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PRENATAL STRESS, GENOMIC VARIATIONS AND RECURRENT RESPIRATORY TRACT INFECTIONS

-the FinnBrain Birth Cohort Study

Laura Korhonen

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To my family

UNIVERSITY OF TURKU
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LAURA KORHONEN: Prenatal stress, genetic variations and recurrent respiratory tract infections -the FinnBrain Birth Cohort Study
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ABSTRACT

Risk profiles associated with many diseases are formed at a very early stage of development, which occur before birth. Exposure of the fetus to maternal psychological distress during pregnancy may alter fetal biology and thus predispose the fetus to subsequent illnesses such as childhood recurrent respiratory infections.

This dissertation is part of the FinnBrain Birth Cohort Study, which recruited 3808 families between years 2012–2015. This prospective cohort-based study followed the symptoms of anxiety and depression in mothers, partnership satisfaction and the occurrence of children's recurrent respiratory tract infections from the 14th week of pregnancy until the child was two years old. Our aim was to investigate whether there was an association between maternal prenatal psychological distress and the occurrence of the child's recurrent respiratory tract infections. In a smaller subset of the Cohort, we investigated the association of maternal prenatal psychological distress on cortisol production in 10-week-old children. We also investigated the relationship between genetic susceptibility to respiratory infections and the interaction between genetic susceptibility and maternal prenatal psychological distress.

In this study, it was shown that in addition to the previously known risk factors (e.g. the number of siblings, shorter duration of breastfeeding), maternal prenatal psychological distress was associated with an increased risk for recurrent respiratory infections in children. In addition, we found that in children, whose mothers had prenatal psychological distress, had a reduced cortisol response in the stress test depending on if the child had a concomitant rhinovirus infection. Genetic variations in the IFI44L gene, part of the type I interferon pathway, associated with an increased susceptibility to respiratory infections and acute otitis media. Interleukin-6 gene variations were associated with an increased risk for recurrent respiratory infections. There was no interaction between these genetic predispositions and maternal stress during pregnancy, but sex differences were noted.

This work provides new preventive strategies for recurrent upper respiratory tract infections in children and offers new insights into the interactions between stress and immune systems.

KEYWORDS: children, cortisol, maternal prenatal stress, otitis media, recurrent respiratory tract infections, single nucleotide polymorphism

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Lastentautioppi
LAURA KORHONEN: Raskaudenaikainen stressi, geneettiset variaatiot ja toistuvat hengitystieinfektiot- FinnBrain-syntymäkohorttitutkimus
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TIIVISTELMÄ

Sairauksien riskiprofiili voi muodostua jo ennen syntymää. Sikiön altistuminen äidin psykologiselle stressille raskauden aikana saattaa muuttaa sikiön biologiaa altistaen myöhemmille sairauksille kuten toistuville hengitystieinfektioille.

Väitöskirja on osa FinnBrain-syntymäkohorttitutkimusta, johon on rekrytoitu 3808 perhettä vuosina 2012–2015. Tässä prospektiivisessa syntymäkohortissa seurattiin äitien ahdistus- ja masennusoireita, parisuhdetyytyväisyyttä 14. raskausviikolta siihen asti, kun lapsi oli kahden vuoden ikäinen. Myös tiedot lapsien toistuvista hengitystieinfektioista kerättiin. Tavoitteenamme oli tutkia äidin raskaudenaikaisen stressin (masennus–ja ahdistusoireilun) yhteyttä lapsen riskiin sairastaa toistuvia hengitystieinfektioita. Pienemmässä kohortin osajoukossa tutkimme äidin raskaudenaikaisen stressin vaikutusta 10 viikon ikäisten lasten kortisolin tuotantoon stressikokeessa. Tutkimme myös geneettistä alttiutta hengitystieinfektioille ja geneettisen alttiuden sekä äidin raskaudenaikaisen stressin yhteisvaikutusta.

Tässä tutkimuksessa osoitettiin, että jo tunnettujen riskitekijöiden (esim. sisarusten lukumäärä, imetyksen lyhyempi kesto) lisäksi äidin raskaudenaikainen stressi oli yhteydessä lapsen suurentuneeseen riskiin sairastaa toistuvia hengitystieinfektioita. Lisäksi havaitsimme, että lapsilla, joiden äideillä oli ollut raskausaikana stressioireita, todettiin alentunut kortisolivaste stressikokeessa, mikäli lapsella oli samanaikainen rinovirusinfektio. Geneettiset vaihtelut tyypin I interferonivasteeseen liittyvässä IFI44L-geenissä lisäsivät alttiutta hengitystieinfektioille ja välikorvatulehduksille. Interleukiini 6-geenin polymorfismi liittyi lapsen lisääntyneeseen riskiin sairastaa toistuvia hengitystieinfektioita. Näillä geneettisillä alttiuksilla sekä äidin raskaudenaikaisella stressillä ei todettu yhteisvaikutusta, mutta sukupuolieroja havaittiin.

Tämä tutkimus tuo uutta tietoa lasten toistuvien hengitystieinfektioiden ennaltaehkäisyyn sekä tuo tietoa stressinsäätely- ja immuunipuolustusjärjestelmien välisen vuorovaikutuksen kehittymisestä.

