi 14 tutkimuskohorttia USA:sta, Suomesta ja Australiasta. Tutkimuksessa on käytetty sekamallista logistista regressioanalyysia tunnistamaan tekijöitä, jotka liittyvät RV-C: suhteellisen osuuden tunnistamiseen RV-A:sta.

COAST-kohortissa RV-A ja RV-C olivat yhtä yleisiä pikkulapsilla, mutta vanhemmilla lapsilla RV-C:n esiintyvyys oli suurempaa kuin RV-A:n esiintyvyys sekä ylähengitysteiden yhteydessä että jo ennalta suunnitelluilla seurantakäynneillä (p<0.001). Kahden vuoden iässä RV-A- tai RV-C-lajien neutraloivien vasta-aineiden esiintyvyys oli matala (5–27 %), mutta 16 ikävuoteen mennessä RV-C-seropositiivisuutta esiintyi enemmän (78% vs. 18%, p<0.0001). Yhdistelmäanalyysissä RV-C RV-A suhde sairauden yhteydessä oli tilastollisesti merkitsevästi riippuvainen iästä, CDHR3-genotyypistä sekä uloshengityksen vinkunaa aiheuttavista sairauksista (p<0.05). Lisäksi tietyt RV-tyypit (esim. C2, C11, A78 ja A12) olivat virulentimpia sekä yleisimmin esiintyviä ajan mittaan.

Tutkimuksen myötä tiedetään enemmän RV-tyyppien esiintyvyydestä ja vasta-ainereaktioista. Parempi ymmärrys iän ja genetiikan suhteen riskissä olevista potilaista voi ohjata rokotteiden tai muiden uusien hoitojen kehitystä.

Avainsanat: rinovirus, genetiikka, CDHR3, epidemiolog

Enhanced Neutralizing Antibody Responses to Rhinovirus C and Age-Dependent Patterns of Infection

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Running title: Determinants of rhinovirus C illnesses in children

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10.11 Pediatrics: Respiratory Infections

ABSTRACT

Rationale: Rhinovirus C (RV-C) can cause asymptomatic infection and respiratory illnesses ranging from the common cold to severe wheezing.

Objectives: To identify how age and other individual-level factors are associated with susceptibility to RV-C illnesses.

Methods: Longitudinal data from the Childhood Origins of ASThma (COAST) birth cohort study were analyzed to determine relationships between age and RV-C infections. Neutralizing antibodies specific for rhinovirus A (RV-A) and RV-C (3 types each) were determined using a novel polymerase chain reaction-based assay. We pooled data from 14 study cohorts in the United States, Finland, and Australia and used mixed-effects logistic regression to identify factors related to the proportion of RV-C versus RV-A detection.

Measurements and Main Results: In COAST, RV-A and RV-C infections were similarly common in infancy, while RV-C was detected much less often than RV-A during both respiratory illnesses and scheduled surveillance visits (p<0.001, chi-square) in older children. The prevalence of neutralizing antibodies to RV-A or RV-C types was low (5%-27%) at age 2 years, but by age 16, RV-C seropositivity was more prevalent (78% vs. 18% for RV-A, p<0.0001). In the pooled analysis, the RV-C to RV-A detection ratio during illnesses was significantly related to age (p<0.0001), *CDHR3* genotype (p<0.05), and wheezing illnesses (p<0.05). Furthermore, certain RV types (e.g., C2, C11, A78, A12) were consistently more virulent and prevalent over time.

Discussion: Knowledge of prevalent RV types, antibody responses, and populations at risk based on age and genetics may guide the development of vaccines or other novel therapies against this important respiratory pathogen.

Abstract: 245 words

INTRODUCTION

Rhinoviruses (RVs) are common causes of upper respiratory tract illnesses and exacerbations of chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease [COPD] (1). In young children, RV infections also cause lower respiratory illnesses that can require hospitalization and even intensive care (2). There are three species of RVs (A, B, and C) based on genetic homology that are further subdivided into more than 160 types (3). Any RV can cause mild or asymptomatic respiratory illness; however, RV-B is less likely to cause severe illnesses or exacerbations of asthma (4, 5). Infections with RV-A and RV-C are common during childhood, but the relative contribution of these viruses to severe respiratory illnesses is controversial. Some studies have shown that RV-C infections are more likely than RV-A infections to cause wheezing illnesses and asthma exacerbations in children (2, 6-8), but other studies have found no species-specific associations (5, 9-11).

