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Associations Between IFI44L Gene Variants and Rates of Respiratory Tract Infections During Early Childhood

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1 **Associations between IFI44L gene variants and rates of respiratory tract infections during**
2 **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

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

77

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

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- 82
- 83 **ABBREVIATIONS**
- 84 DEG: Differentially expressed gene
- 85 GO: Gene Ontology
- 86 IFI44L: Interferon-induced protein 44-like
- 87 IFN: Type I interferon
- 88 ISG: Interferon-stimulated gene
- 89 MBL: Mannose-binding lectin
- 90 mRNA: Messenger RNA
- 91 RTI: Respiratory tract infection
- 92 SNP: Single nucleotide polymorphism
- 93 STEPS: Steps to the Healthy Development and Well-being of Children
- 94 TLR: Toll-like receptor

95 **INTRODUCTION**

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

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

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

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

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

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139 **METHODS**

140 **Study populations**

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

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

147

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

158

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

170

171 Respiratory virus detection

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

182

183 Definitions of respiratory infection outcomes

184 In the STEPS Study, an episode of RTI was defined as the presence of rhinitis or cough, with or
185 without fever or wheezing, documented in the diary by the parents, or as a physician-diagnosed RTI
186 as previously described.³ The number of days with RTI symptoms was analyzed using data filled
187 into daily diaries by the parents. Acute otitis media was diagnosed by a study physician or recorded
188 into the diary or medical records by a physician at an outpatient office or hospital. If there were
189 repeated diagnoses of acute otitis media during continuous respiratory symptoms, parallel diagnoses
190 within 14 days were calculated as one diagnosis. As rhinovirus was the most frequent virus
191 identified in the STEPS Study³, we report separately the rates of rhinovirus-positive RTIs.

192

193 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

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

196

197 **Genetic analysis**

198 In the STEPS Study, DNA was extracted from whole blood according to standard procedures and
199 IFI44L SNPs rs273259 and rs1333969 were analyzed using the Sequenom platform (San Diego,
200 CA) at Genome Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland. In
201 quality control analysis both SNPs had genotype missingness per marker below the threshold of 5%
202 (2.7% and 1.1%, respectively) and both had non-significant Hardy-Weinberg P values ($P = .61$ and
203 $P = .30$, respectively). SNPs rs273259 and rs1333969 show mild linkage disequilibrium ($r^2=0.58$)
204 among the Finnish population.²⁴

205 In the FinnBrain Birth Cohort Study, DNA was extracted from whole blood according
206 to standard procedures and genotyped with Illumina Infinium PsychArray BeadChip comprising
207 603132 SNPs at Estonian Genome Centre, Tartu, Estonia. Quality control was performed with
208 PLINK 1.9 (<http://www.cog-genomics.org/plink/1.9/>).²⁵ Markers were removed for missingness
209 ($>5\%$) and Hardy-Weinberg equilibrium (P value $< 1 \times 10^{-6}$). Individuals were checked for missing
210 genotypes ($>5\%$), relatedness (identical by descent calculation, $PI_HAT>0.2$) and population
211 stratification (multidimensional scaling).

212

213 **Transcriptome analysis**

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

219 expression and transcriptional profiles were compared between 1) major/major genotypes, and 2)
220 other genotypes (including major/minor and minor/minor genotypes). First, we analyzed if the
221 presence of IFI44L polymorphisms influenced IFI44L mRNA expression. To further explore if
222 IFI44L polymorphisms were associated with altered expression of other genes, we performed
223 differential gene expression analysis between the genotypes. Limma²⁷ package in R with false
224 discovery rate (FDR) adjusted *P* value of 0.05 and 1.25 fold change were used to detect
225 differentially expressed genes (DEGs). To assess function of the DEGs and affected pathways, we
226 used Gene Ontology (GO)^{28, 29} biological processes terms and Ingenuity Pathway Analysis (IPA)
227 software (QIAGEN, Redwood City, CA, USA). The data is deposited in the NCBI Gene Expression
228 Omnibus (GEO accession number: XXX).

