Associations between IFI44L gene variants and rates of respiratory tract infections during early childhood

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ABSTRACT

Background. Genetic heterogeneity in type I interferon related gene IFI44L may account for variable susceptibility to respiratory tract infections (RTIs) in children.

Methods. In two prospective, population-based birth cohorts, the STEPS Study and the FinnBrain Birth Cohort Study, IFI44L genotypes for rs273259 and rs1333969 were determined in relation to the development of RTIs until one and two years of age, respectively. At age 3 months, whole blood transcriptional profiles were analyzed and nasal samples were tested for respiratory viruses in a subset of children.

Results. In the STEPS Study (n=1135), IFI44L minor/minor gene variants were associated with lower rates of acute otitis media episodes (adjusted incidence rate ratio [aIRR], 0.77 [95% CI, 0.61-0.96] for rs273259 and 0.74 [0.55-0.99] for rs1333969) and courses of antibiotics for RTIs (aIRR, 0.76 [0.62-0.95] and 0.73 [0.56-0.97], respectively. In the FinnBrain cohort (n=971), IFI44L variants were associated with lower rates of RTIs and courses of antibiotics for RTIs. In respiratory virus-positive 3-month-old children, IFI44L gene variants were associated with decreased expression levels of IFI44L and several other interferon related genes.

Conclusions. Variant forms of IFI44L gene were protective against early-childhood RTIs or acute otitis media, and they attenuated interferon pathway activation by respiratory viruses.

Words 199

***Key words*.** acute otitis media; interferon pathway; polymorphisms; respiratory tract infections; transcriptome

Capsule Summary: Common IFI44L gene variants were protective against early-childhood respiratory tract infections or acute otitis media in two independent birth cohorts. These polymorphisms attenuated interferon pathway activation by respiratory viruses.

Key words: acute otitis media; interferon pathway; polymorphisms; respiratory tract infections; transcriptome

ABBREVIATIONS

DEG: Differentially expressed gene

GO: Gene Ontology

IFI44L: Interferon-induced protein 44-like

IFN: Type I interferon

ISG: Interferon-stimulated gene

MBL: Mannose-binding lectin

mRNA: Messenger RNA

RTI: Respiratory tract infection

SNP: Single nucleotide polymorphism

STEPS: Steps to the Healthy Development and Well-being of Children

TLR: Toll-like receptor

INTRODUCTION

Children younger than 2 years of age have the highest frequency of respiratory tract infections (RTIs) with an average of 6 episodes per year.[1,2,3] RTIs are mostly caused by viruses and are frequently complicated by acute otitis media, where both viruses and bacteria play a role. Some children suffer from higher numbers of RTIs and acute otitis media episodes than others,[4] but reasons for these individual differences are not fully understood.

Risk factors for RTIs include the presence of older siblings, day care attendance, male sex, passive smoke-exposure, and lack of breastfeeding.[2,4,5,6] Recently, genetic susceptibility to RTIs or acute otitis media has been recognized. Single nucleotide polymorphisms (SNPs) and other genetic variants affecting the functions of essential proteins of innate immunity such as toll-like receptors (TLR), mannose-binding lectin (MBL), tumor necrosis factor (TNF) alpha, interleukin (IL)-6, and IL-10 have been associated with increased susceptibility to RTIs.[7,8,9,10,11,12,13] Effects of these common polymorphisms are especially important during early childhood.[14]

Interferon (IFN) pathways are of key importance in innate immune responses. Type I IFNs (including IFN-α and IFN-β) are secreted by infected cells after recognition of microbial (particularly viral) products by cell surface and intracellular pattern recognition receptors.[15] IFNs induce cell-intrinsic antiviral states leading to transcription of IFN-stimulated genes (ISGs) in infected and neighboring cells. Type I IFNs promote antigen presentation and development of antigen-specific T- and B-cell responses but simultaneously restrain pro-inflammatory pathways.[15] The function of several IFN-induced proteins with potential antiviral action and the effects of polymorphisms in ISGs are still poorly characterized. Interferon-induced protein 44-like (IFI44L) protein belongs to the group of proteins encoded by ISGs. IFI44L is transcriptionally induced by type I IFN signaling and is up-regulated in the antiviral response.[16] IFI44L has been reported to have antiviral activity[16,17] but the exact functions of this protein in the innate immune response are not known. Previously reported SNPs rs273259 and rs1333969 in the IFI44L gene have been

suggested to have a functional effect on IFI44L protein.[18,19] However, there is no data on the association of these common gene variants with the susceptibility to RTIs in children.

We aimed to determine the association of variant forms of IFI44L gene polymorphisms rs273259 and rs1333969 with rates of RTIs, acute otitis media episodes, and antibiotic treatment courses during first two years of age. Our study populations were derived from two prospectively followed, independent population-based birth cohorts from Finland, Steps to the Healthy Development and Well-being of Children (STEPS) and the FinnBrain Birth Cohort Study.[20,21] The effect of IFI44L gene polymorphisms on blood messenger RNA (mRNA) transcriptional profiles were analyzed in a subset of children in the FinnBrain Birth Cohort Study.

METHODS

Study populations

This study was conducted within two prospective, population-based birth cohort studies: the STEPS Study and the FinnBrain Birth Cohort Study.[20,21] Children born to their Finnish- or Swedish-speaking mothers were eligible, and no other selection criteria were applied in either study. The STEPS Study and the FinnBrain Birth Cohort Study protocols were approved by the Ethics

Committee of the Hospital District of Southwest Finland. The parents of the participating children gave written informed consent on their child's behalf.

STEPS Study. A cohort of 1827 children born in 2008-2010 in the Hospital District of Southwest Finland were followed for RTIs from birth to two years of age.[3,4] During follow-up, parents documented in a daily diary the presence of respiratory symptoms, physician visits with diagnoses of RTIs, and antibiotic treatments for RTIs. In a subset of children (n = 923; 51%), at the onset of respiratory symptoms, nasal swabs were obtained using flocked nylon swabs (Copan, Brescia, Italy) either at the study clinic or by the parents at home and sent to the laboratory.[3] Children were examined by a study physician during an acute RTI if the parents felt that an evaluation was needed. Blood samples for genetic analyses were obtained at two months of age. Data on emergency department visits and hospitalizations was collected from the Hospital District of Southwest Finland electronic healthcare records.

FinnBrain Birth Cohort Study. A total of 1443 children born between 2011 and 2015 in the Hospital District of Southwest Finland were followed for RTIs from birth to one year of age. Cord blood was collected at birth and used for genetic studies. Data on physician visits for RTIs, physician-diagnosed respiratory infections (RTI, rhinitis, cough, acute otitis media, bronchiolitis, or pneumonia), and antibiotic treatments for RTIs were collected using monthly questionnaires. The final analysis included children with successful genotyping and information on respiratory infections (n = 971 for rs273259 and n = 972 for rs133969). At 3 months of age children were examined by a study physician, and from a subset of 71 children, nasal swabs were collected using flocked nylon swabs (Copan) and blood samples were collected in Tempus tubes (Applied Biosystems, Foster City, CA) for mRNA analysis. At the time of collection of nasal and blood samples infants were afebrile and without signs or symptoms of an RTI.

Respiratory virus detection

Nasal swabs were stored at -80°C until analyses. Swabs were suspended in phosphate buffered saline, and nucleic acids were extracted by NucliSense easyMag (BioMerieux, Boxtel, the Netherlands) or MagnaPure 96 (Roche, Penzberg, Germany) automated extractor. Extracted RNA was reverse transcribed and the cDNA of the STEPS Study samples were amplified using real-time, quantitative reverse- transcription polymerase chain reaction (RT-PCR) for rhinovirus, enteroviruses, and respiratory syncytial virus (RSV).[22, 23] In the FinnBrain Birth Cohort Study, the Anyplex RV16 (Seegene, Seoul, Korea) multiplex PCR assay was performed according to the manufacturer's instructions. This multiplex assay included the detection of adenovirus, bocavirus, coronaviruses, enteroviruses, influenza A and B viruses, metapneumovirus, parainfluenza virus types 1-4, rhinovirus, and RSV A and B.

Definitions of respiratory infection outcomes

In the STEPS Study, an episode of RTI was defined as the presence of rhinitis or cough, with or without fever or wheezing, documented in the diary by the parents, or as a physician-diagnosed RTI as previously described.[3] The number of days with RTI symptoms was analyzed using data filled into daily diaries by the parents. Acute otitis media was diagnosed by a study physician or recorded into the diary or medical records by a physician at an outpatient office or hospital. If there were repeated diagnoses of acute otitis media during continuous respiratory symptoms, parallel diagnoses within 14 days were calculated as one diagnosis. As rhinovirus was the most frequent virus identified in the STEPS Study[3], we report separately the rates of rhinovirus-positive RTIs.