Several factors modify susceptibility to RV-C infections. A coding polymorphism (rs6967330) in the gene for CDHR3, which serves as the RV-C receptor, is associated with greater expression of this protein on the surface of cells and increased susceptibility to both RV-C illnesses and childhood asthma (12, 13). In addition, there is evidence that age might also affect the relative frequency of RV-A versus RV-C infections and illnesses. For example, RV-C was detected more often than RV-A in some studies of young children with respiratory illnesses (14-16), while some studies in adults have reported the opposite (17-19). The reasons for these observations are not fully understood but could be related to a greater naturally acquired immunity to RV-C over time. Infections with RV induce type-specific antibody responses that reduce the risk of reinfections. Natural infections with RV-A and RV-B induce neutralizing antibody

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(nAb) responses that protect against reinfection and persist for at least 2-4 years (20-24). Neutralizing antibody responses to RV-C have not previously been analyzed due to the lack of a suitable *in vitro* system to assay infectivity.

The primary goals of this study were to test the hypothesis that increasing age is associated with reduced frequency and severity of RV-C infections and illnesses, and to determine whether age-related reductions in RV-C illness frequency correspond with increased nAb responses to RV-C infections. We also sought to identify other personal factors that are associated with RV species susceptibility, and to describe relationships among RV species, type, and illness. To accomplish these objectives, we analyzed longitudinal sets of nasal and plasma samples from children from birth to age 18-19 years in the Childhood Origins of ASThma (COAST) birth cohort, which included high-risk children born to parents with allergy and/or asthma (25). In addition, we conducted a multicenter study by pooling data from 14 different studies across numerous cities located in the United States, Finland, and Australia. Some of the results of these studies have been previously reported in the form of an abstract (26).

METHODS

Study Subjects and Study Design

<u>COAST birth cohort study</u>. 289 children from the Madison, Wisconsin, area were enrolled in the COAST study at birth, 259 were followed prospectively to age 6 years and 210 to age 18-19 years, and additional children with asthma were enrolled more recently (25, 27). Families were asked to contact the study center each time the child had respiratory symptoms to enable collection of a

nasal mucus sample for viral diagnostics. Nasal samples were also collected at scheduled study visits (2, 4, 6, 9, 12, 18, and 24 months and then annually), and respiratory illness symptoms were recorded if present. Plasma samples were collected during these annual visits.

Studies included in the pooled analysis. Investigators from 14 different cohort studies that had collected nasal mucus specimens from children during periods of illness and/or health (**Table E1**) contributed data to the pooled analysis. Five of these cohorts are participating in the Environmental Influences on Child Health Outcomes (ECHO) consortium. For each of these cohorts, real-time PCR and partial sequencing was used to identify RV species and type as previously described (28, 29). Each of these studies was approved by the local Human Research Ethics Committees, and participants provided informed consent. In addition to the viral diagnostics, 11 additional variables were included in the analysis. All cohorts had data on age, sex, race, and season of collection; nine cohorts provided data on aeroallergen sensitization (skin test or specific IgE measurement), asthma (parent report of asthma diagnosed by a health care provider), number of older siblings, history of breastfeeding, exposure to daycare, and illness type; and 6 of the 14 cohorts provided genotypes for the *CDHR3* variant rs6967330. These variables were harmonized for the pooled analysis (see online data supplement).

RV Neutralization Assay

RV-A16, -A36, -C2, -C15 and -C41 were clinical isolates cloned in plasmid vectors and produced by reverse genetics methods in WisL cells (human embryonic lung fibroblasts) (30-32); RV-A7 was isolated from nasal secretions and propagated in HeLa cells (31). HeLa-E8–adapted variants of RV-C isolates

(33), possessing K41 mutation in 3A protein, were used for optimal replication in this cell line.

Neutralizing antibodies specific for RV-A and RV-C types were measured using a novel qPCR-based assay. Briefly, RV isolates were preincubated with serial two-fold dilutions of plasma samples, and this mixture was used to inoculate HeLa-E8 cells (34) that were engineered to express CDHR3 (RV-C receptor). Viral replication (progeny yields at 72 hours post-infection) was measured by qPCR. Neutralizing antibody titers (IC_{50}) were calculated by sigmoidal dose-response nonlinear fit analysis of virus replication curves. For the qualitative assay to identify the presence or absence of nAbs, the same infection procedure was used except that the number of plasma serial dilutions was limited to 3 (2-8-fold) (see online data supplement for additional details).

Statistical Analyses

Mixed-effects logistic regression was used to estimate the odds of infection with RV-C alone to infection with RV-A as a function of age (modeled using a natural cubic spline with 4 degrees of freedom). The model included subject as a random effect and age, race, and cohort as fixed effects.

Mixed-effects logistic regression models were used to assess the effects of other covariates (asthma history, allergy history, daycare exposure, number of older siblings, breastfeeding history, race, season of collection, CDHR3 rs6967330 asthma risk genotype, and illness type [**Table E2**]) on the RV-C to RV-A ratio. Additional details are provided in the online supplement.

