Our data are in line with the reported observations in patients with severe inborn or acquired T-cell deficiencies. In patients with recombination-activating gene *(RAG)* and non-homologous end joining gene *(ARTEMIS)* deficiencies, an increased proportion of NK cells had the $CD56^{bright}$ phenotype, and these cells degranulated strongly upon coculture with K562.[7] In HIV patients, an inverse correlation between $CD4^+$ T-cell numbers and $CD56^{bright}$ NK cells was observed. These cells also had a reduced expression of CCR7 and an increased expression of granzyme B.[10]

Together with the changes in the NK-cell compartment in other T-cell–deficient situations, our data demonstrate that $CD56^{bright}$ NK cells form a versatile cell population that can expand and acquire additional effector functions in the absence of T cells. The reactive changes in the NK-cell compartment may represent a compensatory response of NK cells to inflammatory or infectious triggers when T cells are not present to exert their function. The setting of T-cell deficiency provides a unique opportunity to further study the biology of human NK cells and their role in human disease.

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CDHR3 gene variation and childhood bronchiolitis

To the Editor:

In a recent genome-wide association study, we found that a common single nucleotide polymorphism, rs6967330, in the gene cadherin-related family member 3 (*CDHR3*) was associated with an asthma phenotype characterized by recurrent severe exacerbations in early childhood.[1] CDHR3 is a transmembrane protein with a high level of expression in bronchial epithelium and the *CDHR3* variant was not associated with extrapulmonary "atopic" traits, such as allergic sensitization or eczema, suggesting that the underlying mechanism acts locally in the respiratory epithelium, increasing the susceptibility to environmental triggers. Asthma exacerbations in early childhood are predominantly triggered by lower respiratory tract infections,[2] and we hypothesized that the pathogenic mechanism of the *CDHR3* variant is related to increased susceptibility to such infections.

Bronchiolitis is a viral respiratory infection in the first years of life characterized by asthma-like symptoms and associated with increased risk of recurrent asthma-like symptoms and asthma development later in childhood.[3-5] Bronchiolitis thereby has a clinical presentation resembling the phenotype associated with the *CDHR3* variant and provides an opportunity to study the hypothesis that the causal mechanism of the *CDHR3* variant is related to increased susceptibility to viral respiratory infections. We therefore performed a case-control study analyzing the association between the rs6967330 and bronchiolitis in 5 geographically separate cohorts (see Fig E1 in this article's Online Repository at www.jacionline.org) including more than 700 children with bronchiolitis and 1600 healthy controls.

The participating cohorts included 1 Danish prospective birth cohort, the Copenhagen Prospective Studies on Asthma in Childhood 2000 ($COPSAC_{2000}$), and 4 case-control studies of bronchiolitis from Freiburg (Germany), Utrecht (The Netherlands), Kuopio (Finland), and Gothenburg (Sweden). Detailed description of study populations and genotyping methods for each cohort is reported in this article's Online Repository at www.jacionline.org.

We calculated the association of rs6967330 with bronchiolitis using logistic regression in an additive genotype model coding for 0, 1, or 2 doses of the asthma-risk–associated allele A. Meta-analysis estimates were calculated with a random effects model using inverse variance weighting.

TABLE I. Characteristics of bronchiolitis study populations

Characteristic	COPSAC (Denmark)		Kuopio (Finland)*		Gothenburg (Sweden)		Freiburg (Germany)		Utrecht (The Netherlands)		Combined study	
	n	%	n	%	n	%	n	%	n	%	n	%
Total cases	19	100%	63	100%	28	100%	192	100%	441	100%	743	100%
RSV-positive	17	89%	21	33%	10	36%	192	100%	441	100%	681	92%
RSV-negative	2	11%	40	63%	18	64%	0	0%	0	0%	60	8%
Age < 180 d	7	37%	10	16%	5	18%	124	65%	366	83%	512	69%
Age ≥ 180 d	12	63%	51	81%	23	83%	68	35%	75	17%	229	31%
Male sex	13	68%	35	56%	15	54%	124	65%	249	56%	436	59%
Total controls	305		63		29		339		911		1647	
Total population	324		128		57		530		1352		2390	
CDHR3 MAF†		0.177		0.337		0.237		0.170		0.176		0.186

MAF, Minor allele frequency.