In the FinnBrain Birth Cohort study, the definition of an RTI was based on the monthly parental report of physician-diagnosed acute RTIs. Each separate episode in one-month

period, and any episode that continued during the turn of the month, was defined as a separate event.

Genetic analysis

In the STEPS Study, DNA was extracted from whole blood according to standard procedures and IFI44L SNPs rs273259 and rs1333969 were analyzed using the Sequenom platform (San Diego, CA) at Genome Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland. In quality control analysis both SNPs had genotype missingness per marker below the threshold of 5% (2.7% and 1.1%, respectively) and both had non-significant Hardy-Weinberg *P* values (*P* = .61 and *P* = .30, respectively). SNPs rs273259 and rs1333969 show mild linkage disequilibrium (r^2=0.58) among the Finnish population.[24]

In the FinnBrain Birth Cohort Study, DNA was extracted from whole blood according to standard procedures and genotyped with Illumina Infinium PsychArray BeadChip comprising 603132 SNPs at Estonian Genome Centre, Tartu, Estonia. Quality control was performed with PLINK 1.9 (http://www.cog-genomics.org/plink/1.9/).[25] Markers were removed for missingness (>5%) and Hardy-Weinberg equilibrium (*P* value < 1 x 10^{-6}). Individuals were checked for missing genotypes (>5%), relatedness (identical by descent calculation, PI_HAT>0.2) and population stratification (multidimensional scaling).

Transcriptome analysis

Details of the transcriptome analysis are presented in this article's Online Repository. Briefly, whole blood mRNA transcriptional profiles were analyzed in 71 infants from the FinnBrain Birth Cohort Study at 3 months of age (Table E1). RNA was extracted and hybridized to Illumina HT-12 V4 beadchips (Illumina, San Diego, CA). Data was pre-processed and filtered as previously described.[26] As there was low number of infants with minor/minor genotypes (Table E1), mRNA

expression and transcriptional profiles were compared between 1) major/major genotypes, and 2) other genotypes (including major/minor and minor/minor genotypes). First, we analyzed if the presence of IFI44L polymorphisms influenced IFI44L mRNA expression. To further explore if IFI44L polymorphisms were associated with altered expression of other genes, we performed differential gene expression analysis between the genotypes. Limma[27] package in R with false discovery rate (FDR) adjusted *P* value of 0.05 and 1.25 fold change were used to detect differentially expressed genes (DEGs). To assess function of the DEGs and affected pathways, we used Gene Ontology (GO)[28, 29] biological processes terms and Ingenuity Pathway Analysis (IPA) software (QIAGEN, Redwood City, CA, USA). The data is deposited in the NCBI Gene Expression Omnibus (GEO accession number: XXX).

Statistical analysis

In the STEPS Study, the association between the IFI44L genotypes and respiratory infection outcomes in children 0-2 years of age were analyzed using negative binomial regression analysis with natural logarithm of the follow-up time as an offset. Unadjusted and adjusted incidence rate ratios (aIRR, adjusted for sex and the presence of sibling(s) at birth) were reported. In the FinnBrain Birth Cohort Study, the association between the IFI44L genotypes and RTIs and antibiotic treatments for RTIs from birth to 1 year of age were first tested with linear regression analysis implemented with PLINK (Purcell). The number of RTIs was then categorized in four groups: 0, 1-4, 5-10, and >10, and the number of antibiotic courses in three groups: 0, 1-4, and 5 or more. Final analysis with adjustment for sex and the presence of sibling(s) at birth was done using ordinal logistic regression to explore whether the odds of being in a higher category was associated with the heterozygous or homozygous polymorphisms of IFI44L. The selection of sex and presence of sibling(s) as covariates in the final models was based on *a priori* knowledge. We have previously published that, in the STEPS Study, male sex and the presence of older siblings were risk factors for

respiratory infection outcomes, while breastfeeding, parental smoking or daycare attendance were not associated with an increased risk of respiratory infections[13]. Regression analyses were performed using R 3.5.3. Two-tailed *P* values of less than .05 were considered significant.

RESULTS

In the STEPS Study, the final analysis included 1135 children that had genotypes and RTI data, including 738 with data on rhinovirus etiology of RTIs. In the FinnBrain cohort, the final analysis included children with successful genotyping and data on RTIs (n = 970 for rs273259 and n = 971 for rs133969). Background characteristics and allelic distribution of IFI44L polymorphisms in both cohorts are presented in Table 1 and RTI-related outcomes in Table 2.

IFI44L genotypes and respiratory infections in the STEPS Study cohort

Rates of all RTIs and rhinovirus-positive RTIs were similar in the first 2 years of life in children with different IFI44L genotypes (Table 3). Slightly decreased rates of days with RTI symptoms were observed among children with genetic variants. This difference was significant only for children with the CT (major/minor) genotype of rs1333969 compared to those with the CC (major/major) genotype (aIRR, 0.89 [95% CI, 0.81-0.98]).

The minor G allele of the rs273259 polymorphism was associated with decreased rates of acute otitis media both in unadjusted and adjusted analysis (Table 3 and Table E2). Children with a homozygous GG (minor/minor) genotype had lower rates of acute otitis media compared to children with the AA (major/major) genotype (aIRR, 0.77 [95% CI, 0.61-0.96]). Similarly, the minor T allele of the rs1333969 polymorphism was associated with decreased rates of acute otitis media. GG (minor/minor) genotype of the rs273259 and TT (minor/minor) genotype of the rs1333969 were associated with lower rates of antibiotic courses for RTIs compared with children with the respective major/major genotypes (aIRR [95% CI] for rs273259, 0.76 [0.62-0.95],

and for rs1333969, 0.73 [0.56-0.97]).

IFI44L genotypes and respiratory infections in the FinnBrain Study cohort

In ordinal logistic regression analyses adjusted for sex and presence of sibling(s), the G allele of the rs273259 polymorphism in the IFI44L gene was associated with lower number of RTIs during the first year of life. Children with the minor/minor genotype GG had an odds ratio (OR) of 0.64 [95% CI, 0.42-0.97], $P = .04$, and children with the major/minor AG genotype an OR of 0.65 [95% CI, 0.48-0.86], $P = .003$, for RTI frequency compared to the major/major genotype (Table 4). The rs1333969 minor allele T was similarly associated with a decreased frequency of RTIs.

The heterozygous genotypes rs273259 AG and rs1333969 CT were significantly associated with decreased rates of antibiotic courses for RTIs from birth to 1 year of age compared to the major/major genotypes ($P = .02$ and $P = .04$, respectively). Rates of antibiotic courses were also lower in children with minor/minor compared with major/major genotypes of these polymorphisms but differences were not statistically significant.

Effects of IFI44L gene variants on peripheral blood transcription patterns

Blood mRNA transcriptional profiles were analyzed in 71 asymptomatic infants 3 months of age from the FinnBrain Birth Cohort Study. The demographic characteristics, distribution of IFI44L polymorphisms, and virus detections in these children are presented in Table E1. At least one respiratory virus was detected in 25 (35%) of these children, rhinovirus being the most frequently detected.

First, we compared IFI44L expression in all 71 children according to the rs273259 and rs1333969 genotypes and found no differences between the groups (Figure 1, panels A and B). Next, we compared IFI44L expression in a subset of children (n = 25; Table E2) who were positive at least for one respiratory virus. In these virus-positive children, rs1333969 genotype CC

294 (major/major) was associated with higher IFI44L expression (P = .0036). Similar findings were
295 observed with the rs273259 genotype (P = .048) (Figure 1, panels C and D.
296 To explore if different IFI44L genotypes were associated with differences in the
297 expression of other genes we performed differential gene expression analysis among all children.
298 No DEGs were detected when comparing rs273259 and rs1333969 (major/major vs. other
299 genotypes). However, when we included only children with virus detections (n = 25) and compared
300 the rs1333969 CC (major/major) genotype to other genotypes, we identified 116 DEGs. (Table E3,
301 Figure E2). Of these 116 DEGs, 105 (91%) were overexpressed and 11 (9%) were underexpressed
302 in the CC (major/major) genotype. These DEGs were strongly associated with activation of the
303 interferon pathway and immune responses against viruses. Gene set analysis showed that the most
304 significant GO biological process was “type I interferon signaling pathway” with 17/66 overlapping
305 transcripts ($P = 3.3 \times 10^{-21}$; Table E4). Interferon signaling was also the most significant pathway
306 using IPA (12/36 genes; $P = 1.4 \times 10^{-18}$; Figure 2).
307 In similar analysis comparing the rs273259 genotypes among infants with virus
308 detection, we identified 23 DEGs (Table E5, Figure E3). Of these 23 DEGs, 19 (82%) were
309 overexpressed and 4 (18%) were underexpressed in the AA (major/major) genotype. These DEGs
310 included immune response related genes such as interferon gamma (IFNG), granulysin (GNLY),
311 and granzyme A (GZMA). However, no statistically significant GO biological processes or IPA
312 pathways were identified (data not shown). When comparing the DEGs associated with rs1333969
313 (n = 116) and rs273259 (n = 23) genotypes, we found that 16 DEGs were identified in both
314 comparisons (Figure 3).
315 Comparing virus-positive and virus-negative asymptomatic children irrespective of
316 gene polymorphisms, we found no differences in IFI44L expression or in differential gene
317 expression analysis (data not shown).
318

DISCUSSION

In two independent, population-based, prospective birth cohort studies, we analyzed the influence of two common SNPs in IFI44L, a type I interferon gene, on children's susceptibility to RTIs. The variant forms of IFI44L were protective against early-childhood RTIs or acute otitis media in both cohorts. Further, we demonstrated that these gene polymorphisms were associated with altered expression of IFI44L and other transcripts belonging to type I interferon signaling pathways in children with an asymptomatic respiratory virus detection.