RESULTS

RV-A versus RV-C Infections and Illnesses in the COAST Study

In the COAST birth cohort study, we detected and typed RV clinical isolates in surveillance nasal samples collected during scheduled clinic visits (with or without respiratory symptoms) and in samples obtained during times of acute respiratory illnesses (symptom score of ≥ 5 (35)) during four age intervals: ages 0-5 years, 6-12 years, and 13-18 years. Partial sequence analysis confirmed detection of about 94% and 98% of known RV-A and RV-C types, respectively. While RV-A and RV-C infections and illnesses were similarly common in the first 3 years of life, in older children RV-C was detected significantly less often than RV-A during both respiratory illnesses and well visits (p < 0.001, chi-square, **Figure 1)**.

RV Neutralizing Antibody Responses

Both RV-A and RV-C isolates can infect transduced HeLa-E8 cells, which naturally express ICAM-1 and are engineered to express CDHR3 (33). Due to the absence of cytopathic effects (CPE) after infection with "wild-type" RV-C clinical isolates *in vitro*, we developed a novel, qPCR-based RV neutralization assay in HeLa-E8 cells. We then validated the assay by testing pre- and post-infection plasma samples from an RV-A16 experimental inoculation study (36). The nAb titers to RV-A16 determined by the assay correlated well (r_s=0.83, p=0.006) with those obtained by the traditional infectivity assay (endpoint dilution) that is based on CPE in WI-38 cells (**Figure E1**).

We next analyzed nAb responses to RV-A and RV-C types in plasma samples from three age groups (2, 10, and 16 years, same subjects) of COAST study

participants (*n*=20) by qualitative nAb assay. We selected three clinical isolates representing RV-A and RV-C species to use in the neutralization assay. Two isolates from each species were phylogenetically related (A7 and A36, and C15 and C41), and the remaining ones (A16 and C2) were more distant from the first two isolates (**Figure E2**). The results demonstrated marked species-specific differences in the development of nAb responses (**Figure 2**). While nAbs to these RV-A and RV-C types were uncommon at age 2 years (5-27%), by age 10 and 16 years RV-C seropositivity was 70% and 78% compared to only 25% and 18% for RV-A, respectively (p<0.0001). RV-C–specific nAbs were significantly more prevalent compared to nAbs for RV-A in each of the tested age groups (ORs 3.9 [95% CI 1.6 to 9.5] at age 2, 4.0 [95% CI 2.0 to 7.7] at age 10, and 7.1 [95% CI 3.8 to 13.3] at age 16). There was no correlation between the detection frequency of the selected RV-A or RV-C types and the total number of positive nAb responses to them (**Figure E3).**

Neutralizing antibody detected at one timepoint was very likely to persist from one age to the next (**Figure 2**, $p<10^{-7}$, GEE logistic regression model). For RV-A types, positive antibodies persisted from age 2 to age 10 years in 67% (2/3) children and from age 10 to age 16 in 40% (6/15) children. For RV-C types, the corresponding numbers were 94% (15/16) and 90% (38/42) respectively. RV species was not significantly associated with persistence of antibody (p=0.58, Wald test for interaction), although this analysis was underpowered due to the low number of positive results for RV-A types.

Multicenter Analysis of RV-A and RV-C Infections

<u>Results of viral diagnostics</u>. The 14 study cohorts collected 17,664 samples of nasal mucus from study participants and detected at least one RV in 10,185

samples (**Table 1**). Of the 10,185 pooled samples, 6,643 were collected during illnesses and 3,542 were collected during periods of health (e.g., at well-child or routine study visits). Similar numbers of specimens tested positive for RV-A and RV-C. RV-B was detected more often in samples from asymptomatic children compared to samples obtained during illness (857/3,542 [24%] vs. 589/6,643 [9%], two proportion z-test $p < 0.001$).

We detected 178 types, including several provisionally assigned types (designated "pat"), in the pooled analysis. The median detection rate for any given type was 0.45% (range 0.0% to 2.7%). The most frequently detected RV types in illness samples were A78, C02, C11, A12 and A101 in descending order, while 33 of the RV types were detected in <0.1% (6 or fewer) of illness samples (**Table E3**). The rate of detection for the frequently detected types was consistently high over the 22-year sampling period (**Figure E4**). In samples from asymptomatic children, B17, B6, B103, A21, and B91 were detected most frequently in descending order (**Table E3**). We also compared the frequency of types detected during illnesses to that of types detected during healthy periods (**Figure 3**). Twenty-two types (4 A, 18 C) were significantly more likely to be detected during illnesses, while 23 types (4 A, 18 B, 1 C) were more often detected in healthy children.