AVAINSANAT: kortisoli, lapset, toistuvat hengitystieinfektiot, välikorvatulehdus, yhden emäksen polymorfismi, äidin raskaudenaikainen stressi

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Abbreviations

AOM	Acute otitis media
AUC_i	Area under the curve above/below the baseline
CRH	Corticotropin-releasing hormone
DEG	Differentially expressed gene
DOHaD	Developmental Origins of Health and Disease
EPDS	the Edinburgh Postnatal Depression Scale
FinnBrain	Population-based pregnancy cohort of 3837 children
GxE	Gene-Environment
GWAS	Genome-wide association study
HPA	the Hypothalamic-pituitary-adrenal
IFI44L	Interferon-induced protein 44-like
IFN	Type I Interferon
IL-6	Interleukin 6
ISG	Interferon-stimulated gene
NK	Natural killer cell
NPS	Nasopharynx sample
PCR	Polymerase chain reaction
PD	Psychological distress
PRAQ-R2	the Pregnancy-Related Anxiety Questionnaire–Revised2 Scale
PRR	Pattern recognition receptor
RDAS	the Revised Dyadic Adjustment Scale
RRI	Recurrent respiratory tract infections
RTI	Respiratory tract infections
SCL-90	Symptom Checklist-90
sIgA	Secretory immunoglobulin A
SNP	Single-nucleotide polymorphism
STEPS	Steps to the Healthy Development and Well-being of Children
TLR	Toll-like receptor
TNF	Tumor necrosis factor alpha
SSRI	Serotonin reuptake inhibitors
11βHSD2	11β-hydroxysteroid dehydrogenase type 2

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Korhonen LS, Karlsson L, Scheinin NM, Korja R, Tolvanen M, Mertsola J, Peltola V, Karlsson H. Prenatal Maternal Psychological Distress and Offspring Risk for Recurrent Respiratory Infections. *Journal of Pediatrics* 2019; 208: 229-235

II Korhonen LS, Kortesluoma S, Lukkarinen M, Peltola V, Pesonen H, Pelto J, Tuulari JJ, Lukkarinen H, Vuorinen T, Karlsson H, Karlsson L. Prenatal maternal distress associates with a blunted cortisol response in rhinovirus-positive infants. *Psychoneuroendocrinology* 2019; 107:187-190

III Lempainen J,* Korhonen LS,* Kantojärvi K, Heinonen S, Toivonen L, Räty P, Ramilo O, Mejias A, Laine AP, Vuorinen T, Waris M, Karlsson L, Karlsson H, Paunio T, Peltola V. Associations between IFI44L gene variants and rates of respiratory tract infections during early childhood. Submitted

IV Korhonen LS, Lukkarinen M, Kantojärvi K, Räty P, Karlsson H, Paunio T, Peltola V, Karlsson L. Gene Variants, Prenatal Stress, and Risk for Recurrent Respiratory Infections: A Cohort Study. Submitted.

*Equal contribution

1 Introduction

Respiratory tract infections (RTIs) cause most of the morbidity among children under two years of age leading to a health and economic burden on society (Toivonen et al., 2016a; Tang et al., 2017; Fendrick et al., 2003). Approximately 10 % of all children suffer recurrent respiratory tract infections (RRIs). Children with RRIs need recurrent antibiotic treatments and undergo surgical procedures such as tympanostomy tube insertions and adenectomy (Toivonen et al., 2016a). The annual costs of children's RTIs are estimated to be in a similar range as the costs of hypertension and greater than those of asthma (Fendrick et al., 2003). This is due to doctor visits, medical treatments and parents' loss of workdays. It is also self-evident that children suffering from RRIs and acute otitis media (AOM) episodes have a lower quality of life (Jiang et al., 2013; Kujala et al., 2017). Additionally, the parents of children with RRIs often experience a decline in the their quality of life and are likely to spend significant amounts of time away from work (Jiang et al., 2013). Thus, these indirect costs of RRIs can even exceed the direct costs.

In an era of personalized medicine, the unique host response to common infections and pathogens has been recognized. Although the majority of genetic variations does not cause diseases but rather creates personal traits, single nucleotide polymorphisms (SNP) explain a part of the unique response to pathogens and medication. SNPs involved in innate immunity may have an effect on younger children's infections as adaptive and innate immunity matures during infancy. Adaptive immunity is supported by the mothers' immunoglobulin G that is transferred from the mother transplacentally and in breast milk. Females and males differ in their innate immune responses, which implies that some sex differences in innate immunity responses may be encoded on the X chromosome (Klein and Flanagan, 2016). Transcriptional analyses have shown sex differences, for example, in the induction of type I interferon (IFN) responses and synthesis of proinflammatory cytokines (Klein et al., 2010). Still, surprisingly few published papers have addressed the possibility of sex-related vulnerabilities, while studying biological susceptibility to RRIs.

In addition to a genetic predisposition to recurrent infections, the health of child depends on the characteristics of their early environment, such as factors related to

family socioeconomic status or parental health and health behavior. Recently, it has been observed in epidemiological studies that also exposure to maternal psychological stress during gestation may influence immunity and the risk of recurrent infections, while the biological mechanisms behind these observed associations have not been fully studied (Henriksen and Thuen, 2015). One the most actively studied domains is the hypothalamic-pituitary-adrenal (HPA) axis. It has been suggested that alterations in the HPA axis functioning of the mother would play a role in bridging prenatal stress exposures to later offspring health outcomes (Bailey et al., 2003; Beijers et al., 2010; Beijers et al., 2014). A stress-related increase in maternal cortisol potentially increases fetal exposure to cortisol and subsequently affects the development of the fetus' immune domains and the HPA axis (Merlot et al., 2008; Howerton and Bale, 2012; Xiong and Zhang, 2013). If the communication between the HPA axis and immune system is disrupted, changes in anti-viral immune responses may take place (Bailey et al., 2003; Webster and Sternberg, 2004). Bi-directional communication between the neuroendocrine and immune systems plays a significant regulatory role in response to a viral infection (Bailey et al.,2003). During sensitive periods of fetal development, prenatal environmental events, such as exposure to maternal psychological distress and elevated cortisol levels, can have long term consequences on a child's health (Xiong and Zhang, 2013).