In the STEPS Study cohort, we found that the IFI44L gene variants had no effect on the rates of all RTIs or RTIs associated with rhinovirus. However, minor G allele of rs273259 and minor T allele of rs1333969 were associated with a small decrease in the number of days with RTI symptoms per year and with a substantial decrease in the rate of acute otitis media from birth to 2 years of age. In the FinnBrain Birth Cohort Study, we found that the minor alleles of rs273259 and rs1333969 were associated with a decreased RTI frequency during the first year of life. The inconsistencies in the results may be explained by differences in the follow-up period and outcomes between the two birth cohorts. In the STEPS Study, RTI data was largely based on a daily symptom diary kept by parents, documenting also very mild infections. Only 41% of RTIs required a physician visit.[3] The FinnBrain Cohort was followed less intensively, and only RTIs for which a physician visit was needed were recorded. The effect of IFI44L polymorphisms in the STEPS Study on the rate of acute otitis media—which is the most common complication of a viral RTI in children—is in line with an effect on RTIs in the FinnBrain Cohort where acute otitis media was not separately recorded. Associations of minor alleles of IFI44L with a lower rate of antibiotic use for RTIs in both cohorts suggests that these gene variants protect against more severe RTI, which can possibly be complicated with a bacterial infection.

The role of ISGs in the immune defense is poorly described. IFI44L expression increases after infection of dendritic cells with measles virus[30] or airway epithelial cells with RSV

344 or influenza virus.[31] Upregulation of ISGs including IFI44L has been associated with the
345 persistence of hepatitis E virus.[17] IFI44L is shown to negatively modulate innate immune responses
346 in the context of a viral infection, and decreasing IFI44L expression impairs viral replication.[32]
347 Moreover, IFI44L had direct antiviral effects towards hepatitis C virus in a large-scale ISG screen.[16]
348 H28, a mouse homolog for human IFI44L gene, affects the susceptibility to viral myocarditis in a
349 mouse model.[33] Determination of the transcription activation of IFI44L has been recently suggested
350 as a diagnostic tool to discriminate between viral and bacterial infections.[34,35] These findings
351 illustrate the importance of a balanced regulation of IFI44L in the immune response against viral
352 infections. With this background it is rather surprising that IFI44L polymorphisms were associated
353 with acute otitis media and physician-diagnosed RTIs, but not with the rates of documented
354 rhinovirus infections. Acute otitis media in children can be caused by viruses, bacteria, or both, and
355 it almost always develops during or after a viral infection.[36] Our results suggest that an appropriate
356 level of IFI44L activation is important for young children in order to contain viral RTIs and prevent
357 development of acute otitis media.

358 Previous data on functional effects of polymorphisms in the IFI44L gene is limited.
359 An association between major alleles in IFI44L rs273259 and intronic rs1333973 and elevated
360 levels of measles-specific neutralizing antibodies in children has been reported.[18] The IFI44L
361 rs273259 and rs1333973 polymorphisms are in complete linkage disequilibrium (r^2 1.0) and thus
362 most likely represent the effect of the same gene loci.[24] A tendency for febrile seizures following
363 measles, mumps, and rubella vaccination has been associated with the major A allele of rs273259.[18]
364 These reports suggest that the major allele of rs273259 associates with strong immune or
365 inflammatory responses after a stimulus such as a live vaccine.

366 We used transcriptional profiling at the age of 3 months to better understand whether
367 these polymorphisms influenced gene expression. Differences were seen only in children positive
368 for a respiratory virus, although they had no apparent symptoms of an infection at the time of

sample collection. Compared to children with major/major genotypes under this natural immunologic stimulus, children with major/minor or minor/minor genotypes had weaker transcription activity of IFI44L and, also, of expression of other type I interferon signaling pathway genes. Although the numbers of subjects were limited, these results suggest that a relatively weak interferon response could be beneficial in terms of development of symptomatic RTIs or acute otitis media. Symptoms of respiratory virus infections are largely mediated by the host response, which may explain these findings.

Strengths of this study include large study populations from two independent birth cohorts, detailed follow-up of RTIs particularly in the STEPS Study, and search for functional effects of IFI44L polymorphisms by global transcriptome analysis in children with or without a virus infection. Our study also has limitations. Clinical outcomes were partly different between the two cohorts, and in the Finnbrain Cohort study follow-up was not as detailed as in the STEPS Study. However, data such as antibiotic use for RTIs was similar in both cohorts and the findings support each other. Both cohorts were from Finland and corresponding data from other populations would be informative. Transcriptome data was available from a subset of children and larger studies are needed to validate the findings.

In conclusion, we report an effect of IFI44L rs273259 and 1333969 polymorphisms on susceptibility to RTIs in early childhood. Minor alleles associated with lower rates of RTIs or acute otitis media and with weaker interferon response. The exact mechanisms how the polymorphisms affect the immune functions need further investigation.

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Figure legends

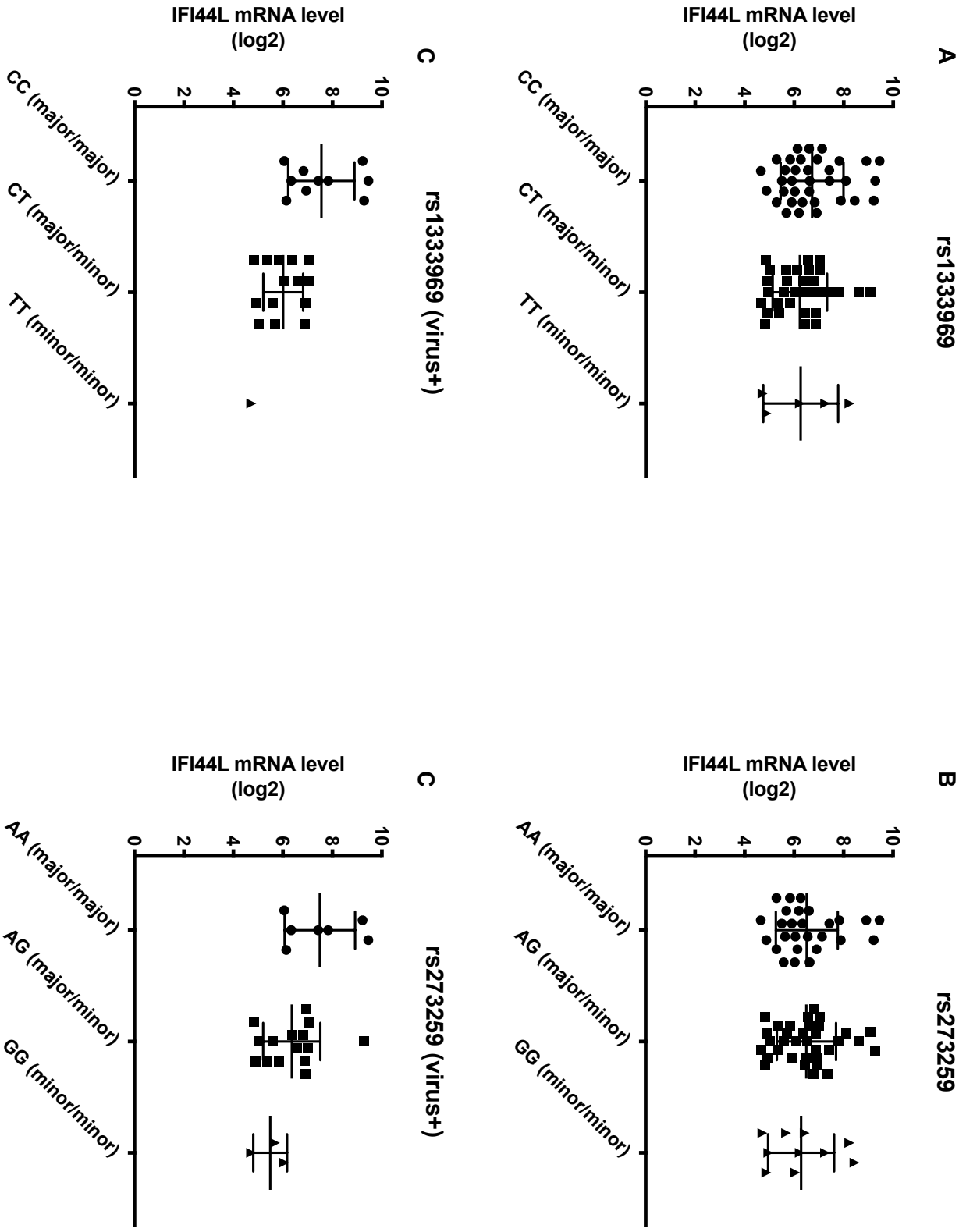
Figure 1. Expression values of IFI44L according to IFI44L polymorphisms and virus detection. In 71 children with transcriptome data available there were no differences in IFI44L expression according to rs1333969 (panel A) or rs273259 (panel B) genotype. However, in a subgroup of children with respiratory virus detection at the time of mRNA sampling (n = 25), IFI44L expression differed according to the genotype (panels C and D). In virus-positive children rs1333969 major/major genotype was associated with higher IFI44L overexpression compared to other genotypes (major/minor and minor/minor genotypes combined) (panel C; Mann-Whitney P = .0036). Similar finding was observed with rs273259 (panel D; P = .048).