229

230 **Statistical analysis**

231 In the STEPS Study, the association between the IFI44L genotypes and respiratory infection
232 outcomes in children 0-2 years of age were analyzed using negative binomial regression analysis
233 with natural logarithm of the follow-up time as an offset. Unadjusted and adjusted incidence rate
234 ratios (aIRR, adjusted for sex and the presence of sibling(s) at birth) were reported. In the FinnBrain
235 Birth Cohort Study, the association between the IFI44L genotypes and RTIs and antibiotic
236 treatments for RTIs from birth to 1 year of age were first tested with linear regression analysis
237 implemented with PLINK (Purcell). The number of RTIs was then categorized in four groups: 0, 1-
238 4, 5-10, and >10, and the number of antibiotic courses in three groups: 0, 1-4, and 5 or more. Final
239 analysis with adjustment for sex and the presence of sibling(s) at birth was done using ordinal
240 logistic regression to explore whether the odds of being in a higher category was associated with the
241 heterozygous or homozygous polymorphisms of IFI44L. The selection of sex and presence of
242 sibling(s) as covariates in the final models was based on *a priori* knowledge. We have previously
243 published that, in the STEPS Study, male sex and the presence of older siblings were risk factors for

244 respiratory infection outcomes, while breastfeeding, parental smoking or daycare attendance were
245 not associated with an increased risk of respiratory infections¹³. Regression analyses were
246 performed using R 3.5.3. Two-tailed *P* values of less than .05 were considered significant.

247

248 **RESULTS**

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

254

255 **IFI44L genotypes and respiratory infections in the STEPS Study cohort**

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

261 The minor G allele of the rs273259 polymorphism was associated with decreased
262 rates of acute otitis media both in unadjusted and adjusted analysis (Table 3 and Table E2).

263 Children with a homozygous GG (minor/minor) genotype had lower rates of acute otitis media
264 compared to children with the AA (major/major) genotype (aIRR, 0.77 [95% CI, 0.61-0.96]).

265 Similarly, the minor T allele of the rs1333969 polymorphism was associated with decreased rates of
266 acute otitis media. GG (minor/minor) genotype of the rs273259 and TT (minor/minor) genotype of
267 the rs1333969 were associated with lower rates of antibiotic courses for RTIs compared with
268 children with the respective major/major genotypes (aIRR [95% CI] for rs273259, 0.76 [0.62-0.95],

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

270

271 **IFI44L genotypes and respiratory infections in the FinnBrain Study cohort**

272 In ordinal logistic regression analyses adjusted for sex and presence of sibling(s), the G allele of the
273 rs273259 polymorphism in the IFI44L gene was associated with lower number of RTIs during the
274 first year of life. Children with the minor/minor genotype GG had an odds ratio (OR) of 0.64 [95%
275 CI, 0.42-0.97], $P = .04$, and children with the major/minor AG genotype an OR of 0.65 [95% CI,
276 0.48-0.86], $P = .003$, for RTI frequency compared to the major/major genotype (Table 4). The
277 rs1333969 minor allele T was similarly associated with a decreased frequency of RTIs.

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

283

284 **Effects of IFI44L gene variants on peripheral blood transcription patterns**

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

290 First, we compared IFI44L expression in all 71 children according to the rs273259
291 and rs1333969 genotypes and found no differences between the groups (Figure 1, panels A and B).
292 Next, we compared IFI44L expression in a subset of children ($n = 25$; Table E2) who were positive
293 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

319 **DISCUSSION**

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

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

342 The role of ISGs in the immune defense is poorly described. IFI44L expression
343 increases after infection of dendritic cells with measles virus³⁰ 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

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

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

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

389

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491 **Figure legends**492 **Figure 1. Expression values of IFI44L according to IFI44L polymorphisms and virus**

493 **detection.** In 71 children with transcriptome data available there were no differences in IFI44L
494 expression according to rs1333969 (panel A) or rs273259 (panel B) genotype. However, in a
495 subgroup of children with respiratory virus detection at the time of mRNA sampling ($n = 25$),
496 IFI44L expression differed according to the genotype (panels C and D). In virus-positive children
497 rs1333969 major/major genotype was associated with higher IFI44L overexpression compared to
498 other genotypes (major/minor and minor/minor genotypes combined) (panel C; Mann-Whitney $P =$
499 $.0036$). Similar finding was observed with rs273259 (panel D; $P = .048$).