In a transgenerational, prospective and observational population-based birth cohort study, we first analyzed novel risk factors for RRIs in children aged from 12 to 24 months. Furthermore, maternal psychological distress during pregnancy and the genetic variants of innate immunity and gene - environment interactions between the two were investigated. Since previous studies in which the effects of the child's sex have been taken into account are few in number, the possibility of sexually dimorphic responses was investigated. More specifically, the influence of prenatal maternal psychological distress on child HPA axis function in the context of subclinical rhinovirus infection was studied. Finally, regarding the genetic variants of innate immunity, another independent population-based birth cohort (the Steps to the Healthy Development and Well-being of Children, STEPS) was used in combination with the FinnBrain Cohort to study specifically the role of Interferon-induced protein 44-like (IFI44L) polymorphisms in the susceptibility to RTIs in early childhood. The effect of these polymorphisms on blood messenger RNA (mRNA) transcriptional profiles were further analyzed in a subset of children from the FinnBrain Cohort.

2 Review of the Literature

2.1 Respiratory tract infections in children

RTIs are the most common infectious diseases among infants and toddlers with significant morbidity and mortality (Toivonen et al., 2016a; Monto and Sullivan, 1993; Wardlaw et al., 2006). Usually children have 6–8 RTIs per year, but it seems that boys have more RTIs than girls during the first years of life (Monto and U.B, 1974). Recent studies have presented lower prevalence of RTIs, and Toivonen and colleagues evaluated 8847 RTIs in children under 2 years of age. Their average annual incidence was 5.9 RTIs/child (95% C.I. 5.7-6.1)(Toivonen et al., 2016b). RTIs present seasonally, and in the Northern Hemisphere, they occur predominantly during autumn and winter, while in tropical areas during the rainy season (Heikkinen and Järvinen, 2003). Most RTIs are caused by respiratory viruses circulating in the community. For example, viruses spread from siblings to younger children and from child to child in daycare. Rhinovirus is the most common cause of RTIs in children, followed by coronaviruses, influenza viruses, respiratory syncytial virus and parainfluenza viruses (Heikkinen and Järvinen, 2003; Toivonen et al., 2016b). Clinical presentation of rhinovirus infection can often be asymptomatic, and it does not cause long-term viral persistence in the respiratory tract. Respiratory viruses increase the susceptibility to secondary bacterial infections with several mechanisms like compromised physical and immunological barriers. There is also evidence of a synergistic effect between viruses and bacteria in the pathogenesis of respiratory infections (Bellinghausen et al., 2016).

RTIs pose a substantial disease burden for individuals, as they are often complicated by AOM and antibiotic use is common, even in the pneumococcal conjugate vaccine era (Chonmaitree et al., 2016). Bronchiolitis and wheezing are also associated with RTIs and may lead to hospitalization and the development of childhood asthma (Jartti and Gern, 2017). Viral infections have been shown to be a prevalent risk factor for wheezing among children hospitalized before 3 years of age and the interaction between asthma and virus-induced inflammation may be synergistic rather than additive (Heymann et al., 2004). It has been reported that 10% of children under two years of age suffer from recurrent upper and lower RTIs (Toivonen et al., 2016a).

2.2 Definition of recurrent respiratory infections

Defining RRIs is a complex issue, as there is no clear consensus about how frequent infections should be considered as "recurrent" infections. Very different definitions of RRIs exist in the literature, and they can be generic or restrictive (Jesenak et al., 2011). Studies have defined RRIs by the number of infection episodes per year. Usually six or greater number of respiratory infections per year are considered as recurrent infections or one or more respiratory infections per month involving the upper airways from September to April (Jesenak et al., 2011). One definition is the occurrence of eight or more documented airway infections per year in children up to three years of age or of six or more in children older than three years of age, assuming that the child has no diagnosis of an underlying long-term illness that predisposes to infections (Schaad et al., 2015). In a birth cohort study, STEPS, 1089 children were followed to 2 years of age for respiratory infections by a daily symptom diary. In all, 10% of children with the highest number of annual respiratory illness days were defined to have RRIs. These children had a median of 9.6 acute respiratory infections per year (Toivonen et al., 2016a).

Other definitions have been more focused on diagnoses, for example, acute otitis media (AOM) or lower respiratory infections (Nokso-Koivisto et al., 2002; de Benedictis and Bush, 2018). As for the frequency of infections, it has been defined, that three AOM episodes within 6 months or four episodes within 12 months stand for recurrent infections (Schaad et al., 2015). RRIs may overlap with each other or be prolonged, which makes it difficult to report the exact number of infections, or for example, AOM diagnoses. Between individuals, the severity of symptoms and complications vary as some children may have several RTIs without developing AOM, whereas others have several antibiotic treatments due to bacterial complications of RTIs.

2.3 Risk factors and morbidity of recurrent respiratory infections

Parental smoking during and after pregnancy, a lower socioeconomic status of the family, a shorter duration of breastfeeding, a greater number of siblings and outside-home daycare have all been identified as environmental risk factors for RTIs in children and are noted in Table 1 (Cohen, 1999; Schuez-Havupalo et al., 2017). These risk factors can be independent but are more likely to occur simultaneously forming a compounded effect of environmental and sociocultural exposures and therefore can be modifiable. These risk factors highlight the importance of preventive healthcare. Indeed, children's health is linked to their parents' physical

and emotional health, social conditions and child-rearing practices (Schor and American Academy of Pediatrics Task Force on the Family, 2003).

Table 1. Studies assessing risk factors for respiratory tract infections in children.