Figure 2. rs1333969 genotype of ILI44L affects expression of other transcripts in the interferon signaling pathway. We performed global transcriptome analysis in respiratory virus-positive (n = 25) children and detected 116 genes that were differentially expressed between the rs1333969 major/major and other (major/minor and minor/minor) genotypes. Using gene set analysis approach and Ingenuity Pathway Analysis (IPA) software, we detected that interferon signaling pathway was the most affected pathway with 12/36 overlapping genes ($P = 1.4 \times 10^{-18}$). Differentially expressed transcripts in the interferon type II and type I signaling pathways are highlighted with purple.

Figure 3. Venn diagram presenting the number of differentially expressed and overlapping genes in children with different genotypes of rs1333969 and rs273259 polymorphisms. Differential expression analysis was performed comparing major/major genotype to other genotypes (major/minor and minor/minor). False discovery rate adjusted P value .05 and 1.25 fold change were used as cut-offs for differentially expressed genes.

516

517 Figure 1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rs1333969
IFI44L mRNA level
(log2)
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2
4
6
8
10
CC (major/major)
CT (major/minor)
TT (minor/minor)
C
rs1333969 (virus+)
IFI44L mRNA level
(log2)
0
2
4
6
8
10
CC (major/major)
CT (major/minor)
TT (minor/minor)
B
rs273259
IFI44L mRNA level
(log2)
0
2
4
6
8
10
AA (major/major)
AG (major/minor)
GG (minor/minor)
C
rs273259 (virus+)
IFI44L mRNA level
(log2)
0
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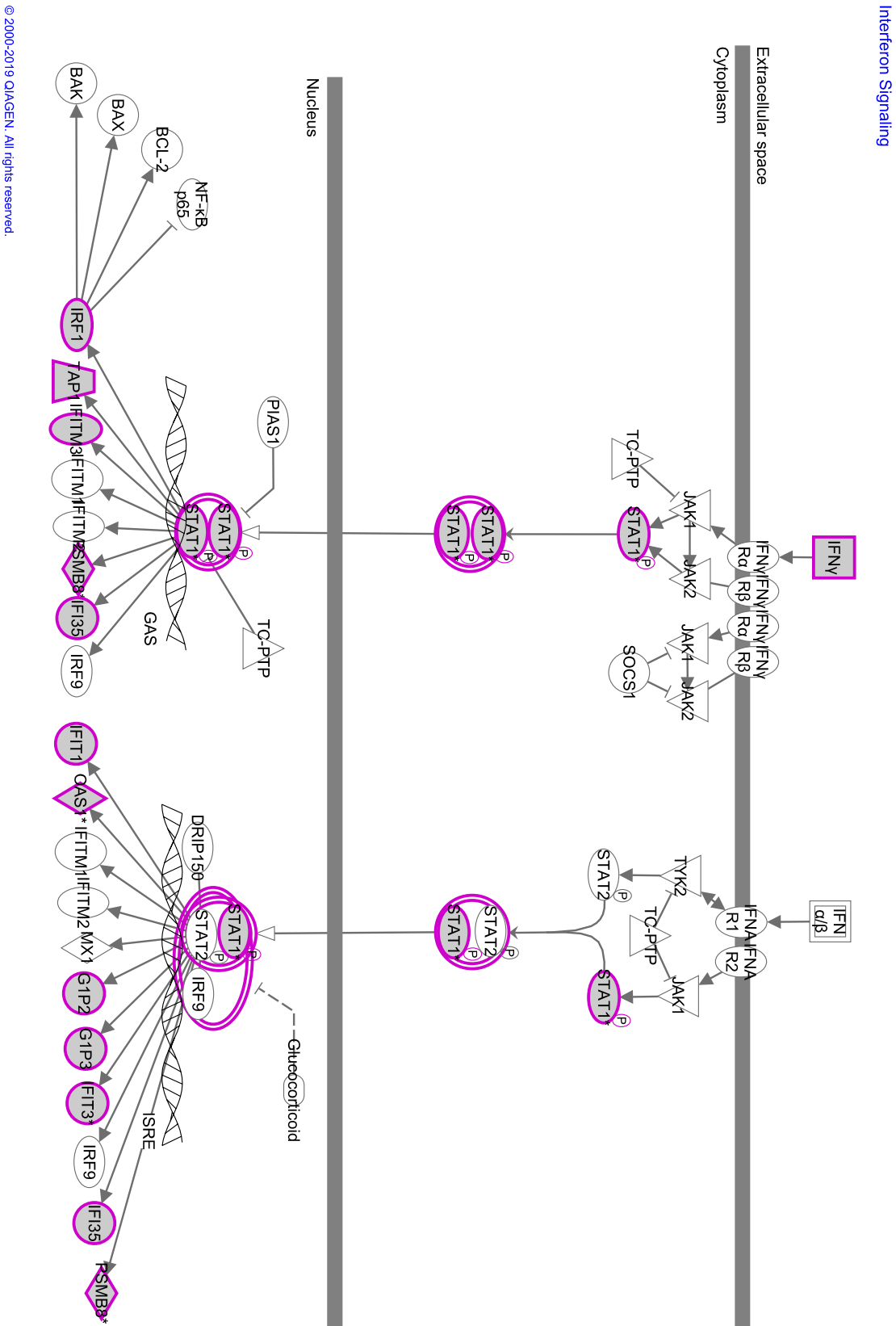
AA (major/major)
AG (major/minor)
GG (minor/minor)

519 Figure 2.



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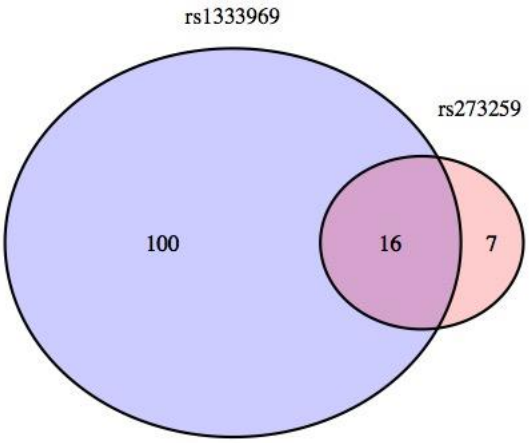

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Table I. Background characteristics and allelic distribution of IFI44L polymorphisms in children in the STEPS Study and in the FinnBrain Birth Cohort Study

	STEPS Study, No. (%)	FinnBrain Cohort, No. (%)
Female	612 (53.9)	464 (47.8)
Older siblings	612 (53.9)	463 (49.3)[a]
IFI44L rs273259		
GG	147 (13.0)	133 (14.0)[b]
AG	497 (43.8)	441 (45.0)
AA	491 (43.3)	396 (41.0)
IFI44L rs1333969		
TT	80 (7.0)	79 (8.0)
CT	414 (36.5)	357 (37.0)
CC	641 (56.6)	535 (55.0)

[a] Data is missing for 31 children

[b] N=970

Table II. Clinical outcomes in the STEPS Study and in the FinnBrain Birth Cohort Study

Clinical outcomes	
STEPS Study, n = 1135	
Length of follow-up, years, median (IQR)	2.0 (1.5-2.0)
RTIs during age 0-2 years, incidence rate per child-year (95% CI)[a]	
RTI episodes	5.9 (5.7-6.0)
Days with RTI symptoms	50.4 (48.2-52.7)
Rhinovirus-positive RTIs	2.0 (1.9-2.1)
Acute otitis media episodes	1.0 (0.9-1.0)
Antibiotic courses for RTIs	1.3 (1.2-1.4)
FinnBrain cohort, n = 972	
Number of RTIs during age 0-1 year, No. (%)[b]	
0	627 (64.5)
1–4	281 (29.0)
5–10	61 (6.2)
>10	3 (0.3)
Number of antibiotic treatments for RTIs during age 0-1 year, No. (%)	
0	710 (73.0)
1–4	235 (24.0)
>4	27 (3.0)

CI, confidence interval; IQR, interquartile range; RTI, respiratory tract infection.