500

501 **Figure 2. rs1333969 genotype of IFI44L affects expression of other transcripts in the**

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

509

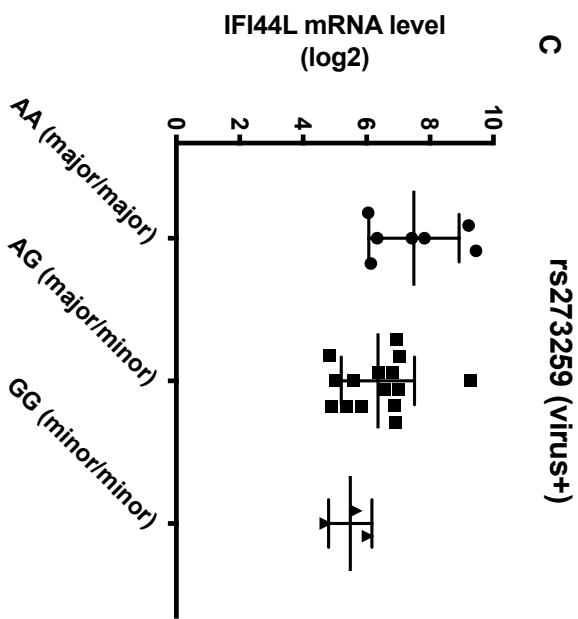
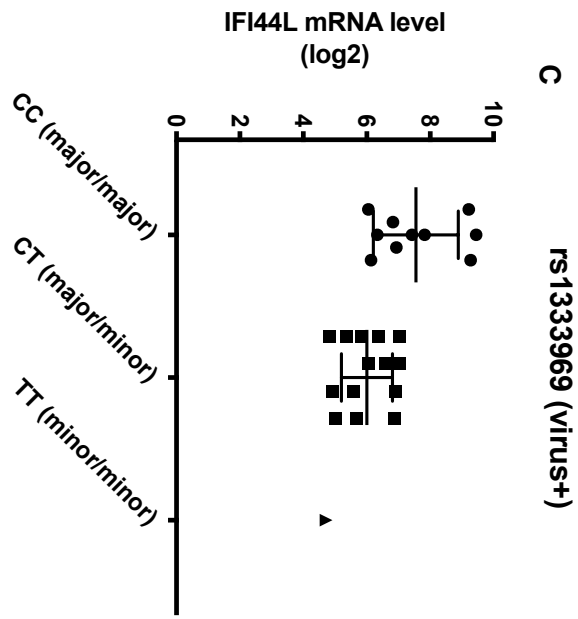