*Two bronchiolitis cases from Kuopio cases had missing information on age-at-diagnosis and RSV status. For details, see Table E1 in this article's Online Repository at www.jacionline.org.

†MAF of combined study population including both cases and controls.

TABLE II. Association between CDHR3 gene variation (rs6967330, A allele) and bronchiolitis subtypes

Bronchiolitis type	COPSAC (Denmark)	Kuopio (Finland)	Gothenburg (Sweden)	Freiburg (Germany)	Utrecht (The Netherlands)	Meta-analysis			
						I^2	Heterogeneity *P* value	OR (95% CI)	*P* value
All bronchiolitis	2.4 (1.2-4.6)	1.8 (1.0-3.3)	4.0 (1.4-12.6)	0.9 (0.7-1.3)	1.0 (0.8-1.2)	74.6%	<.01	1.4 (1.0-2.2)	.08
RSV-positive	2.5 (1.2-4.9)	1.3 (0.6-2.9)	3.3 (0.8-16.0)	0.9 (0.7-1.3)	1.0 (0.8-1.2)	57.6%	.05	1.2 (0.9-1.7)	.26
RSV-negative	*	2.1 (1.1-4.1)	4.4 (1.3-16.6)	*	*	**10.3%**	**.29**	**2.5 (1.3-4.8)**	**.0058**
Age < 180 d	3.2 (1.1-9.1)	0.6 (0.2-1.9)	3.3 (0.5-28.9)	1.0 (0.7-1.4)	1.0 (0.8-1.2)	44.7%	.12	1.1 (0.8-1.6)	.62
Age ≥ 180 d	1.9 (0.8-4.5)	2.1 (1.2-4.1)	4.2 (1.3-14.0)	0.9 (0.5-1.4)	0.9 (0.6-1.4)	64.9%	.02	1.5 (0.9-2.4)	.12

Significant meta-analysis result is indicated by boldface.

*No or too few cases to perform analysis.

Participant and genotype characteristics are presented for each study in Table I. The Freiburg and Utrecht cohorts were restricted to respiratory syncytial virus (RSV)-positive cases, whereas COPSAC had 11% RSV-negative cases and Kuopio and Gothenburg populations had 63% and 64% RSV-negative cases, respectively. Age-at-diagnosis had a marked uneven distribution, with the percentage of cases with age-at-diagnosis below 180 days varying from 83% in the Utrecht cohort to 16% in the Kuopio cohort (see Fig E2 in this article's Online Repository at www.jacionline.org). Sex was equally distributed among study populations.

There was no statistically significant association between rs6967330 and bronchiolitis in the overall meta-analysis (odds ratio [OR], 1.4; 95% CI, 1.0-2.2; $P = .08$) (Table II; see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). However, a statistically significant association was seen in 3 of the 5 study populations and there was strong evidence of heterogeneity between studies ($P = .0034$; $I^2 = 74.6\%$).

Restricting our analysis to only RSV-positive bronchiolitis showed an even weaker association (OR, 1.2; 95% CI, 0.9-1.7; $P = .26$) (Fig E3). In contrast, there was a large effect size and statistically significant association between the variant and RSV-negative bronchiolitis (OR, 2.5; 95% CI, 1.3-4.8; $P < .01$) (Fig E3, *C*), and this result was also significant after adjustment (Bonferroni correction) for the 5 analyses performed in the study. However, this analysis only included 58 cases and 92 controls from 2 studies.

Age-stratified analyses showed a slightly higher effect estimate in the analysis restricted to children who were older than 6 months at hospitalization but this association was not statistically significant (Table II). An age-stratified analysis restricted to RSV-positive cases showed no indication of stronger association in older children (OR, 1.1; 95% CI, 0.8-1.6; $P = .44$). Adjustment for sex did not change the results (Fig E3, *D*).

A major strength of our study is the sample size, including a large proportion of RSV-positive cases, allowing us to make firm conclusion about association to this, the most common, bronchiolitis presentation. However, it is a limitation of our study that we only included few children with non-RSV–triggered bronchiolitis and that we did not have information on other specific triggers of bronchiolitis than RSV, particularly human rhinovirus and parainfluenza virus, which are detected in a sizable number of children hospitalized with bronchiolitis.[6] A further limitation to this, and other studies of bronchiolitis, is the lack of a universal bronchiolitis definition.[6] This might cause heterogeneity in case definition between studies and countries,[7] which is also evident in the present study.