[a] Includes all RTIs with or without a physician visit. Incidence rates were calculated using negative binomial distribution and log-link with natural logarithm of the follow-up time as an offset.

[b] Includes only RTIs that necessitated a physician visit.

Table III. Association between IFI44L polymorphisms and rates of respiratory tract infections (RTIs) and related outcomes during age 0-2 years in the STEPS Study children (n = 1135)[a]

	IFI44L gene polymorphism	Genotype (No.)	Incidence rate per child-year (95% CI)[a]	Incidence rate ratio (95% CI)[a]	P
RTIs	rs273259	AA (491)	6.0 (5.7-6.3)	reference	
		AG (497)	5.8 (5.6-6.1)	0.98 (0.92-1.04)	.46
		GG (147)	5.7 (5.3-6.2)	0.97 (0.89-1.06)	.56
	rs1333969	CC (641)	6.0 (5.7-6.2)	reference	
		CT (414)	5.7 (5.4-6.0)	0.96 (0.90-1.01)	.14
		TT (80)	6.0 (5.4-6.7)	1.04 (0.93-1.16)	.46
Rhinovirus-positive RTIs	rs273259	AA (319)	2.1 (1.9-2.2)	reference	
		AG (321)	1.9 (1.7-2.0)	0.92 (0.83-1.03)	.16
		GG (96)	2.1 (1.8-2.5)	1.05 (0.89-1.24)	.54
	rs1333969	CC (409)	2.0 (1.9-2.2)	reference	
		CT (269)	1.9 (1.8-2.1)	0.95 (0.85-1.06)	.35
		TT (58)	2.2 (1.8-2.6)	1.12 (0.93-1.36)	.24
Days with RTI symptoms	rs273259	AA (445)	52.2 (48.7-55.9)	reference	
		AG (457)	50.0 (46.8-53.6)	0.96 (0.87-1.06)	.41
		GG (134)	45.6 (40.3-51.8)	0.90 (0.78-1.04)	.13
	rs1333969	CC (579)	52.7 (49.6-56.0)	reference	
		CT (384)	47.2 (43.9-50.9)	0.89 (0.81-0.98)	.02
		TT (73)	48.8 (41.3-58.1)	0.96 (0.81-1.15)	.67
Acute otitis media episodes	rs273259	AA (491)	1.1 (1.0-1.2)	reference	
		AG (497)	0.9 (0.8-1.0)	0.87 (0.75-1.01)	.07
		GG (147)	0.8 (0.6-1.0)	0.77 (0.61-0.96)	.02
	rs1333969	CC (641)	1.0 (1.0-1.2)	reference	
		CT (414)	0.9 (0.8-1.0)	0.87 (0.75-1.01)	.06
		TT (80)	0.7 (0.6-1.0)	0.74 (0.55-0.99)	.04
Antibiotic courses for RTIs	rs273259	AA (491)	1.4 (1.3-1.6)	reference	
		AG (497)	1.3 (1.1-1.4)	0.89 (0.78-1.03)	.13
		GG (147)	1.0 (0.9-1.3)	0.76 (0.62-0.95)	.02
	rs1333969	CC (641)	1.4 (1.3-1.5)	reference	
		CT (414)	1.3 (1.1-1.4)	0.91 (0.79-1.05)	.18
		TT (80)	1.0 (0.7-1.3)	0.73 (0.56-0.97)	.03

CI, confidence interval; RTI, respiratory tract infection.

[a] Incidence rates were analysed using negative binomial regression analysis with natural logarithm of the follow-up time as an offset, adjusting for sex and the presence of sibling(s) at birth.

Table IV. Associations between IFI44L polymorphisms and the frequency of respiratory tract infections (RTIs) and antibiotic treatments for respiratory infections in the FinnBrain cohort (n = 971 for rs133969 and n = 970 for rs273259)

	IFI44L gene polymorphism	**Genotype (No.)**	**OR (95% CI)[a]**	**P**
RTIs[b]	rs273259	AA (396)	reference	
		AG (441)	0.65 (0.48-0.86)	.003
		GG (133)	0.64 (0.42-0.97)	.04
	rs1333969	CC (535)	reference	
		CT (357)	0.70 (0.53-0.94)	.02
		TT (79)	0.67 (0.40-1.09)	.11
Antibiotic courses for RTIs[c]	rs273259	AA (396)	reference	
		AG (441)	0.68 (0.50-0.93)	.02
		GG (133)	0.76 (0.48-1.18)	.23
	rs1333969	CC (535)	reference	
		CT (357)	0.72 (0.52-0.98)	.04
		TT (79)	0.92 (0.53-1.53)	.75

CI, confidence interval; OR, odds ratio; RTI, respiratory tract infection.

[a] Ordinal logistic regression adjusted for sex and presence of sibling(s) at birth.

[b] The frequency of acute respiratory infections from birth to 1 year of age was categorized in four groups: 0, 1-4, 5-10, and >10 respiratory tract infections.

[c] The number of antibiotic courses from birth to 1 year of age was categorized in three groups: 0, 1-4, and 5 or more antibiotic treatments.

Online repository

Associations between IFI44L gene variants and rates of respiratory tract infections during early childhood

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Statistical analysis

In the STEPS Study, the association between IFI44L polymorphisms rs273259 and rs1333969 and the incidence rate of respiratory tract infections (RTIs), rhinovirus-positive RTIs, days with RTI symptoms, acute otitis media episodes, and antibiotic treatments for RTIs during age 0-2 years were analyzed using negative binomial regression analysis with natural logarithm of the follow-up time as an offset, adjusting for sex and the presence of sibling(s) at birth (using R version 3.5.3). In the FinnBrain Birth Cohort Study, the associations between single nucleotide polymorphisms (SNPs) rs273259 and rs1333969 and RTIs as well as antibiotic courses for RTIs were tested with linear regression analysis implemented with PLINK.[1] Final analysis with adjustment for sex and the presence of sibling(s) at birth was done using ordinal logistic regression. The ordinal logistic regression analyses were performed in R 3.5.3[2] using the polr function in the MASS package.[3] Two-tailed *P* values were reported, with $P < 0.05$ considered statistically significant.

Transcriptome analysis

mRNA samples

Data on mRNA transcriptional profiles was available from a subset of 81 children in the FinnBrain study cohort. Four samples were excluded due to low sample quality and 6 samples were excluded due to missing data on IFI44L polymorphisms. Subsequently, samples from 71 children were included in the downstream analyses. (Table S2).

mRNA sample collection and data pre-processing

Blood samples were collected during a pre-scheduled study visit at 3 months of age. 1 ml of blood was drawn in Tempus tubes (Applied Biosystems, Foster City, CA) and stored in -20 °C. RNA was extracted and hybridized to Illumina HT-12 V4 beadchips (Illumina, San Diego, CA). After hybridization beadchips were scanned on Illumina Beadstation 500 and Illumina GenomeStudio software (Illumina, San Diego, CA) was used to subtract background and for average signal intensity scaling (average normalization). All raw expression values <10 were set to 10 and the data was log2-transformed.

Data analysis

As the number of children with minor/minor genotypes was low (Table S2), minor/minor homozygotes (rs1333969 TT and rs273259 GG) and major/minor heterozygotes (rs1333969 CT and rs273259 AG) were combined in a single class ("other genotype") for each of the studied polymorphism and gene expression analyses were performed by comparing major/major genotypes to "other genotypes" (including major/minor and minor/minor).

IFI44L expression

HT-12 V4 beadchips contain two probes targeting IFI44L (ILMN_1723912 and ILMN_1835092). The expression values of these two probes were highly correlated (Spearman r 0.906, P <0.0001, Figure S1) and the mean of the two probes was used as expression value for IFI44L. Expression

values were analyzed according to genotype and virus detection and compared by the Mann-Whitney test. Analysis was performed using GraphPad Prism software version 8.0.0 (Graphpad, San Diego, CA).

Differential gene expression analysis

Data was first filtered by including only transcripts that were 'present' (signal precision <0.01) in ≥10% of the samples (PAL10%, 18,636 transcripts) in the downstream analyses. limma[4] package and R[2] version 3.5.1 were used to detect differentially expressed genes (DEGs) between the groups. False discovery rate (FDR) corrected *P* value 0.05 and 1.25 fold change were used as cut-offs for DEGs. Gene set analysis was performed for functional characterization of DEGs by analyzing Gene Ontology (GO)[5,6] biological processes terms associated with DEG lists. For this we used PANTHER overreprentation test (available at http://geneontology.org/) with Homo sapiens reference gene list and Fisher's exact test with Benjamini-Hochberg multiple test correction. Ingenuity Pathway Analysis software (QIAGEN, Redwood City, CA, USA) was used to further explore affected pathways.