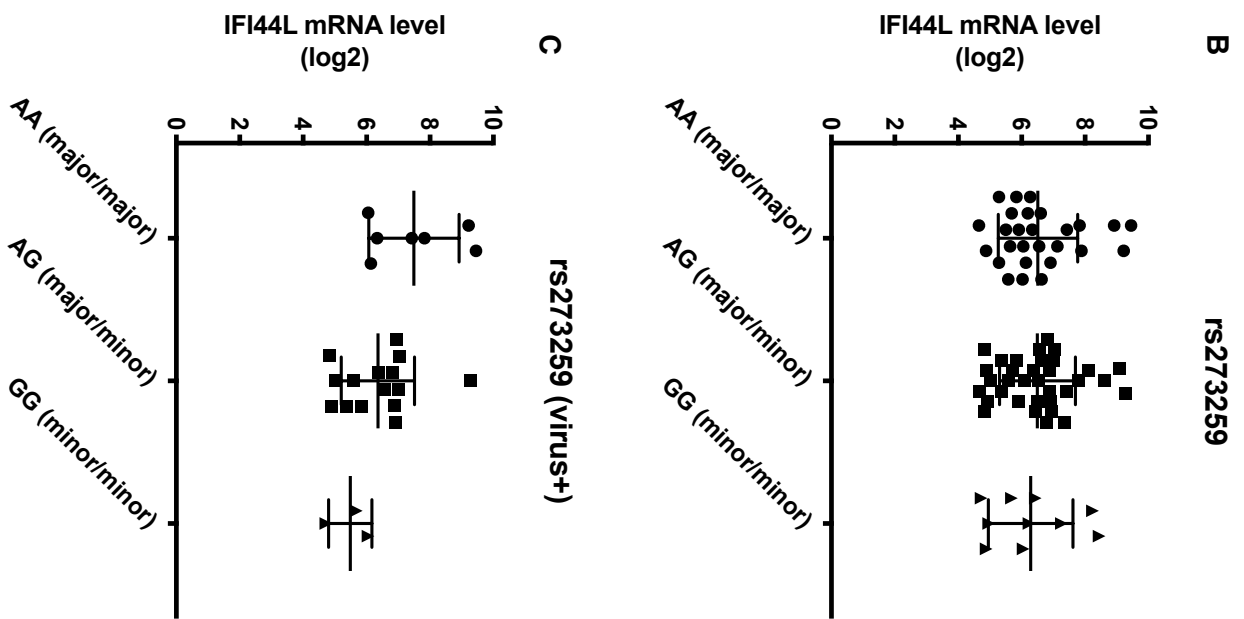
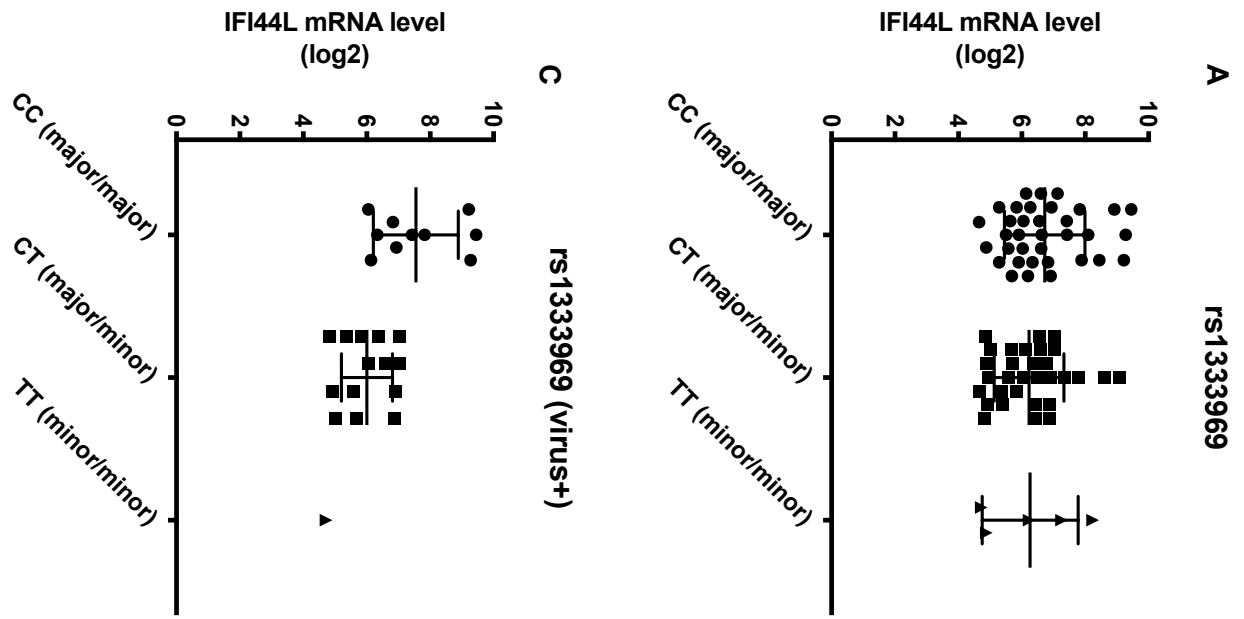
510 **Figure 3. Venn diagram presenting the number of differentially expressed and overlapping**
511 **genes in children with different genotypes of rs1333969 and rs273259 polymorphisms.**