We found that the *CDHR3* asthma-risk variant at rs6967330 was not associated with bronchiolitis in general. Particularly, there was no association with bronchiolitis triggered by RSV infection. This lack of association was found in 4 of 5 study groups, and was not modified by age at onset of bronchiolitis. However, our results suggest that the *CDHR3* gene variant could be associated with bronchiolitis triggered by infectious agents other than RSV. For this bronchiolitis subtype, we found a considerably higher effect estimate and statistical significance in spite of a lower number of cases, indicating that the mechanism associated with the *CDHR3* variant might be

virus-specific. This is supported by a recent experimental study reporting that CDHR3 functions as a rhinovirus C receptor and specifically that the *CDHR3* asthma-risk variant analyzed in our study (A allele at rs6967330) increases rhinovirus C binding and replication.[8] A rhinovirus C–related mechanism fits well with our observations because rhinoviruses are common triggers of non-RSV bronchiolitis.[6] Our data are also in line with a recent report of association between the *CDHR3* variant and chronic rhinosinusitis, which is often triggered by rhinoviruses.[9] However, further clinical studies on the association between *CDHR3* genotype and bronchiolitis triggered by specific infectious agents, including rhinovirus subtypes, are needed to confirm such a disease mechanism.

Bronchiolitis is classically considered a single disease entity, although large variation in the clinical presentation might suggest that it is in fact a heterogeneous syndrome.[5,7] Our results support such heterogeneity by showing heterogeneity in gene association between studies and bronchiolitis subtypes. A hypothesis-free clustering approach including more than 2600 bronchiolitis cases found that the bronchiolitis syndrome is likely to contain 3 to 4 separate phenotypes.[7] One of the phenotypes identified in the above-mentioned study was dominated by rhinovirus infection, had an older age at presentation, and was more frequently associated with recurrent wheezing. This phenotype resembles our RSV-negative subgroup and we hypothesize that *CDHR3* variation could partly explain clinical bronchiolitis heterogeneity through association with a specific underlying mechanism increasing the risk of rhinovirus-induced bronchiolitis and recurrent asthmatic symptoms (see Fig E4 in this article's Online Repository at www.jacionline.org).

In summary, the *CDHR3* asthma-risk variant at rs6967330 was not associated with severe RSV bronchiolitis. However, our data indicated association with a bronchiolitis subtype triggered by other infectious agents, which should be addressed in future studies.

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STUDY POPULATIONS AND GENOTYPING METHODS

The following extended description of study methods is reported in accordance with the STREGA (Strengthening the Reporting of Genetic Association studies) recommendations,[E1] an extension of the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology). Summary statistics with information on unsuccessful genotyping and missing data are reported in Table E1.

$COPSAC_{2000}$

Study population. Four hundred eleven children born to mothers with a history of physician-diagnosed asthma were enrolled at age 1 month in the $COPSAC_{2000}$ prospective birth cohort study.[E2] Key exclusion criteria were symptoms of lower airway infection or neonatal mechanical ventilation before inclusion, gestational age of less than 36 weeks, and any congenital abnormality or systemic illness. The birth cohort was prospectively monitored closely for respiratory symptoms with daily diary cards from age 1 month and clinical examinations performed by the COPSAC physicians at the research clinic every 6 months and in cases of acute respiratory symptoms. *Acute severe bronchiolitis* was defined as an acute respiratory tract illness with onset before the age of 2 years based on symptoms of coryza progressing over a few days to cough, tachypnea, chest retractions, and auscultative widespread crepitation and/or rhonchi.[E3,E4] For this meta-analysis, bronchiolitis occurring before age 2 years and requiring hospitalization were included as cases and children without such episode and with full follow-up to age 2 years were used as controls. RSV diagnostics were performed according to local hospital procedures and hospital records were retrieved and reviewed to verify respiratory symptoms compatible with the above-mentioned criteria for diagnosing acute severe bronchiolitis.[E3,E4]

Genotyping methods. High-throughput genome-wide single-nucleotide polymorphism (SNP) genotyping was performed on all samples simultaneously using the Illumina Infinium II HumanHap550 v1, v3, or Quad BeadChip platform (Illumina, San Diego, Calif) at the Children's Hospital of Philadelphia's Center for Applied Genomics. Rs6967330 had a call rate of 100% and $P = .8875$ for a test against Hardy-Weinberg equilibrium.