Risk factor for RTIs	OR (95%CI)	Study population N	Age years	Study
Duration of breast feeding > 3 months	0.5 (0.3–0.9)	273	< 3	Nicolai et al., 2017
	0.96 (0.93–0.99)	367	≤ 1	Chonmaitree et al., 2016
Number of siblings	3.0 (1.8–5.2)	273	< 3	Nicolai et al., 2017
	1.07 (1.01–1.14)	367	≤ 1	Chonmaitree et al., 2016
Outside-home daycare	1.74 (1.44–2.11)	367	≤ 1	Chonmaitree et al., 2016
Parental smoking	1.74 (1.06–2.87)	1129	< 9	Jedrychowski and Flak, 1997
	2.3 (2.0–4.2)	273	< 3	Nicolai et al., 2017
Lower socioeconomic status	1.5 (1.1–2.0)	5989	≤ 5	Bor et al., 1993

Respiratory tract infection (RTI)

AOM is one of the most common childhood infectious diseases and is often preceded by a respiratory infection. RRIs and recurrent or prolonged otitis media infections overlap with each other. Duration of breastfeeding, out-of- home day care and parental smoking were identified as significant risk factors for AOMs in the meta-analysis by Uhari and colleagues. The authors implied that consideration should be given to these risk factors to reduce AOM episodes and antibiotic treatments in children (Uhari et al., 1996). Regarding a child's own genetic traits, some of the children may be more vulnerable than others to environmental risk factors regarding RTIs and RTI-related morbidity. In a study including two birth cohorts, the 17q21 risk SNPs and, in particular, rhinovirus- derived early respiratory wheezing proceeded the development of asthma in childhood (Caliskan M et al., 2013). SNPs at the 17q21 locus have been associated in retrospective reports with respiratory illnesses in early life. Reports have shown that the associations between 17q21 region genetic variants and RTIs or asthma are further enhanced among children exposed to environmental tobacco smoke (Smit et al., 2010).

2.4 Pathophysiology and genetics of recurrent respiratory infections

Children under two years of age have a predisposition to frequent RTIs, as the innate and adaptive immune responses are immature, and maternal antibodies derived through the placenta decrease (Tregoning and Schwarze, 2010). Viral infections activate the innate immune system as a first line defense in minutes. The key function of the innate immunity is to detect viral components such as viral RNA or DNA or viral intermediate products with pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). Infected cells produce IFNs and other pro-inflammatory cytokines, for example, tumor necrosis factor alpha (TNF) and interleukin-6 (IL-6) to prevent viral invasion or replication before more specific protection by the adaptive immune system is generated (Koyama et al., 2008).

A single-nucleotide polymorphism (SNP) refers to a genetic polymorphism based on a single base pair exchange in adenine (A), thymine (T), cytosine (C) or guanine (G) causing DNA sequence variation. Almost all common SNPs have only two alleles, for example a C and G. In order for such a variation to be defined as a SNP, the less common allele must occur in the population at a frequency of at least 1%. SNPs represent around 90% of the differences among the genomes of human individuals and occur in coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes. The most common allele is named as a "major" or "wild type" and the less frequent allele as "minor", which frequency is often reported as minor allele frequency (MAF). Although the majority of variations in the DNA sequences does not cause diseases but rather creates personal traits, SNPs explain part of the unique response to pathogens. Individual responses to common pathogens vary, and SNPs involved in innate immunity may have an effect on younger children's infections. Genetic variants affecting the function of the innate immune system have been linked to infection susceptibility particularly in young children. Factors of innate immunity with genetic variation include, for example, PRRs, such as the family of TLRs (Mittal et al., 2014) and mannose-binding lectin (MBL) (Cedzynski et al., 2004; Eisen, 2010). These PRRs recognize pathogens or specific microbial structures leading to the induction of inflammatory pathways in cells. IFNs are secreted by infected cells after recognition of microbial products, for example, viral components such as viral RNA or DNA, by PRRs (Ivashkiv and Donlin, 2014). IFNs promote antigen presentation and NK cell functioning, and this leads to transcription of interferon-stimulated genes (ISGs) in infected cells. Interferon-induced protein 44-like (IFI44L) protein belongs to the group of proteins encoded by ISGs. IFI44L is transcriptionally induced by IFNs and has antiviral specificity (Schoggins et al., 2011). Cytokines participate in cell signaling involved in the innate and adaptive immunity and play a role in children's immunocompetence. Polymorphisms in TNF, IL-6 and IL-10 genes have been

reported to affect the susceptibility to RTIs (Rose-John et al., 2017; Emonts et al., 2007; Revai et al., 2009). Revai et al, studied 242 children, who were followed over 2689 patient-months and genotyped. 1235 RTIs occurred, and 392 (32%) were complicated by AOM. Children, who had an IL-6 (-174) gene variant, had a higher susceptibility to RTIs during the study period and were more likely to meet established otitis susceptibility criteria than children with the wildtype gene. The presence of a TNFα (-308) polymorphism was associated with an increased risk for AOM after an episode of respiratory tract infection (Revai et al., 2009).

Moreover, few genome-wide association studies (GWAS) have been performed for childhood infections. A study by Tian et al suggested associations between genes of innate immunity and common infections such as tonsillitis and middle ear infections. Genes involved in embryonic development also associated with infections, for example, the missense mutation (rs72646967-C, N397H) in TBX1, which is essential for inner ear development associated with a lower risk of AOMs (Tian et al., 2017). Furthermore, individuals have sex-specific genetic traits and innate immunity responses may be encoded on the X chromosome (Klein and Flanagan, 2016). Male sex has been found to be risk factor for respiratory tract infection recurrence (Anders et al., 2015).