512 Differential expression analysis was performed comparing major/major genotype to other
513 genotypes (major/minor and minor/minor). False discovery rate adjusted P value $.05$ and 1.25 fold
514 change were used as cut-offs for differentially expressed genes.

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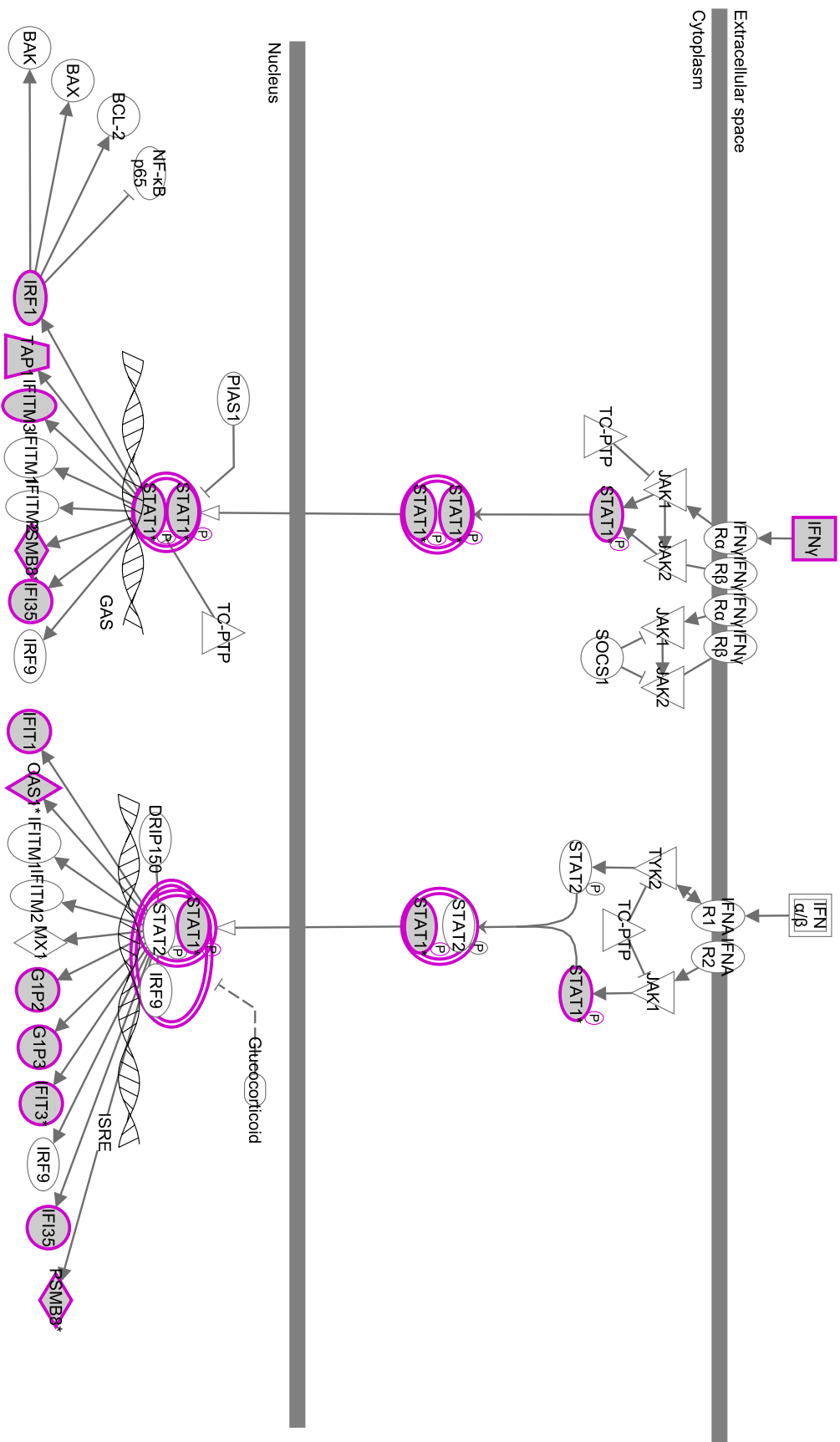
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517 Figure 1



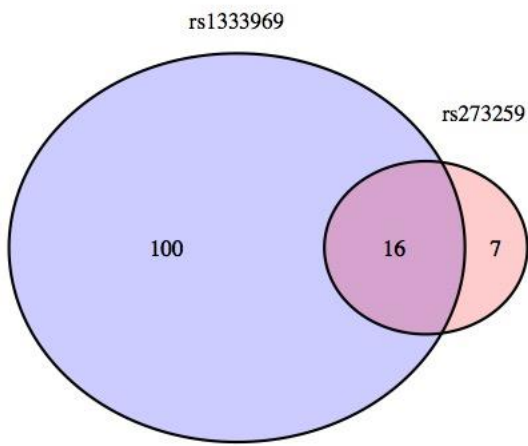
519 Figure 2.