Freiburg

Study population. The population was recruited at the University Children's Hospital, Freiburg, Germany, and at the Community Children's Hospital of Freiburg, St Josefs Hospital between September 1998 and March 2005. Infants were eligible for the study if they had been hospitalized because of RSV infection within their first 2 years of life. Infection with RSV was confirmed by antigen test and/or RSV-specific PCR. According to the case definition, these children had symptoms of bronchiolitis such as wheezing and tachypnea and needed either supplementary oxygen and/or gavage feeding and/or intravenous fluids. Children with congenital heart defects, immune deficiency, or chromosomal aberrations were excluded.

Genotyping methods. DNA samples were collected either by blood taking or by buccal swabs with sterile cotton sticks. In total 154 children were included. The control population consisted of 396 healthy children with no history of underlying chronic disease, aged 4 to 14 years. DNA was extracted from peripheral blood leucocytes or buccal smears following standard protocols and then column-purified (DNA midikit, Qiagen, Germany). Before extraction, blood samples or buccal swabs were stored at −70°C. Genotyping of rs6967330 was performed commercially by LGC Genomics (LGC Genomics, Hoddesdon, UK). The call rate was 90% for the control samples and 93% for the case samples. The allelic frequency of the SNP was in Hardy-Weinberg equilibrium.

Kuopio and Gothenburg

Study populations. The study populations of 93 bronchiolitis cases and 94 controls were collected in the area of Kuopio, Finland, and Gothenburg, Sweden. The inclusion criteria for the affected children were hospitalization for severe bronchiolitis, an age of less than 24 months at the time of diagnosis, and native Finnish or Swedish ethnicity. Bronchiolitis was defined as wheezing, either audible wheezing or a prolonged expirium, during respiratory infection. RSV infection/viral etiology was confirmed by immunofluorescent or radioimmunoassays, viral isolation, PCR of nasopharyngeal cells, or serum antibody assays.

Study subjects from Gothenburg comprised a prospective cohort of children who were admitted with a first wheezing episode (wheezing bronchitis/acute bronchiolitis) severe enough to require in-hospital treatment and age-matched controls (±2 months).[E5] Hospitalizations occurred between March 1984 and November 1985.

Study subjects from Kuopio comprised 2 prospective birth cohorts that were hospitalized for bronchiolitis in the period 1981 to 1982 and 1992 to 1993 at Kuopio University Hospital and age-matched controls (±2 months).[E6] The presence of a chronic pulmonary disease, including asthma, was an exclusion criterion for cases in Kuopio. For controls, wheezing or any hospitalization before the age of 24 months was an exclusion criterion.

Genotyping methods. Genomic DNA was extracted from whole blood samples with the UltraClean Blood DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, Calif). Before extraction, blood samples were stored at −70°C. Genome-wide genotyping was performed for the populations from Gothenburg and Kuopio simultaneously using the HumanOmniExpress BeadChip (Illumina, San Diego, Calif). The Technology Centre at the Institute for Molecular Medicine Finland, University of Helsinki, did the genotyping. Samples with a genotype call rate of less than 97% were removed. The SNP rs6967330 was imputed and the imputation info score was 0.97. The data were prephased with SHAPEIT2, followed by genotype imputation performed with IMPUTE2. Call rate for rs6967330 was 98.5% in the population of Kuopio and 96.6% in Gothenburg. Allelic frequencies were tested against Hardy-Weinberg equilibrium, with $P = 1$ and $P = .0252$ for controls and cases, respectively, in the Gothenburg population and with $P = .3515$ and $P = .7932$ for controls and cases, respectively, in the Kuopio population.

Utrecht

Study population. The Utrecht cohort has been described previously.[E7] In short, we selected previously healthy infants hospitalized for RSV-associated lower respiratory tract illness in the first year of life. Children with previous airway morbidity or airway medication were excluded. An unselected control population of 1030 Dutch persons born in the Netherlands was randomly taken from a healthy baby clinic (referred to as the Regenboog study[E8]).