2.5 Prenatal stress

Maternal stress is a common environmental risk factor for the fetus, as stress is increasingly becoming a common part of our everyday living. More than a quarter of people in a developed country reported daily stress levels to be high or severe (Connor et al., 2019). Prenatal maternal stress is associated with later mental health problems of offspring during adulthood such as depression and personality disorders (Thompson et al., 2001; Brannigan et al., 2019). The Developmental Origins of Health and Disease (DOHaD) theory is applicable in psychiatry/psychology, because the environment of early childhood plays a role in the determinants of mental health (O'Donnell and Meaney, 2017). Barker and his colleagues made the first observations that a lower birth weight is a risk factor for the development of cardiovascular and metabolic diseases such as arterial hypertension, coronary heart disease, obesity and type 2 diabetes (Barker and Osmond, 1986). The hypothesis was that birth weight is an indicator of intrauterine conditions and that variation in fetal growth reflects variation in how the fetus adapts to subsequent extrauterine life. Additionally, according to this theory, the set point of physiological and metabolic responses is programmed during gestation. Later, epidemiological studies showed that, during sensitive periods of fetal development, prenatal environmental events are important factors for shaping the risk for morbidity later in life together with genetic risks and postnatal exposures (Daskalakis et al., 2013).

The central nervous system, autonomic nervous system, neuro-endocrine, HPA axis, cardiovascular and immune systems have their highest plasticity during early development, when the organ systems are still immature making them vulnerable to the influences of prenatal stress exposure (Bale, 2015; Griffiths and Hunter, 2014; Harris and Seckl, 2011;Meaney et al., 2007; Seckl, 2004; Xiong and Zhang, 2013). Maternal prenatal stress has been linked with alterations in HPA axis function, changes in glucocorticoid receptor sensitivity, changes in proteins and neurotransmitters involved in neuronal development and function in the central nervous system of the fetus (Van den Bergh et al., 2017). These changes lay the foundation for susceptibility to somatic diseases and mental health problems in their interactions with genetic liabilities and postnatal challenges (Van den Bergh et al., 2017). During embryonic and fetal development, tissues and organs are created in a chronological sequence. This leads to specific time points in which some cell components become more sensitive than others. The recognition of these windows of vulnerability is fundamental considering the complexity of the fetal development (Veru et al., 2014). The definition of prenatal stress has included various sources of stress, for example, the couples' relationship satisfaction and stressful life events, daily hassles and natural disasters such as the 1998 Quebec ice storm (Beijers et al., 2010; Henriksen and Thuen, 2015; Veru et al., 2015). Maternal psychological distress as source of prenatal stress has comprised maternal symptoms of depression, anxiety and pregnancy-specific anxiety. These different sources of stress have resulted in the biological heterogeneity and variety among the phenotypes of prenatal stress (Mustonen et al., 2018). For example, depression and anxiety may occur through different biological pathways.

2.6 Prenatal maternal psychological distress and recurrent respiratory infections

As previously stated, the psychological well-being of the mother affects the child's health. Most the studies showing a link between the maternal psychological health and a child's RTIs have focused on the postnatal period (Horwitz et al., 1993; K Louhi-Pirkanniemi et al., 2004). Nevertheless there are few studies demonstrating a link between prenatal maternal psychological distress and infection related outcomes (Marion Tegethoff et al., 2011; Nielsen et al., 201; Beijers et al., 2010; Henriksen and Thuen, 2015). In a Danish nation-wide cohort, Nielsen et al found, an association between maternal prenatal stress, which is defined as maternal exposure to a stressful life event during pregnancy or in the 3-year period before conception, and the risk for hospitalizations because of infectious diseases during childhood. Beijers and colleagues found that maternal pregnancy-related anxiety by self-reporting as well as by mothers' circadian cortisol from saliva samples predicted a significant

proportion of variance in the offspring respiratory illness during the first year of life. In addition, maternal scoring in The Pregnancy-Related Anxiety Questionnaire (PRAQ) first subscale; being the *Fear of Giving Birth*, was related to an offspring's greater use of antibiotics during their first year of life (Beijers et al., 2010). During pregnancy, the parental relationship is often the main source of psychological support for both adults. Thus, the parental relationship can be viewed as one of the fundamental sources of stress regulation. In the Norwegian Mother and Child Cohort Study 2015, including 100,027 children, prenatal maternal relationship dissatisfaction and stressful life events were significantly associated with an increased frequency of infectious diseases in the offspring after controlling for the most important confounding factors (Henriksen and Thuen, 2015).

Although it is impossible to completely disentangle and distinguish the behavioral and genetic aspects from fetal programming, maternal psychological distress during pregnancy is reportedly an independent risk factor for respiratory illness and infectious disease hospitalization in the offspring (Beijers et al., 2010; Henriksen and Thuen, 2015; Nielsen et al., 2011). Indeed, prenatal and early life stress exposures have potential effects on the development of the child's immune system.

2.7 Prenatal maternal psychological distress and offspring's immunity

Human and animal studies do suggest that maternal prenatal stress has long-lasting effects on immunity. In addition to infection-related outcomes, maternal long-term prenatal psychological stress is associated with childhood asthma and allergy as well as inflammatory bowel disease (Andersson et al., 2016; Aujnarain et al., 2013). Although knowledge on the possible mechanisms underlying these relations is limited, several potential mechanisms involved have been proposed (Beijers et al., 2014).