Analysis of factors related to RV-A and RV-C infections. Consistent with findings in COAST, increasing age was strongly correlated with the ratio of RV-C to RV-A infections in the pooled data (**Figure 4 and Figure E5**). RV-C infections were more prevalent in young children up until about age 5 years, followed by a steady decline in the ratio such that RV-A infections predominated in adolescents. In order to evaluate other potential predictors of the RV-C to RV-A ratio, we performed logistic regression on each of the covariates

separately while adjusting for age and participant (some studies included multiple samples per participant). Because there was significant variety among the cohort studies in age and covariates measured, the analyses were performed in discrete age intervals (**Figure 5**). Two covariates were significant predictors of increased RV-C infections compared to RV-A infections. The *CDHR3* rs6967330 asthma risk allele (A) significantly increased the risk for RV-C illness (**Figure 6A**). In addition, the RV-C to RV-A ratio was related to the presence of lower respiratory illness (LRI) with wheezing but not LRI without wheezing (**Figures 6B and 6C**).

Season of collection was also related to the RV-C to RV-A ratio; children were more likely to be infected with RV-C versus RV-A in fall and winter compared to spring for 5-6 of the 12 age windows (**Figure E6**). Other personal factors, including race, aeroallergen sensitization, asthma history, history of breastfeeding, exposure to daycare, and number of older siblings, were not consistently related to the RV-C to RV-A ratio (**Figure E7** and data not shown).

DISCUSSION

RV infections are important causes of LRI and wheezing during infancy and in children and adults with asthma and other chronic respiratory diseases, and understanding the contributions of different viruses is needed to guide efforts to develop specific treatments. While there is consensus that RV-B is less likely to cause severe respiratory illnesses, the relative roles of RV-A and RV-C have been uncertain. In the COAST birth cohort study, RV-A and RV-C infections and illnesses were both common in the first 3 years of life, but RV-C was less often detected than RV-A in older children during both respiratory illnesses and scheduled surveillance visits. Analysis of nAb responses to selected RV-A and

RV-C types in the COAST cohort demonstrated progressively greater frequency of protective responses to RV-C over time, suggesting that species-specific differences in the development of nAb responses may contribute to the frequency of illnesses with RV-C versus RV-A. Pooling data from 14 cohorts confirmed that RV-C illnesses are more prevalent in preschoolers. These data also provide evidence that RV-C (compared to RV-A) is specifically associated with wheezing LRI, and confirm that a single nucleotide polymorphism (rs6967330) in the *CDHR3* gene promotes RV-C infections and illnesses throughout childhood.

Previous studies have demonstrated that RV infection rates peak during infancy and gradually decline with age (37). In parallel, nAb responses to RV-A and RV-B types generally increase during childhood and early adult years and plateau with nAb to approximately 40% of RV types after 35-40 years. RV infections continue at a lower rate throughout adult life, suggesting that protective immunity is either not fully developed to each virus type (low nAb titers) or is not maintained (38). Our new data provide definitive evidence that, in comparison to RV-A, young children have similar rates of RV-C infections but proportionally higher rates of RV-C illnesses, and that increasing age is associated with steady reductions in RV-C detection rates during sickness or health. While some previous studies have detected this trend, this pooled data analysis estimated the odds of detecting RV-C compared to RV-A over a broad age range and in a large and diverse study population with over 10,000 nasal samples typed.

The significant age-related decline in the RV-C to RV-A ratio during RV infections was associated with marked increases in titers of nAb specific for RV-C types. Notably, this was true for RV-C types that were commonly detected

(RV-C2 and RV-C15), and also for RV-C types that were less commonly detected (RV-C41). While this finding needs to be confirmed by testing for nAb responses to other RV-C types, this raises the possibility that RV-C infections induce antibody responses that can cross-neutralize other RV-C types.

Prior studies of antibody responses to RV-C measured IgG antibody binding to synthetic viral peptides by ELISA. Interestingly, antibody responses specific for RV-A and RV-C peptides were found to be highly cross-reactive (39-41). In addition, serum IgG1 antibody titers to RV-C were significantly lower than those to RV-A and RV-B suggesting that humoral immune response to RV-C may be muted (40, 41). In contrast, a recent study utilizing an RV peptide array that included representative capsid protein sequences from all three species reported higher antibody levels to RV-A and RV-C compared to RV-B in both healthy and asthmatic children (42). The authors also reported that asthma was associated with higher antibody levels that did not seem to confer enhanced protection. Antibody responses to viral capsid proteins (VP1) of RV-A and RV-C were also associated with severity of symptoms of previous respiratory illnesses (39, 43).