Genotyping methods. DNA was isolated from blood samples or buccal swabs as previously described.[E9] Before extraction, samples were stored at −20°C. Shortly DNA was isolated from blood samples or, when blood was not available, from buccal swabs, using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). For the control population (REGENBOOG samples), genomic DNA was extracted from buffy coats by digestion with proteinase K, followed by salting out with potassium acetate and chloroform/isoamyl alcohol extraction. The DNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Mass). Extracted DNA samples were diluted with Tris-EDTA buffer to 7 ng/μl and sent on dry ice to LGC Genomics (Hoddesdon, UK) for genotyping with the KASPar technology. For rs6967330, call rate was 97% and the allelic frequency of the SNP was in Hardy-Weinberg equilibrium ($P > .05$).

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FIG E1. Geographical distribution of study populations.

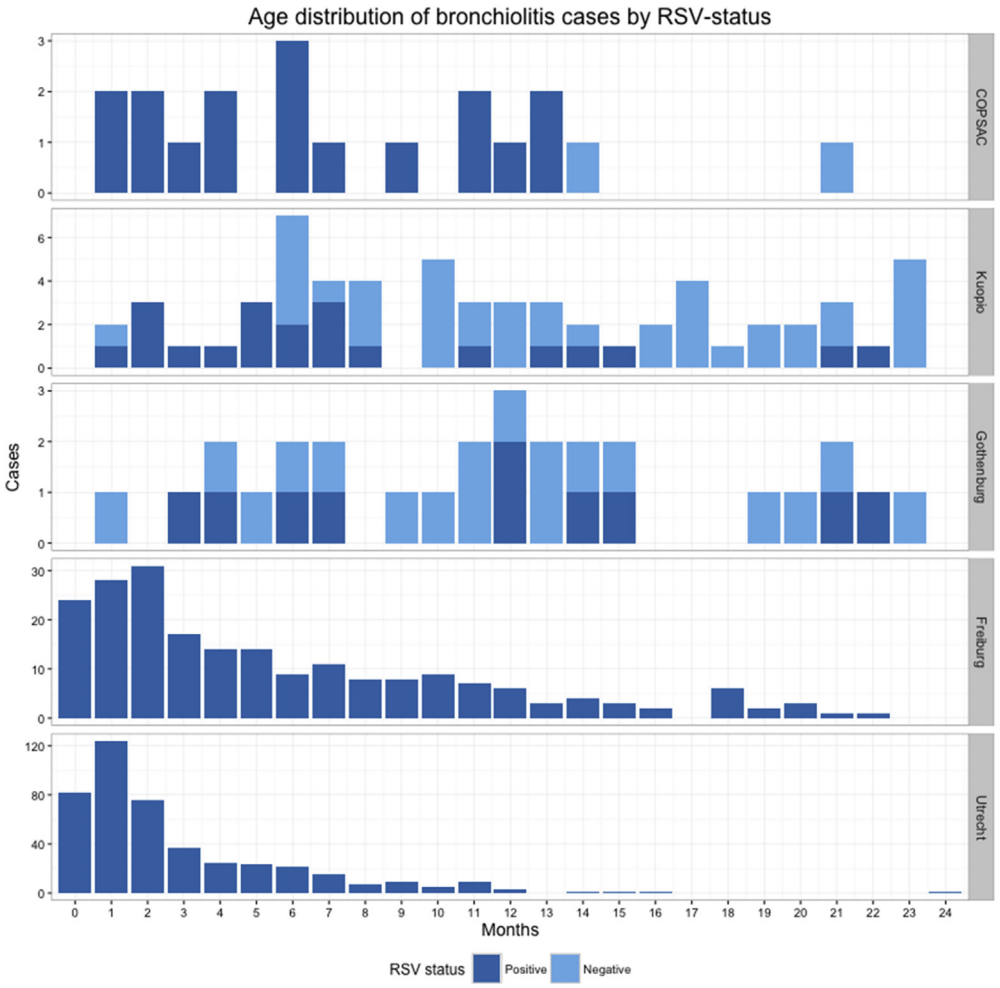

FIG E2. Age distribution of study populations.