The stress response is mainly mediated through two physiological mechanisms, the sympatho-adreno-medullary (SAM) system and the HPA axis. The adrenal cortex produces cortisol and cortisol can cross the placenta at high levels. The access of glucocorticoids to the fetus is regulated by the placental enzyme 11-β-hydroxysteroid dehydrogenase type 2 (11βHSD2). Maternal psychological stress during pregnancy can lead to secretion of catecholamines, which can lead to a decrease in the activity of 11βHSD (O'Donnell and Meaney, 2017). Maternal plasma cortisol levels have been shown to correlate with fetal cortisol levels, and maternal cortisol accounts for about 40% of the variance in fetal cortisol concentrations (Gitau et al., 1998). The relationships between the neuroendocrine and immune systems are mediated by hormones, neurotransmitters as well as cytokines. Maternal chronic

stress is related to a less well-functioning immune system, higher susceptibility to infections and generally a lower quality of physical health with altered health behaviors that includes eating, sleeping, and exercise (Beijers et al., 2014). Psychosocial stress has also been related to unbalances in the intestinal microbiota (Beijers et al., 2014).

During sensitive periods of fetal development, maternal stress hormones can affect the development of the fetal immune system or other physiological systems that regulate the immune response such as the HPA axis (Merlot et al., 2008; O'Donnell and Meaney, 2017). These changes may be due to gene-environmental interactions and alterations in epigenetic pathways. The ontogeny of the immune system consists of a series of coordinated and sequential processes. During fetal development, gene expression is constantly altered modifying the profile of receptor molecules in all cells and their responsiveness to environmental influences including hormones, cytokines and toxicants (Munitic et al., 2004). HPA axis hormones can have an effect on the gene expression profile, and when the developing immune system is exposed to excess glucocorticoids during the critical periods, even a permanent alteration of physiology and later health can take place (Xiong and Zhang, 2013). As mentioned before, the specific time frames are very important during ontogenesis and studies should state these windows of vulnerability (Merlot et al., 2008; Veru et al., 2014). Regarding to prenatal maternal psychological distress and its effects on infant's immunity, the findings are heterogeneous. This is partly due to the varying definitions of prenatal stress and partly due to the fact that not all studies have taken in to account the time of the stress exposure during pregnancy. Also, animal studies have shown that chronic maternal stress during pregnancy has a different effect on offspring immunity compared to one-time exposure.

2.7.1 Animal studies

Animal studies have shown that prenatal stress affects the development of the fetal immune system (Bellinger et al., 2008; Couret et al., 2009). Findings are incoherent, as the duration of the prenatal stressor and/or time of stress exposure varies. Also, different types of stressor have been used, usually being either neurogenic with exposure to light, electric shocks or saline injection, for example, or psychogenic with housing that is unfamiliar conspecific, crowded or having a resident-intruder confrontation (Veru et al., 2014). Depending on the type of stressor, the pregnant animal's nervous system processes these harmful stimuli differently and produces a distinctive neuroendocrine response (Veru et al., 2014). This response is modulated by pregnancy-related changes in the mechanisms of the neuroendocrine immune response. IL-6 has been suggested to be one of the mediators in the programming of immune ontogeny. It has been shown that, during mid-gestation, IL-6 is capable of

crossing the placenta (Dahlgren et al., 2006). Mediators of the neuroendocrine-immune system can work directly or indirectly (e.g., through placental changes). Findings are different between the species as primates, for example, are the only species that produce placental corticotropin releasing hormone (CRH) during pregnancy (Merlot et al., 2008). Also, sex of the offspring have an effect on outcomes (Veru et al., 2014). Overall, prenatal maternal stress can result in maladaptive programming of immune function, as prenatal maternal stress increases production of Th2 relative to Th1 cytokines that dampen cellular immunity (Veru et al., 2014). Signs of immune system formation in animal studies include decreased cytotoxicity of natural killer (NK) cells and decreased cytokine secretion (Kay et al., 1998) (Coe et al., 2002). Couret et al. studied the consequences of a repeated social stress applied during late gestation of the pregnant gilt on the immune system and HPA axis activity of the piglets from birth to two months of age. Prenatal maternal stress decreased the total numbers of white blood cells, lymphocytes and granulocytes, the CD4 (+)/CD8(+) T cell ratio and lipopolysaccharide -induced TNF production. There is also evidence that the effects of prenatal stress on the immune system extend into adulthood (Merlot et al., 2008).

2.7.2 Human studies

Although epidemiological studies have proven that there is a link between prenatal stress and alterations in an infant's immunity, studies on biological mechanisms behind this association are rare. The most frequently mentioned biological mechanisms are: 1) immunomodulation due to modification of ontogeny of the immune system and 2) changes in communication between the HPA axis and immune system. Emerging data additionally suggests that alterations in gut immunity and microbial composition in the offspring as well as in the mother play a role (Figure 1).