Previous studies of antibody responses to RV-C did not include measurement of nAbs (40-42). Infectivity assays in HeLa-H1 cells or lung fibroblasts, which were used for RV-A and RV-B neutralization (44), are not suitable for RV-C because these cells lack CDHR3 expression required for viral binding and replication (34, 45, 46). We developed a novel qPCR based neutralization assay for both RV-A and RV-C to analyze nAbs in the absence of visible cytopathic effects; the results of the new assay correlated closely with those of a standard CPE-based assay (36). Our findings demonstrate that natural humoral immunity to RV-C develops at an accelerated rate compared to RV-A.

These findings suggest that RV-C types are more immunogenic compared to RV-A, or are more likely to induce cross-neutralizing antibody. Neutralizing antigenic sites in RV-A and RV-B are located at the highest points of the capsid surface in hypervariable regions of VP1, VP2 and VP3 (47-50). Little is known about neutralizing sites in RV-C, which has unique structural features and receptor interactions (51, 52). The first extracellular domain (EC1) of CDHR3 binds to residues located in a shallow groove formed primarily by VP2 and VP3, with some contribution from VP1 (52). The accessibility of the binding site suggests that certain epitopes in this region could elicit nAbs that block receptor binding. In addition, our data confirm that many RV-C types are more virulent compared to other RV types, and previous studies have demonstrated that viremia (detection of viral RNA in serum specimens) is much more common with RV-C infections compared to infections with other RV species (53, 54). Virulence of RV-C types could also be enhanced by a muted T helper-1 response to RV-C versus RV-A, which could impair antiviral responses (55). Because symptom severity and peak viral titer during acute infections are positively related to induction of RV-specific nAb responses (26, 56), the increased virulence of RV-C could contribute to the greater prevalence and perhaps duration of nAb responses.

The pooled analysis provided descriptive information on over 10,000 samples that tested positive for RV. Several RV types (RV-C2, RV-A78, RV-C11, and RV-A12) were frequently detected and found at high rates throughout most of the years during the two decade period of analysis. Similarly, a recent meta-analysis found that RV-A12, RV-A78, RV-C15, and RV-C2 were reported in more published studies than any other RV-A or RV-C types (57). The consistency of these findings suggests that there are certain RV types such as

RV-C2 and RV-A78 that are either more virulent or more readily transmitted. Notably, the affinity of CDHR3 binding for four RV-C types was recently estimated by measuring how multiple single mutations in a soluble EC1 domain affected its ability to inhibit viral replication (52). The results indicate that CDHR3 binding is tightest for C02, and progressively less for C15, C41, and C45. This estimate of binding affinity corresponded with the frequency with which these viruses were detected in our pooled analysis of illness samples, suggesting that the strength of viral binding to CDHR3 could contribute to virulence and/or transmission. Direct measurements of binding of RV-C types to CDHR3 are needed to test this hypothesis.

This study has several strengths and some limitations to consider when interpreting the results. Longitudinal sampling in COAST from birth to adolescence allowed assessments of relative contributions of RV types across childhood within the same children. The pooled sample analysis provided a large sample and included studies that were diverse in terms of geography, climates, years of sampling, ethnicity, and home and community environments. Several studies collected samples from children with and without illness, which enabled analysis across illness and health. Viral typing was performed by partial sequencing using the same methods. Limitations included the need to harmonize variables such as illness type that were defined differently by each study cohort, which could have led to reduced precision. Several of the studies collected samples and information over a relatively short age range, which necessitated analyzing risk factors for RV-C and RV-A infections in age windows so that age would not be confounded with study site. Finally, we measured neutralizing antibody responses to 6 RV types, and data for additional RV types would be helpful to confirm our findings and to identify viral

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characteristics besides species that can influence the development of neutralizing antibody responses.

In conclusion, this study and others identify young children and those who are genetically predisposed as populations at high-risk for RV-C wheezing illnesses, who could potentially benefit from preventive approaches such as vaccination. Obstacles to the development of RV-specific vaccines have included the large number of RV types and a paucity of information about RV-C epidemiology and protective serologic responses. To overcome these limitations, this study has identified key subsets of RV-C types that are more prevalent and more virulent, and have demonstrated that RV-C infections elicit potent and durable nAb responses. Therefore, building on these data it may now be more feasible to develop an RV-C vaccine for use early in life to prevent rhinovirus-provoked wheezing illnesses and reduce a major health burden in high-risk children.