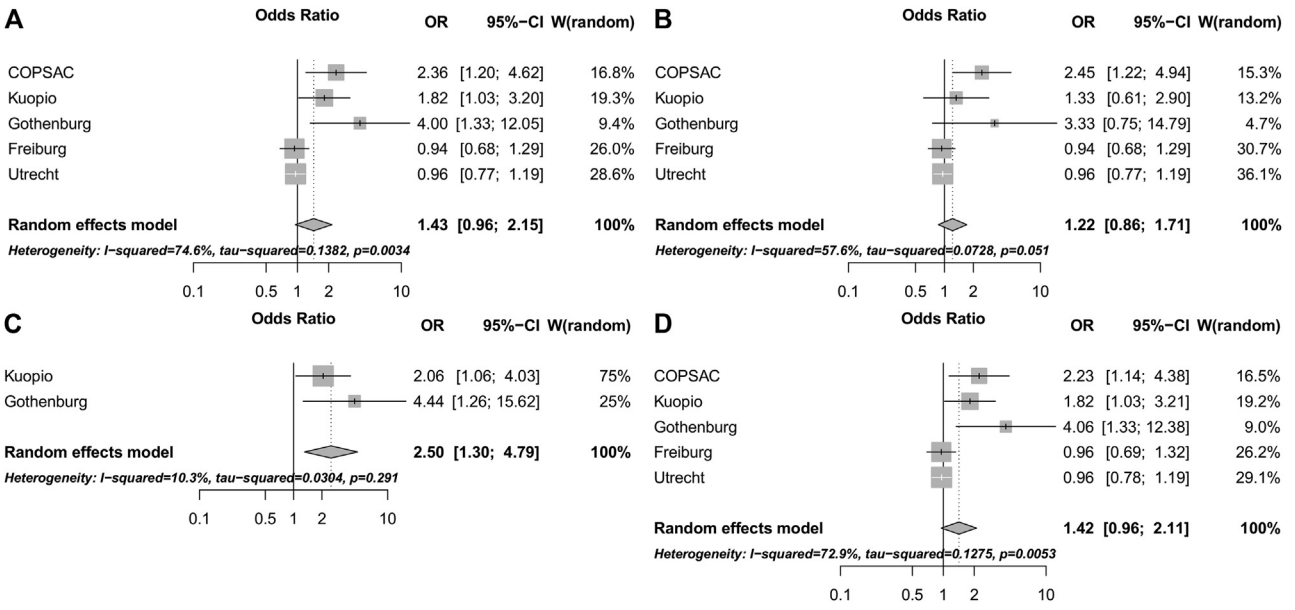

FIG E3. Forest plot of association of CDHR3 gene variation (rs6967330, A allele) with bronchiolitis overall **(A)**, RSV-positive bronchiolitis **(B)**, RSV-negative bronchiolitis **(C)**, and bronchiolitis overall adjusted for sex **(D)**.