Interferon Signaling



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521 Figure 3.



522

523

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 rs1333969 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 3.5.3² 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 $\geq 10\%$ of the samples (PAL10%, 18,636 transcripts) in the downstream analyses. limma⁴ package and R² 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 overrepresentation 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.

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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.

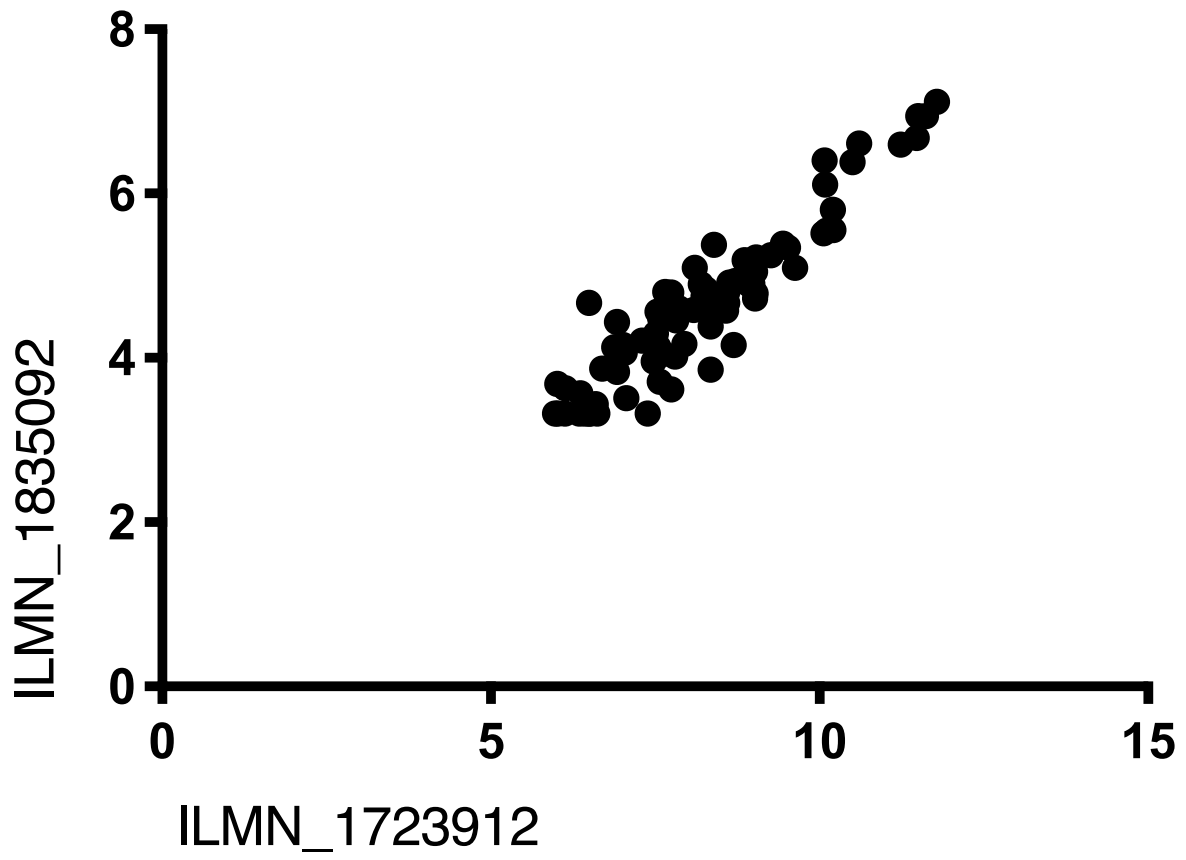


Figure E2.