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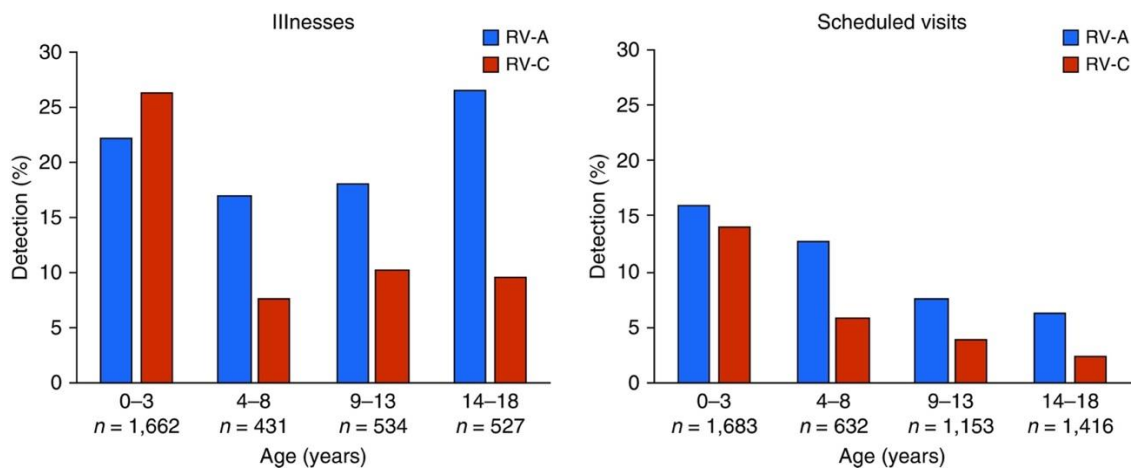
Figure 1. Relationship of age to frequency of rhinovirus (RV)-C and RV-A detection during illnesses in the COAST birth cohort. RV types and species (A, B, and C) were determined in nasal samples by partial sequencing. Detection rates (%) of RV-A and RV-C at different ages are shown as bar plots. Odds ratios (ORs) refer to odds of RV-C relative to RV-A at older ages compared to younger ages. Groups were compared using Fisher's exact test. P values of < 0.05 were considered to indicate statistical significance. Illnesses: OR = 0.31 (95% CI 0.21-0.45), $p < 0.0001$; scheduled visits: OR = 0.31 (0.14-0.64), $p = 0.0006$. N represents the number of samples analyzed for each age category.
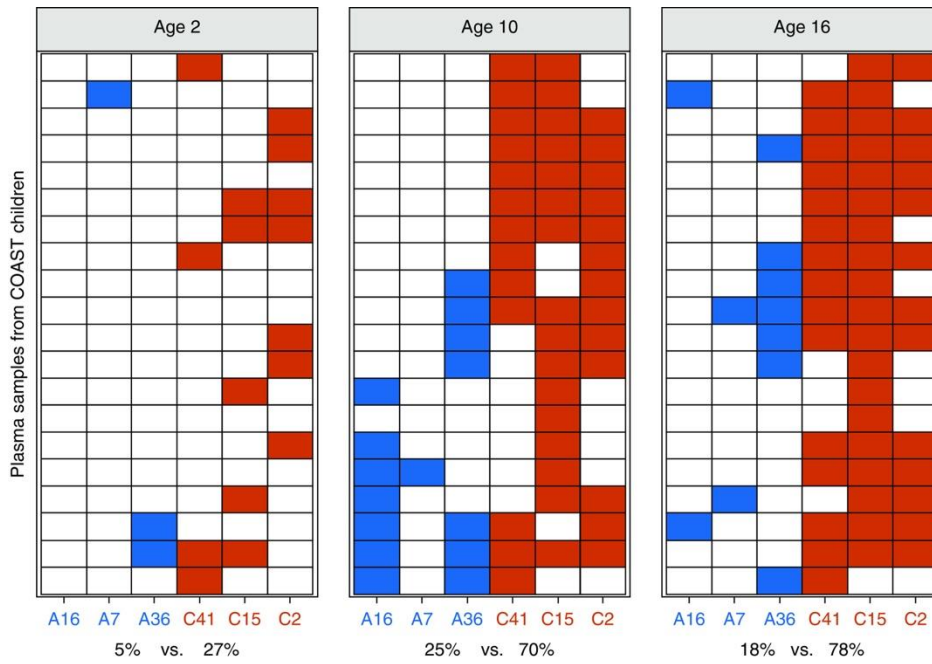
Figure 2. Neutralizing antibody (nAb) responses to selected RV-A and RV-C types in the COAST study children. Heatmaps show the presence of nAbs to indicated RV-A (blue) and RV-C (red) types in plasma (ages 2, 10, and 16 years, each row represents serial sampling from the same subjects) from COAST study participants (n = 20). Odds ratios (ORs) refer to odds of finding nAbs to RV-C relative to RV-A at different ages. Age 2: OR = 3.9 (95% CI 1.6 to 9.5); age 10: OR = 4.0 (95% CI 2.0 to 7.7); age 16: OR = 7.1 (95% CI 3.8 to 13.3). The differences in nAb responses to RV-A versus RV-C were highly significant at all ages tested. (p < 0.0001, GEE logistic regression).
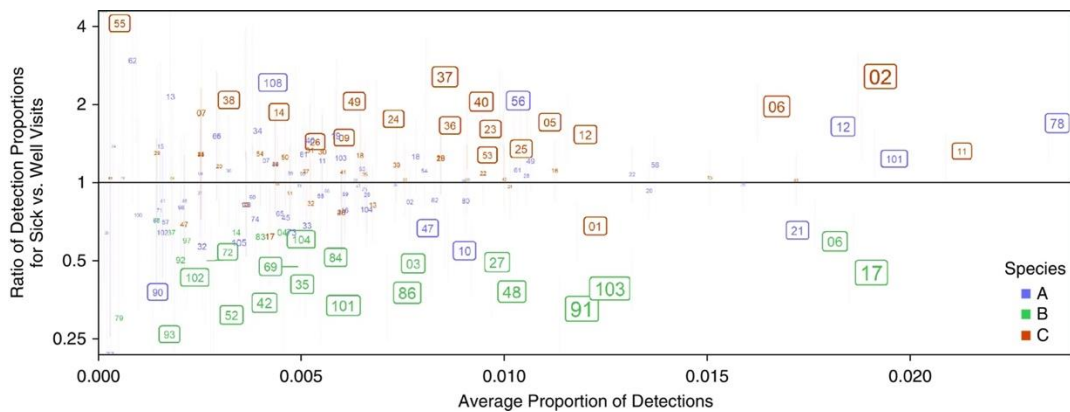