E References

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E6. The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res [Internet]. 2019 [cited 2019 Mar 17];47:D330–8. Available from: https://academic.oup.com/nar/article/47/D1/D330/5160994

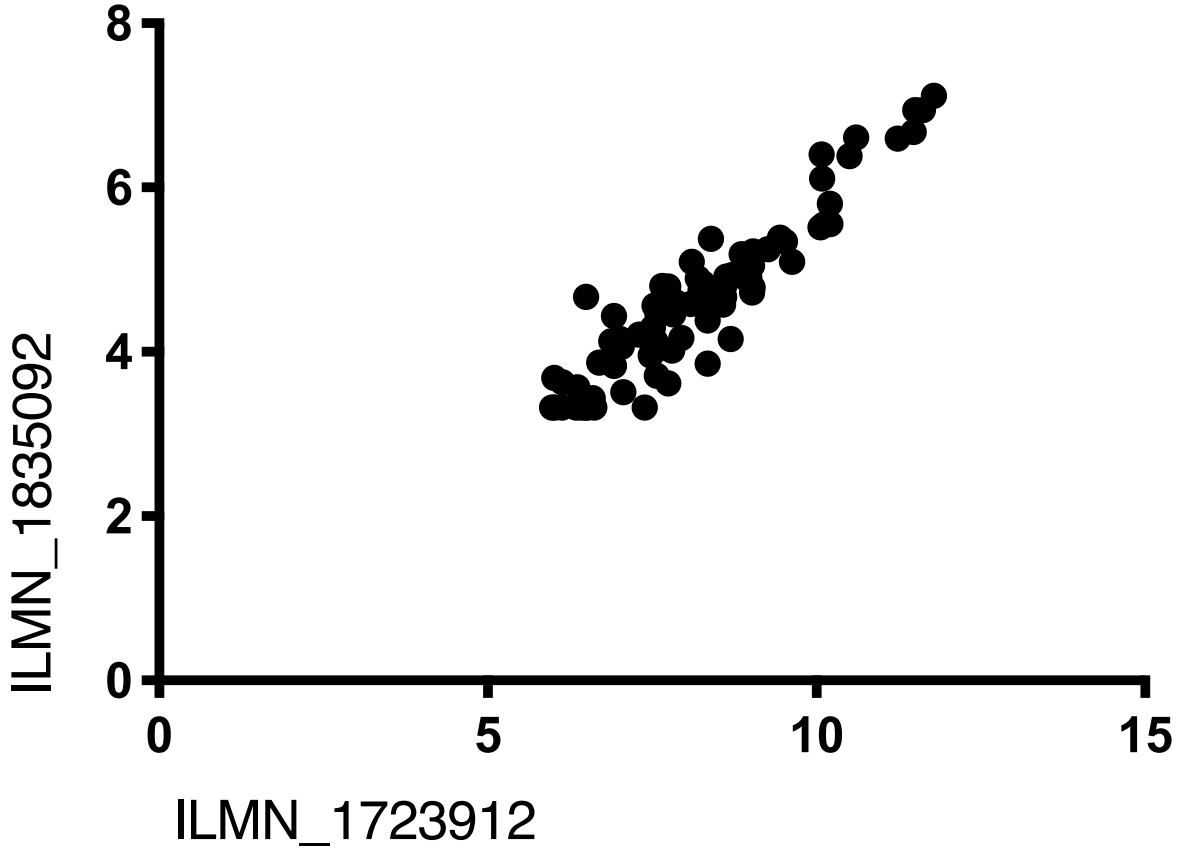
Figure E1. Correlation of the expression values of two probes targeting IFI44L in the Illumina HT-12 V4 beadchips (ILMN_1723912 and ILMN_1835092) in 71 children with transcriptome data available. As the two expression values had a strong correlation (Spearman r 0.906, P <0.0001), a mean value of the two probes was used in downstream analyses. All values are log2 transformed.

Figure E2. Heatmap visualising the expression levels of 134 probes targeting 116 differentially expressed genes (DEGs) between rs1333969 CC (major/major) and other genotypes in children with viral detections (n=25). False discovery rate corrected *P* value 0.05 and 1.25 fold change were used as cut-offs to detect DEGs. DEGs are listed in Supplementary Table 3. Expression values are log2 transformed and normalized to median of the class "other genotype" (including CT [major/minor] and TT [minor/minor] genotypes). Samples are clustered using hierarchical clustering and Euclidean distance and colored according to rs1333969 genotype: CC (major/major)=green; CT (major/minor)=grey; TT (minor/minor)=magenta.
DEG, differentially expressed gene

Figure E3. Heatmap visualising the expression levels of 24 probes targeting 23 differentially expressed genes (DEGs) between rs273259 AA (major/major) and other genotypes in children with viral detections (n=25). False discovery rate corrected *P* value 0.05 and 1.25 fold change were used as cut-offs to detect DEGs. DEGs are listed in Supplementary Table 5. Expression values are log2 transformed and normalized to median of the class "other genotype" (including AG [major/minor] and GG [minor/minor] genotypes). Samples are clustered using hierarchical clustering and Euclidean distance and colored according to rs273259 genotype: AA (major/major)=green; AG (major/minor)= grey; GG (minor/minor) magenta.

DEG, differentially expressed gene

Figure E1.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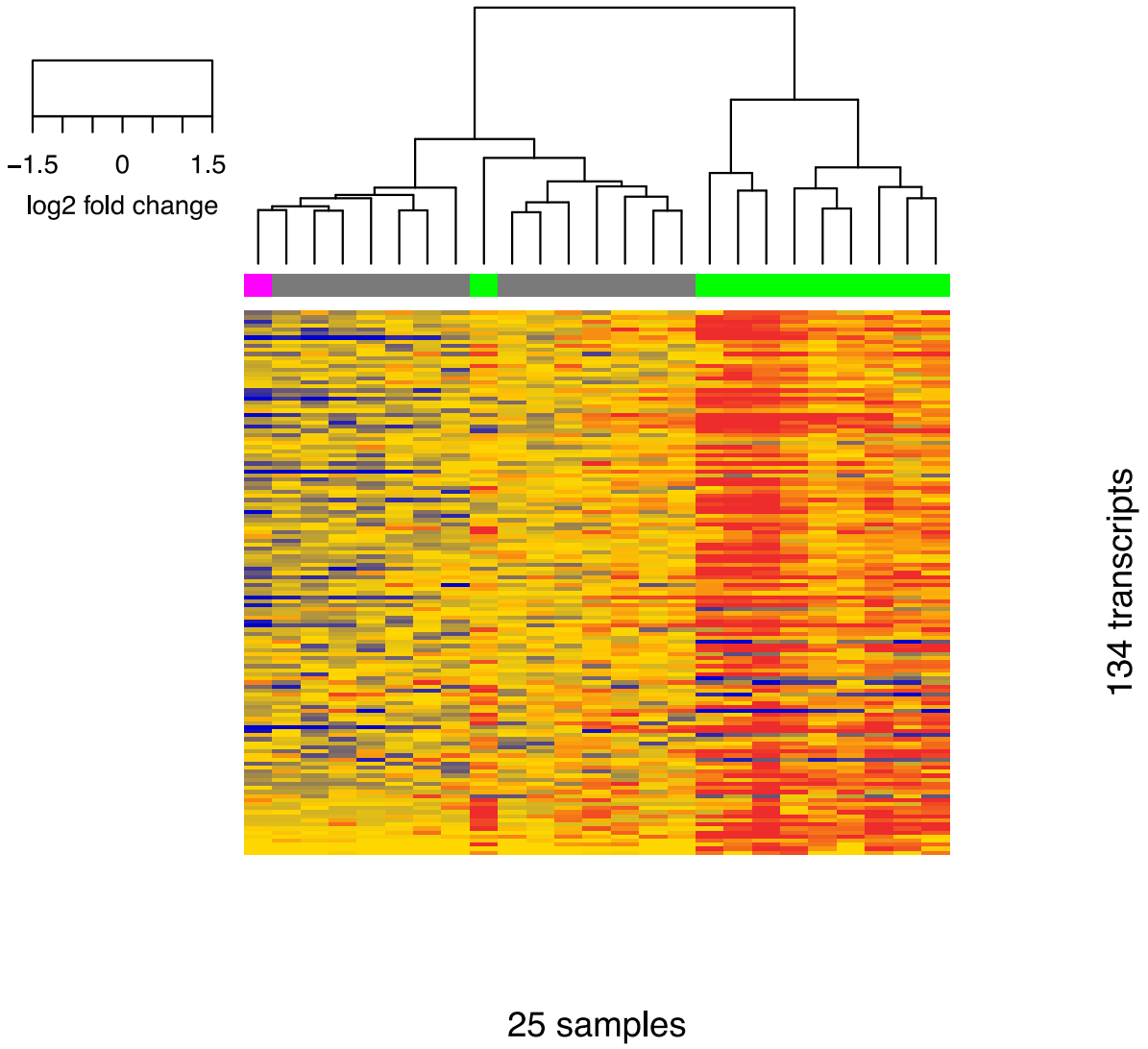
ILMN_1723912