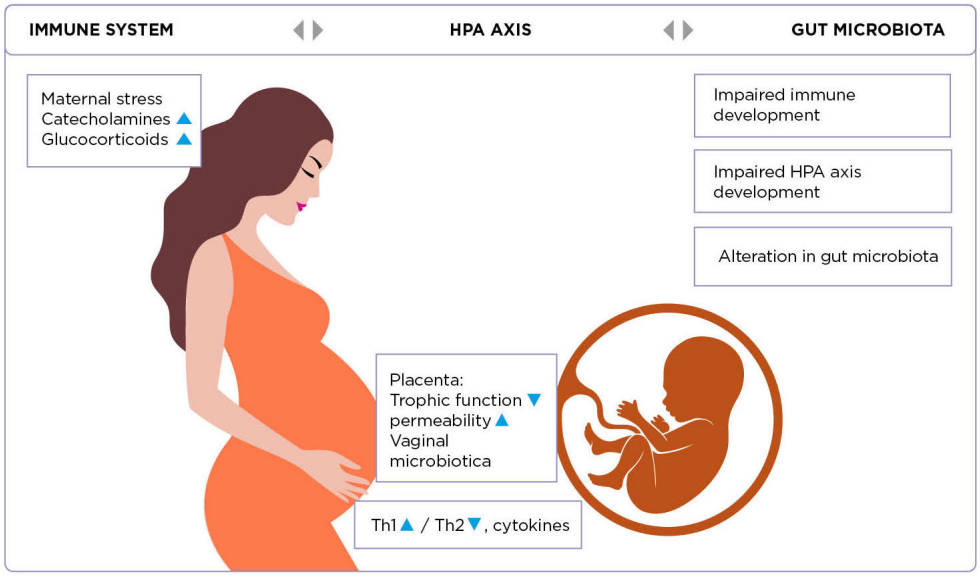

Figure 1. Maternal prenatal stress related to the current hypothesis of the effects on placenta and fetus. Suggested mediating mechanisms are the developmental changes in the immune system and in the HPA axis. Also, alterations in gut immunity and microbial composition in the offspring as well as in the mother are associated with maternal prenatal stress.

In a subsample of 403 term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, infants born to mothers with pre- and postnatal depressive symptoms had significantly lower median secretory Immunoglobulin A (sIgA) concentrations than those in the reference group after controlling for breastfeeding status, infant age, antibiotics exposure and other covariates. Secretory IgA plays a critical role in infant gut mucosal immunity (Kang et al., 2018). In another study, the development of the infant intestinal microbiota was followed until 4 months of age in a healthy cohort of 56 vaginally born Dutch infants. Results suggested that maternal prenatal stress, measured by either self-reports or elevated basal maternal salivary cortisol concentrations or both, was associated with the infants' microbiota composition. Infants of mothers with high cumulative stress (i.e., high self-reported stress and high cortisol concentrations) during pregnancy had significantly higher amounts of proteobacterial groups and lower number of lactic acid bacteria, which are characteristics of a potentially increased level of inflammation. Furthermore, this aberrant colonization pattern was related to an increased number of maternally reported infant gastrointestinal symptoms and allergic reactions (Zijlmans et al., 2015).

The Project Ice Storm included mothers that were pregnant during the 1998 Quebec ice storm. Blood samples from 37 of their children, when they were 13 years old, were obtained to measure cell population percentages and mitogen-induced

cytokine production. They found that the mothers' objective degree of prenatal maternal stress exposure significantly predicted reductions in total and CD4+ lymphocyte proportions; increases in TNF-α, IL-1β and IL-6 levels, and an enhancement of the Th2 cytokines IL-4 and IL-13. Sex and timing of exposure during gestation were also associated with some outcomes (Veru et al., 2015). They also showed that DNA methylation meditates the effect of exposure to prenatal maternal stress on cytokine production, that favors a Th2 shift (Cao-Lei et al., 2016). The Urban Environment and Childhood Asthma Study (N = 557 families) demonstrated that prenatal stress was associated with altered innate and adaptive immune responses in cord blood mononuclear cells suggesting stress-induced perinatal immunomodulation (Wright et al., 2010). In a small prospective study of 27 mother-newborn dyads, the authors found a significant, independent and linear effect of pregnancy-specific stress on newborn leukocyte telomere length that accounted for 25% of the variance (Entringer et al., 2013).

Activation of the HPA axis is an important regulatory mechanism for the inflammatory response to an infectious challenge. Almost all immune cells have receptors for HPA hormones (Glaser and Kiecolt-Glaser, 2005). In contrast to the traditional view of glucocorticoids as immunosuppressant hormones, they are more accurately conceptualized as immunomodulatory hormones that can both stimulate as well as suppress immune function (McEwen, 2018). Huda et al found an association of pain-induced cortisol responsiveness with thymic function and vaccine responses in 1-to -15-week-old infants compromising 153 male infants and 153 female infants and showed that cortisol responsiveness was negatively associated with thymus size (measured by ultrasound) at all ages ($P < 0.01$) in boys. Cortisol responsiveness was negatively associated with naive helper T-cell concentrations in both sexes at both 6 and 15 weeks of age (Huda et al., 2019).

2.8 Prenatal maternal psychological distress: effects on offspring's HPA axis function

Psychosocial stress triggers a series of behavioral, neural, hormonal and molecular responses that are useful for survival that allow appropriate adaptation during a time frame (Zannas and Chrousos, 2017). A primary actor of the stress response is the HPA axis, which is activated by hypothalamic secretion of corticotropin-releasing hormone (CRH) and arginine vasopressin in the brain leading to adrenal secretion of glucocorticoids in the systemic circulation. At the molecular level, the effects of HPA axis activation and glucocorticoid signaling are largely mediated by the glucocorticoid receptor, a ligand-dependent transcription factor that drives the genomic actions of glucocorticoids in essentially all of body tissues (Zannas and Chrousos, 2017). In animal models of prenatal stress, glucocorticoid exposure, both

endogenous and exogenous, influences the development of the fetal HPA axis function, and this in turn leads to adaptation at every level of development from cellular to behavioral (Cottrell and Seckl, 2009; Harris and Seckl, 2011). According to the fetal programming hypothesis, glucocorticoids transmitted from mother to fetus during gestation communicate useful information about the external world, and this information is used for adaption, survival and success in the postnatal environment (Bateson et al., 2004).