Figure 3. Frequency of detection for each RV type and ratio of detection proportions for sick versus well visits in the pooled dataset. The species is

color-coded, and individual types are numbered within each species. The size of the plotting characters is proportional to the negative logarithm of the p value, and plotting characters in boxes are significant at the 5% level (unadjusted for multiple comparisons). Error bars represent 95% confidence intervals for the ratio of detection proportions.
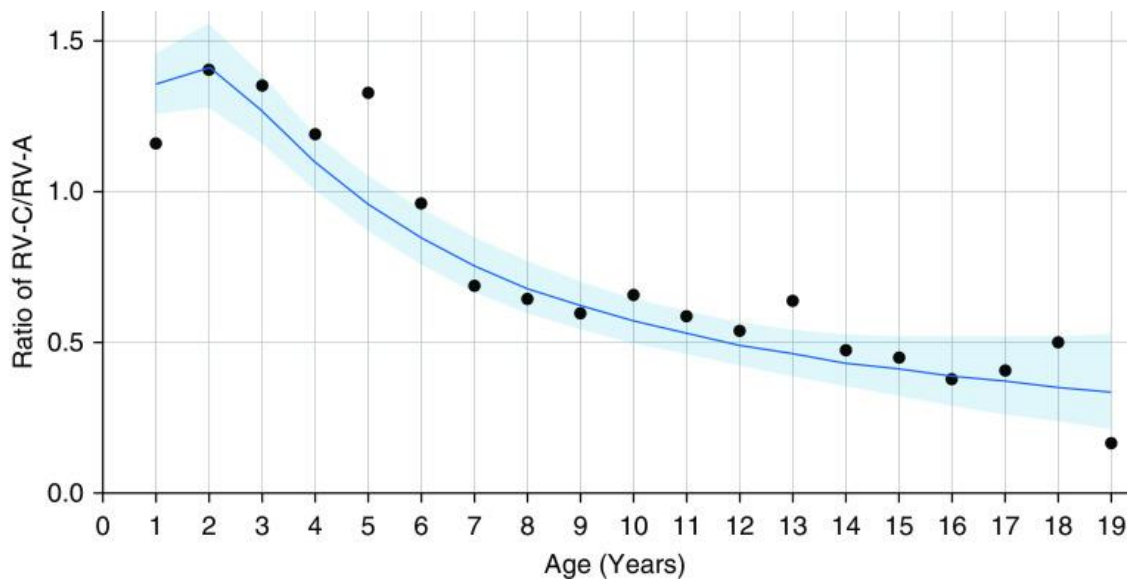
Figure 4. Ratio of RV-C to RV-A in illness samples from the pooled dataset with respect to age. Individual points represent the RV-C to RV-A detection ratio for each year of age. The line and shaded area represent the logistic regression model fitted to the sample-level data and the associated 95% confidence intervals, respectively.
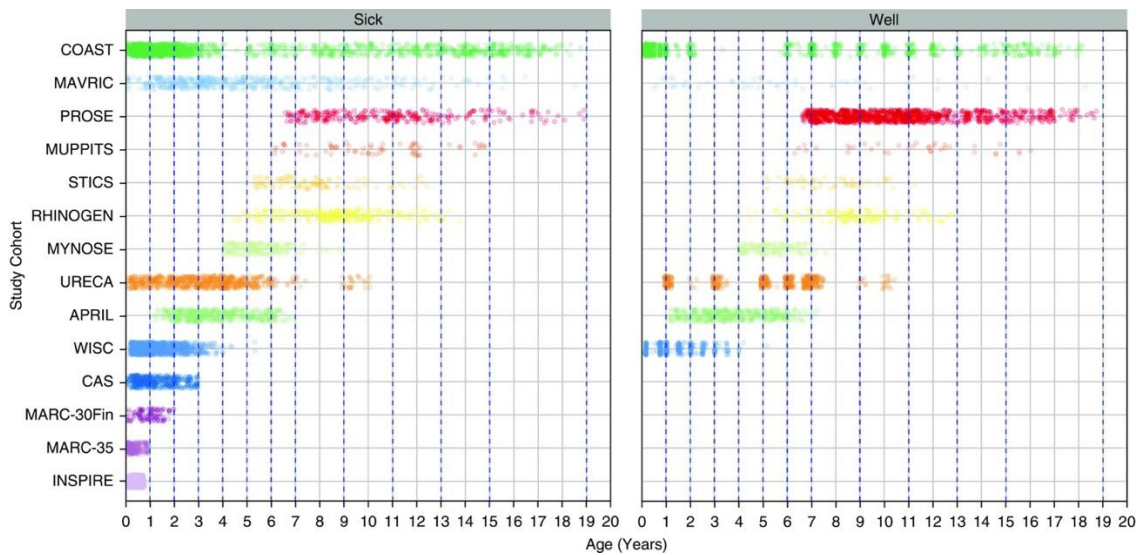