Figure E2.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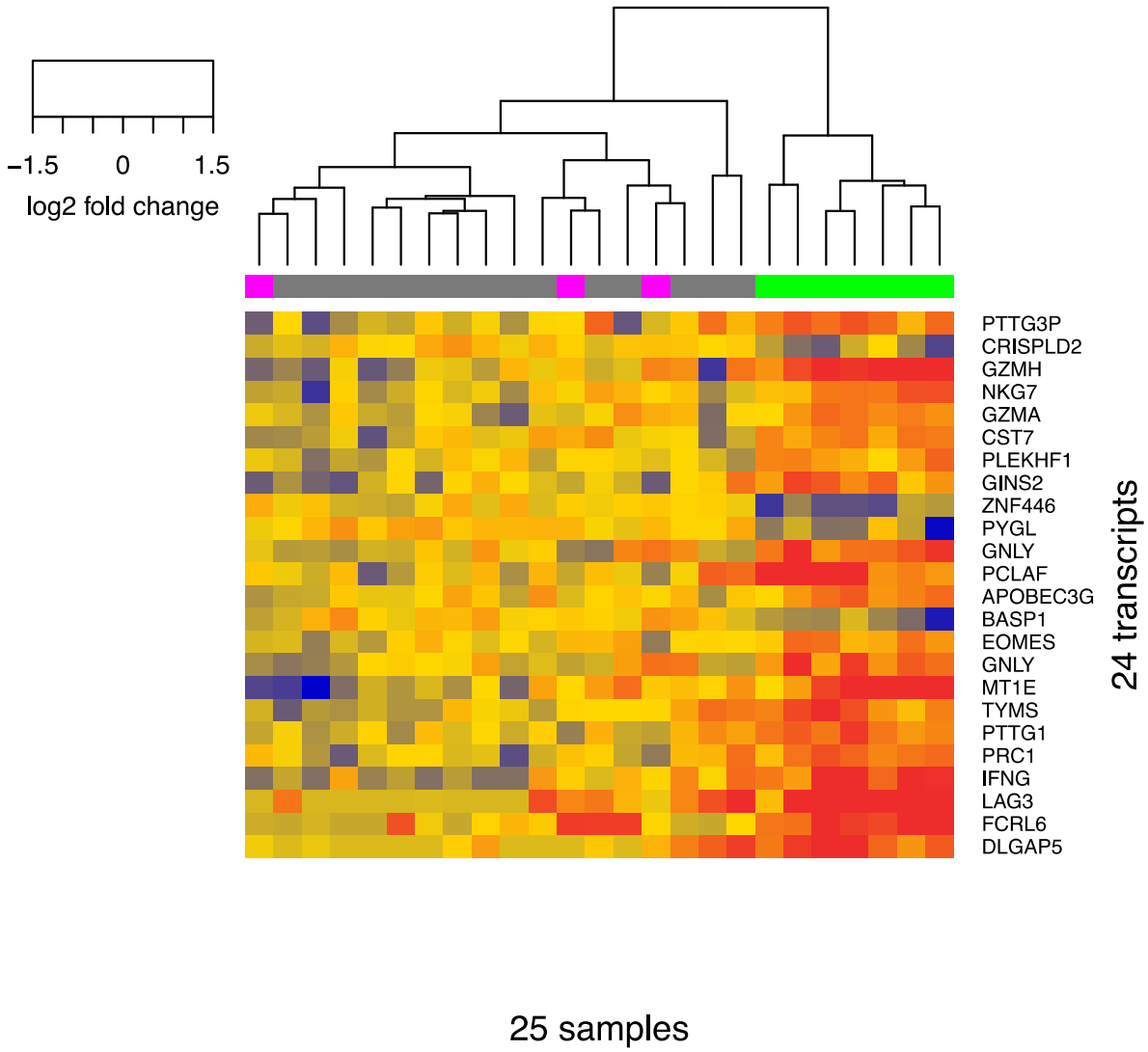
0
1.5
log2 fold change
134 transcripts
25 samples

Figure E3.


−1.5
0
1.5
log2 fold change
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CRISPLD2
GZMH
NKG7
GZMA
CST7
PLEKHF1
GINS2
ZNF446
PYGL
GNLY
PCLAF
APOBEC3G
BASP1
EOMES
GNLY
MT1E
TYMS
PTTG1
PRC1
IFNG
LAG3
FCRL6
DLGAP5
24 transcripts
25 samples

Table E1. Background characteristics, allelic distribution of IFI44L polymorphisms, and respiratory viruses detected at 3 months of age in children included in transcriptome analysis in the FinnBrain Birth Cohort Study.

	FinnBrain Cohort, No. (%)
Female	36 (51)
Older siblings	31 (44)
IFI44L rs273259	
GG	11 (15)
AG	33 (47)
AA	27 (38)
IFI44L rs1333969	
TT	6 (8)
CT	30 (42)
CC	35 (50)
Detection of ≥1 respiratory virus*	25 (36)
Rhinovirus	16 (23)
Adenovirus	3 (4)
Coronavirus	3 (4)
Respiratory syncytial virus	2 (3)
Bocavirus	1 (1)
Influenza A virus	1 (1)

* Data on respiratory viruses was available from 70/71 children. 1 child had two viruses (rhinovirus and adenovirus) detected concomitantly.

Table E2. Unadjusted analysis of association between IFI44L polymorphisms with rates of respiratory tract infections (RTIs) and related outcomes during age 0-2 years in the STEPS Study children (n=1135)[a]

	IFI44L polymorphism	Genotype (No.)	Incidence rate per child-year (95% CI)[a]	Unadjusted incidence rate ratio (95% CI)[a]	P
RTIs	rs273259	GG (147)	5.7 (5.3-6.2)	0.95 (0.87-1.04)	.28
		AG (497)	5.8 (5.6-6.1)	0.97 (0.91-1.03)	.35
		AA (491)	6.0 (5.7-6.3)	reference	
	rs1333969	TT (80)	6.0 (5.4-6.7)	1.01 (0.90-1.13)	.91
		CT (414)	5.7 (5.4-6.0)	0.95 (0.90-1.01)	.12
		CC (641)	6.0 (5.7-6.2)	reference	
Rhinovirus-positive	rs273259	GG (96)	2.1 (1.8-2.5)	1.04 (0.88-1.23)	.62
RTIs		AG (321)	1.9 (1.7-2.0)	0.92 (0.83-1.03)	.17
		AA (319)	2.1 (1.9-2.2)	reference	
	rs1333969	TT (58)	2.2 (1.8-2.6)	1.10 (0.90-1.33)	.36
		CT (269)	1.9 (1.8-2.1)	0.96 (0.86-1.07)	.46
		CC (409)	2.0 (1.9-2.2)	reference	
Days with RTI	rs273259	GG (134)	45.6 (40.3-51.8)	0.87 (0.76-1.01)	.06
symptoms		AG (457)	50.0 (46.8-53.6)	0.96 (0.87-1.06)	.40
		AA (445)	52.2 (48.7-55.9)	reference	
	rs1333969	TT (73)	48.8 (41.3-58.1)	0.93 (0.78-1.11)	.41
		CT (384)	47.2 (43.9-50.9)	0.90 (0.81-0.99)	.03
		CC (579)	52.7 (49.6-56.0)	reference	
Acute otitis media	rs273259	GG (147)	0.8 (0.6-1.0)	0.74 (0.59-0.93)	.01
episodes		AG (497)	0.9 (0.8-1.0)	0.86 (0.74-1.00)	.05
		AA (491)	1.1 (1.0-1.2)	reference	
	rs1333969	TT (80)	0.7 (0.6-1.0)	0.70 (0.52-0.94)	.02
		CT (414)	0.9 (0.8-1.0)	0.87 (0.75-1.01)	.07
		CC (641)	1.0 (1.0-1.2)	reference	
Antibiotic courses for	rs273259	GG (147)	1.0 (0.9-1.3)	0.73 (0.59-0.91)	.01
RTIs		AG (497)	1.3 (1.1-1.4)	0.88 (0.77-1.02)	.10
		AA (491)	1.4 (1.3-1.6)	reference	
	rs1333969	TT (80)	1.0 (0.7-1.3)	0.70 (0.53-0.92)	.01
		CT (414)	1.3 (1.1-1.4)	0.90 (0.78-1.05)	.18
		CC (641)	1.4 (1.3-1.5)	reference	

CI, confidence interval; RTI, respiratory tract infection.