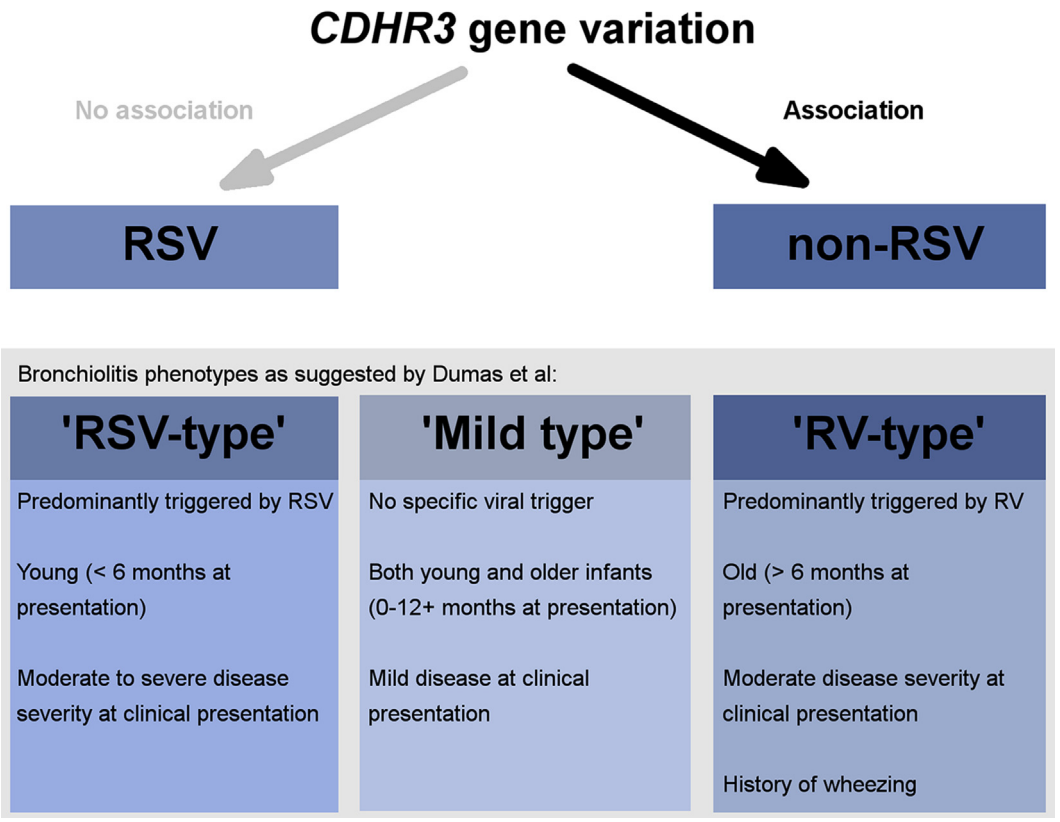

FIG E4. Graphical illustration of the relationship between *CDHR3* gene variation and bronchiolitis subtypes in the present study *(top part)* and resemblance with clinical bronchiolitis phenotypes suggested in a previously published clustering analysis[E10] *(bottom part)*.

TABLE E1. Summary statistics with information on unsuccessful genotyping and missing data, in accordance with STREGA guidelines[E1]

	Sex			Age (d)			RSV					Sex			
Cases by genotype	**Male**	**Female**	**Missing**	**<180**	**≥180**	**Missing**	**Positive**	**Negative**	**Missing**	**All**	**Controls by genotype**	**Male**	**Female**	**Missing**	**All**
COPSAC summary statistics															
GG	5	4	0	3	6	0	8	1	0	9	GG	99	116	0	215
AG	5	2	0	2	5	0	6	1	0	7	AG	38	40	0	78
AA	3	0	0	2	1	0	3	0	0	3	AA	7	5	0	12
Missing	0	0	0	0	0	0	0	0	0	0	Missing	0	0	0	0
Total	13	6	0	7	12	0	17	2	0	19	Total	144	161	0	305
Kuopio summary statistics															
GG	13	9	0	7	15	0	10	12	0	22	GG	16	15	0	31
AG	17	15	0	2	29	1	8	23	1	32	AG	19	10	0	29
AA	6	3	0	1	7	1	3	5	1	9	AA	1	2	0	3
Missing	0	1	0	0	1	0	0	1	0	1	Missing	0	1	0	1
Total	36	28	0	10	52	2	21	41	2	64	Total	36	28	0	64
Gothenburg summary statistics															
GG	7	3	0	2	8	0	4	6	0	10	GG	7	13	0	20
AG	8	10	0	3	15	0	6	12	0	18	AG	5	4	0	9
AA	0	0	0	0	0	0	0	0	0	0	AA	0	0	0	0
Missing	1	0	0	0	1	0	0	1	0	1	Missing	1	1	0	2
Total	16	13	0	5	24	0	10	19	0	29	Total	13	18	0	31
Freiburg summary statistics															
GG	85	50	0	87	48	0	135	0	0	135	GG	138	98	1	237
AG	36	15	0	36	15	0	51	0	0	51	AG	44	41	1	86
AA	3	3	0	3	3	0	6	0	0	6	AA	6	10	0	16
Missing	8	10	1	9	9	1	24	0	0	24	Missing	27	25	1	53
Total	132	78	1	135	75	1	216	0	0	216	Total	215	174	3	392
Utrecht summary statistics															
GG	168	131	1	248	51	1	300	0	0	300	GG	353	263	0	616
AG	74	58	0	109	23	0	132	0	0	132	AG	150	116	0	266
AA	7	3	0	9	1	0	10	0	0	10	AA	17	12	0	29
Missing	16	7	0	19	4	0	23	0	0	23	Missing	10	9	0	19
Total	265	199	1	385	79	1	465	0	0	465	Total	530	400	0	930

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