Figure 5. Age distribution of 14 study cohorts included in pooled sample analysis. Symbols represent samples included in this analysis by cohort and the age of sample acquisition. Age windows were used in the multivariable analyses, and start and end points were assigned based on ages at which a study or studies started contributing samples or stopped contributing samples to the overall pool.
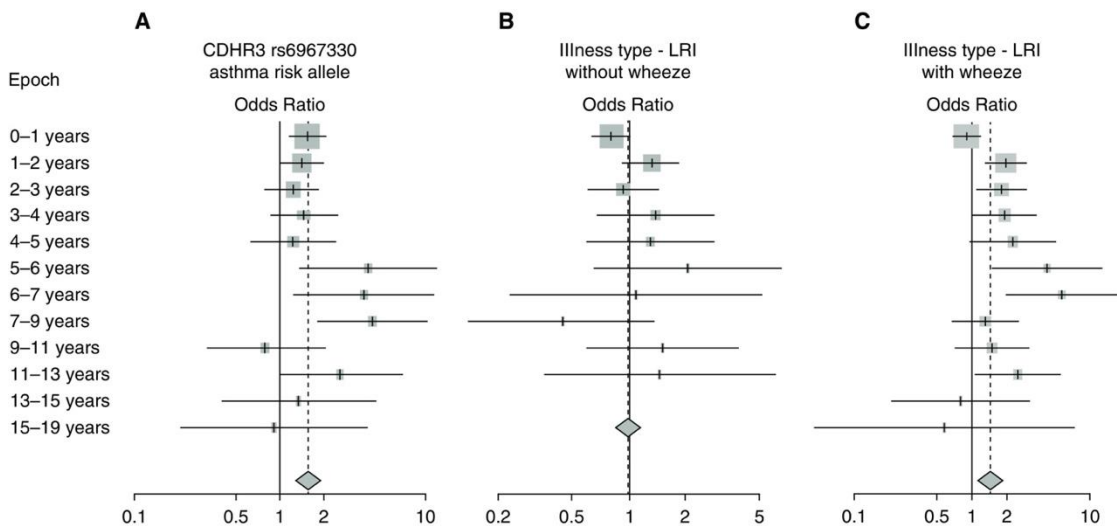
Figure 6. Analysis of covariates related to the RV-C to RV-A ratio. Odds ratios (logistic regression) were calculated to compare the RV-C/RV-A ratio to the *CDHR3* asthma risk allele (panel A, rs6967330$_{G\rightarrow A}$, 1 or 2 risk alleles compared to 0) and to illness type (B, LRI vs. URI; C, wheezing LRI vs. URI). Odds ratios for each age interval were calculated, along with an overall odds ratio including

all ages. Whisker bars represent 95% confidence intervals. Abbreviation: wLRI,

LRI with wheezing.

Table 1. Viruses detected in rhinovirus (RV)-positive samples.

Virus	Sample Type		
	All	**Sick**	**Well**
Solo RV-A	4379	2890	1489
Solo RV-B	1197	453	744
Solo RV-C	4235	3067	1168
Mixed RV	374	233	141
Total	**10185**	**6643**	**3542**