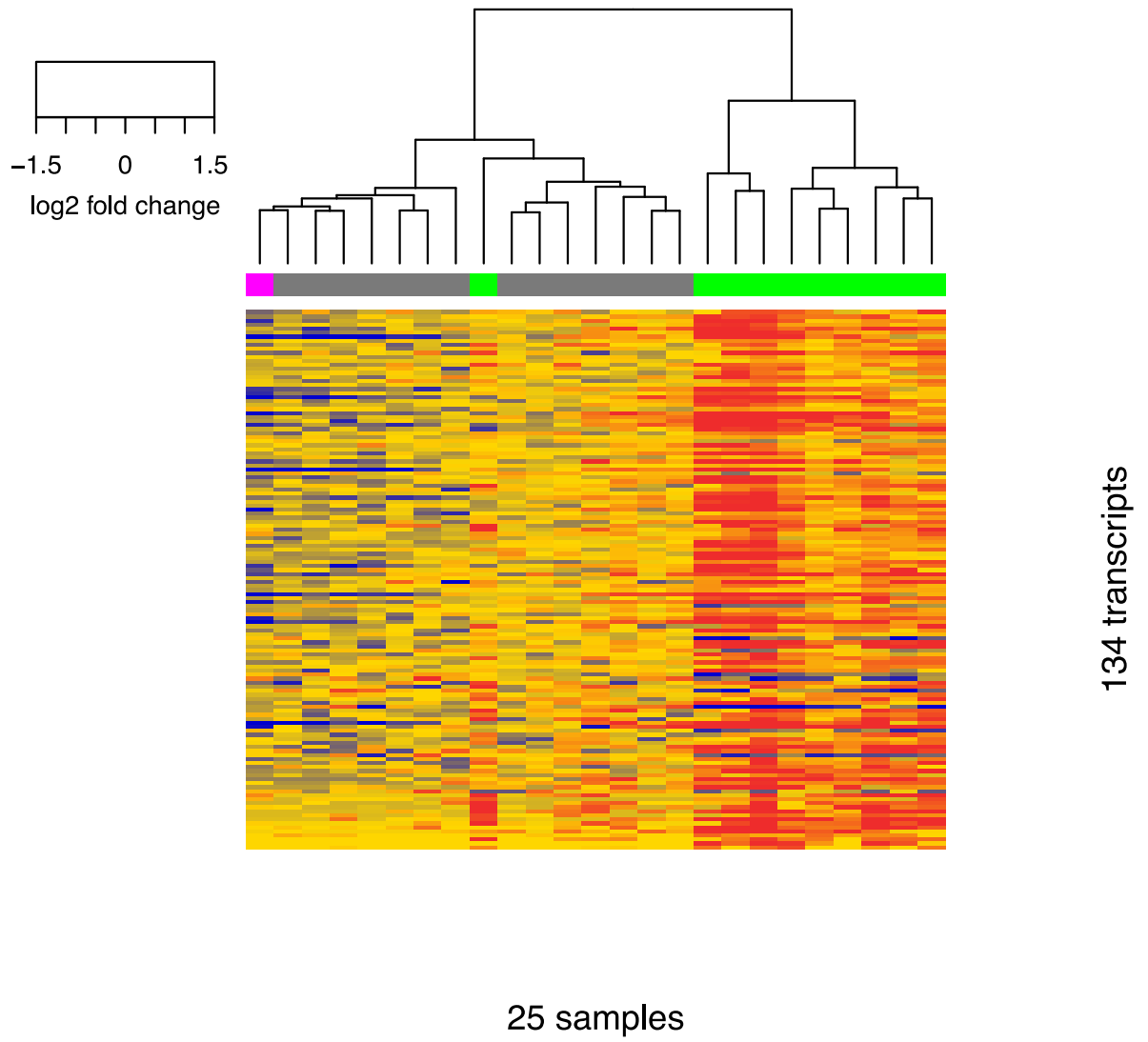


Figure E3.

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 RTIs	rs273259	GG (96)	2.1 (1.8-2.5)	1.04 (0.88-1.23)	.62
		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 symptoms	rs273259	GG (134)	45.6 (40.3-51.8)	0.87 (0.76-1.01)	.06
		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 episodes	rs273259	GG (147)	0.8 (0.6-1.0)	0.74 (0.59-0.93)	.01
		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 RTIs	rs273259	GG (147)	1.0 (0.9-1.3)	0.73 (0.59-0.91)	.01
		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	+
GPLY	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	-