[a] Incidence rates were analysed using negative binomial regression analysis with natural logarithm of the follow-up time as an offset.

Table E3. List of 116 differentially expressed genes (DEGs) between rs1333969 CC (major/major) and other genotypes in children with viral detections (n=25). False discovery rate corrected *P* value 0.05 and 1.25 fold change were used as cut-offs to detect DEGs. Along with the Gene symbol, also the Illumina probe identifier and expression level in rs1333969 CC (major/major) is presented. "+" denotes higher expression level and "-" lower expression level compared to other genotypes.

Gene Symbol	Illumina probe identifier	Expression level in rs1333969 CC (major/major) genotype
AIM2	ILMN_1681301	+
ANKRD22	ILMN_2132599	+
APOBEC3G	ILMN_1802106	+
APOL3	ILMN_1756862	+
ASCL2	ILMN_1723412	+
ATF3	ILMN_2374865	+
AURKA	ILMN_1680955	+
BATF	ILMN_1668822	+
BATF2	ILMN_1690241	+
BIRC5	ILMN_2349459	+
BLVRA	ILMN_1691436	+
BST2	ILMN_3259146	+
C3orf14	ILMN_2224486	+
CCNA2	ILMN_1786125	+
CCNB2	ILMN_1801939	+
CD48	ILMN_2061043	+
CDC20	ILMN_1663390	+
CDC45	ILMN_1670238	+
CENPE	ILMN_1716279	+
CEP55	ILMN_1747016	+
CKS2	ILMN_1756326	+
CST7	ILMN_1679826	+
CXCL10	ILMN_1791759	+
DHX58	ILMN_1678422	+
DLGAP5	ILMN_3239771	+
DTX3L	ILMN_1784380	+
EPSTI1	ILMN_2388547	+
FABP5	ILMN_3266606	+
FASLG	ILMN_1781824	+
FBXO6	ILMN_1701455	+
FGFBP2	ILMN_1761945	+
GBP1	ILMN_2148785	+
GBP2	ILMN_1774077	+
GBP4	ILMN_1771385	+
GBP5	ILMN_2114568	+
GCH1	ILMN_1812759	+
GINS2	ILMN_1809590	+
GNLY	ILMN_1790692	+
GZMA	ILMN_1779324	+
GZMB	ILMN_2109489	+
GZMH	ILMN_1731233	+
HCST	ILMN_2396991	+
HERC5	ILMN_1729749	+
HMMR	ILMN_2409220	+
HSPB11	ILMN_1681340	+

IDH2	ILMN_1751753	+
IFI35	ILMN_1745374	+
IFI44	ILMN_1760062	+
IFI44L	ILMN_1723912	+
IFI6	ILMN_2347798	+
IFIT1	ILMN_1707695	+
IFIT2	ILMN_1739428	+
IFIT3	ILMN_1701789	+
IFITM3	ILMN_1805750	+
IFNG	ILMN_2207291	+
IRF1	ILMN_1708375	+
IRF7	ILMN_2349061	+
ISG15	ILMN_2054019	+
KDELC2	ILMN_1651557	+
KIAA0101	ILMN_2285996	+
KIF2C	ILMN_1685916	+
LAG3	ILMN_1813338	+
LAP3	ILMN_3295494	+
LY6E	ILMN_1695404	+
MT1A	ILMN_1691156	+
MT1E	ILMN_2173611	+
MT1F	ILMN_1718766	+
MT1IP	ILMN_2136089	+
MT2A	ILMN_1686664	+
NCAPG	ILMN_1751444	+
NKG7	ILMN_1682993	+
OAS1	ILMN_2410826	+
OAS3	ILMN_1745397	+
OASL	ILMN_1674811	+
PARP12	ILMN_1718558	+
PARP9	ILMN_1731224	+
PARPBP	ILMN_1727055	+
PI4K2B	ILMN_1815134	+
POLE2	ILMN_1774336	+
PRC1	ILMN_1728934	+
PSMA3	ILMN_2387553	+
PSMB8	ILMN_2390299	+
PSMB9	ILMN_2376108	+
PSME2	ILMN_1786612	+
PTTG1	ILMN_2042771	+
PTTG3P	ILMN_2049021	+
RARRES3	ILMN_1701613	+
RSAD2	ILMN_1657871	+
RTP4	ILMN_2173975	+
SCO2	ILMN_1701621	+
SERPING1	ILMN_1670305	+
SGOL1	ILMN_1730825	+
SP140	ILMN_2246882	+
SPATS2L	ILMN_1683678	+
STAT1	ILMN_1777325	+
TAP1	ILMN_1751079	+
TIMM23	ILMN_1664231	+
TK1	ILMN_1806037	+
TRIM22	ILMN_1779252	+
TYMP	ILMN_3223126	+
TYMS	ILMN_1806040	+
UBE2C	ILMN_2301083	+
UBE2L6	ILMN_1769520	+

USP18	ILMN_3240420	+
VAMP5	ILMN_1809467	+
ADAM19	ILMN_1713751	-
ALOX15	ILMN_1783443	-
CAMK2G	ILMN_2359601	-
DPEP2	ILMN_1689160	-
EMR4P	ILMN_3243190	-
LZTR1	ILMN_1805161	-
MEGF6	ILMN_3241441	-
OVGP1	ILMN_1734542	-
PRSS33	ILMN_1736831	-
PVALB	ILMN_2069224	-
RELL1	ILMN_3233388	-

Table E4. Gene Ontology (GO) biological process terms associated with the 116 differentially expressed genes (DEG) between IFI44L rs1333969 genotypes. Association between the GO biological process terms and the DEG lists is presented as an overlap between the GO terms and the DEG list and with a *P* value determining the probability that the overlap would be explained by chance alone. *P* values are calculated using Fisher's exact test with Benjamini-Hochberg multiple test correction.

GO Biological Process Term	Overlap	Adjusted P value
Type I interferon signaling pathway (GO:0060337)	17/66	3.3E-21
Cellular response to type I interferon (GO:0071357)	17/66	3.3E-21
Cytokine-mediated signaling pathway (GO:0019221)	31/634	9.9E-18
Cellular response to interferon-gamma (GO:0071346)	12/117	8.0E-10
Positive regulation of defense response to virus by host (GO:0002230)	6/22	2.2E-07
Interferon-gamma-mediated signaling pathway (GO:0060333)	12/71	2.1E-12
Regulation of defense response to virus by host (GO:0050691)	7/31	4.7E-08
Negative regulation of viral genome replication (GO:0045071)	9/51	2.3E-09
Regulation of nuclease activity (GO:0032069)	3/7	2.6E-04
Negative regulation of viral life cycle (GO:1903901)	9/62	1.2E-08
Regulation of mitotic sister chromatid separation (GO:0010965)	4/16	1.2E-04
Regulation of viral genome replication (GO:0045069)	9/64	1.5E-08
Response to interferon-beta (GO:0035456)	4/20	2.4E-04

Anaphase-promoting complex-dependent catabolic process (GO:0031145)	8/80	1.9E-06
Regulation of mitotic cell cycle phase transition (GO:1901990)	9/185	1.0E-04
Granzyme-mediated apoptotic signaling pathway (GO:0008626)	2/7	9.6E-03
Cellular response to zinc ion (GO:0071294)	4/20	2.4E-04
Positive regulation of ubiquitin protein ligase activity (GO:1904668)	6/83	3.4E-04
Response to copper ion (GO:0046688)	4/26	5.1E-04

Table E5. List of 24 differentially expressed genes (DEGs) between rs273259 AA (major/major) and other genotypes in children with viral detections (n=25). False discovery rate corrected *P* value 0.05 and 1.25 fold change were used as cut-offs to detect DEGs. Along with the Gene symbol, also the Illumina probe identifier and expression level in rs273259 AA (major/major) is presented. "+" denotes higher expression level and "-" lower expression level compared to other genotypes.

Gene Symbol	Illumina probe identifier	Expression level in rs273259 AA (major/major) genotype
APOBEC3G	ILMN_1802106	+
CST7	ILMN_1679826	+
DLGAP5	ILMN_1749829	+
EOMES	ILMN_1760509	+
FCRL6	ILMN_2074762	+
GINS2	ILMN_1809590	+
GNLY	ILMN_1708779	+
GZMA	ILMN_1779324	+
GZMH	ILMN_1731233	+
IFNG	ILMN_2207291	+
KIAA0101	ILMN_2285996	+
LAG3	ILMN_1813338	+
MT1E	ILMN_2173611	+
NKG7	ILMN_1682993	+
PLEKHF1	ILMN_1708041	+
PRC1	ILMN_1728934	+
PTTG1	ILMN_2042771	+
PTTG3P	ILMN_2049021	+
TYMS	ILMN_1806040	+
BASP1	ILMN_1651826	-
CRISPLD2	ILMN_1790689	-
PYGL	ILMN_1696187	-
ZNF446	ILMN_1743767	-