In a systematic review by Mustonen et al., associations between maternal prenatal self-reported symptoms of different types of prenatal psychological distress and hair cortisol concentrations across pregnancy were shown. The existing data implied that in a population with low levels of self-reported prenatal psychological distress, the associations with hair-derived cortisol concentrations were rather weak, but this association might be strengthened when studying populations with a greater variance in psychological distress and a higher frequency of elevated levels of psychological distress (Mustonen et al., 2018). It might be that there is not a clear correlation between self-reported questionnaire scores on maternal prenatal stress and cortisol concentrations in blood, saliva, hair or urine. Behavioral phenotypes may have different biological pathways. Still, self-report assessments of prenatal maternal psychosocial stress can be predictors of the future health of the child and may correlate well with biological measures. A study by Kane et all. with 448 women showed that higher mean levels of pregnancy-specific anxiety over the course of pregnancy predicted higher maternal saliva cortisol traits compared to lower pregnancy anxiety (Kane et al., 2014). Moreover, chronicity of depressive symptoms seems to be related to elevated hair cortisol levels during pregnancy (Mustonen et al., 2019).

2.8.1 Animal studies

In animal models, high maternal cortisol levels have been primary mediators in programming of the offspring's HPA function (Kapoor et al., 2006). In rats, maternal prenatal stress and high fetal corticosterone levels cause downregulation of fetal glucocorticoid and mineralocorticoid receptors and impair the HPA axis feedback control during both infancy and adulthood. The impairment in this HPA axis activity can be prevented by maternal adrenalectomy and mimicked by administration of glucocorticoids (Weinstock, 2005).

Animal models do suggest that sex hormones modulate the effect of stress in reprogramming of the HPA axis. Previous studies have shown that glucocorticoid levels are higher in females than in males after HPA axis stimulation (Kudielka and Kirschbaum, 2005; Yoshimura et al., 2003). Animal studies have convincingly proven that estradiol affects the functioning of the HPA axis and has modulatory

effects on mineralocorticoid and glucocorticoid receptors (Kudielka and Kirschbaum, 2005; Viau and Meaney, 1991; Carey et al., 1995; Peiffer et al., 1991). By binding to estrogen-responsive elements on the CRH gene, estradiol may directly enhance CRH gene transcription (Gray et al., 2017). Androgens have specific and in partially opposite effects on HPA axis regulation, but it should be taken in to account that testosterone can be metabolized to estrogen by aromatization in both nervous and peripheral tissues and thus have estrogen-like effects (Kudielka and Kirschbaum, 2005;Viau and Meaney, 1996; Chowen et al., 1990).

Less severe protocols of prenatal stress result in a longer duration of corticosterone recovery after acute stress in adult females but not in adult males in rodents (Gray et al., 2017; Weinstock et al., 1992; McCormick et al., 1995). After more severe prenatal stress, adult male and female rodents show a longer duration of corticosterone secretion when exposed to acute stress (Gray et al., 2017; Maccari et al., 1995). This suggests that females are sensitive to less severe prenatal stress, whereas males require more severe prenatal stress to reprogram the HPA axis (Gray et al., 2017).

2.8.2 Human studies

The fetus is exposed to elevated levels of endogenous glucocorticoids in conditions when the levels of glucocorticoid are elevated in the mother due to stress or when the placental 11βHSD barrier decreases. During normal pregnancy, glucocorticoid concentrations in the maternal circulation are markedly higher than those in the fetal circulation (Benediktsson et al., 1997). During human pregnancy, the expression and activity of 11βHSD steadily increases as gestation progresses, and then decreases near term (at 38–40 weeks) (Schoof et al., 2001; Xiong and Zhang, 2013). Also, increased maternal cortisol concentrations may rise placental CRH concentrations. The placenta synthesizes and releases large amounts of CRH into the maternal and fetal circulations. Placental CRH concentrations have been related to impaired fetal growth (Beijers et al., 2014; Wadhwa et al., 2004). Studies have shown that adverse fetal programming may occur very early in pregnancy, even before neuroendocrine systems have developed and not only on or near birth (Speirs et al., 2004). The stress response is mediated also by the sympatho-adreno-medullary pathway, which is called the SAM system. Adrenaline is often associated with an acute stress, whereas noradrenaline is released to stabilize the stress response (Beijers et al., 2014; Sapolsky et al., 2000). Catecholamines may influence both the placenta and the fetus. A mother's elevated catecholamines concentrations lead to constriction of placental blood vessels, a decreasing fetal supply of nutrients and oxygen and elevated fetal catecholamine concentrations (Beijers et al., 2014). Also catecholamines may down-regulate human placental 11βHSD during gestation (Schoof et al., 2001). Studies on

psychosocial stress and catecholamine levels in pregnancy are few in number. The study of Petraglia and colleagues did not find a significant relationship between psychosocial stress and catecholamine levels in pregnant women at 28 gestational weeks (gwks) (Petraglia et al., 2001).

Many effects of *in utero* exposure to maternal stress on offspring development are believed to manifest as hyperreactivity or hyporeactivity due to alterations in the infant's HPA axis (Giesbrecht et al., 2017). Relatively few human studies with high quality on cortisol measurements and appropriate numbers of controls for potential confounding variables in statistical analyses have assessed the associations between prenatal cortisol exposure and children's HPA axis function (Giesbrecht et al., 2017). Despite the high quality of these studies, the results are heterogeneous. Three studies reported higher cortisol levels and hyperreactivity of the HPA axis after prenatal maternal psychological distress exposure (Davis et al., 2011; Gutteling et al., 2005; Gutteling et al., 2004). One reported higher cortisol levels in offspring but a blunted response of the HPA axis in a stress test (O'Connor et al., 2013). Two studies found no association (de Weerth et al., 2013; Tollenaar et al., 2011). Possible factors explaining the differences in the results, such as the timing of the maternal cortisol measure, varied in both within the day and within pregnancy measurements. Also, there were differences in the methods on how the cortisol exposure was measured. When saliva cortisol samples are collected over time, the area under the curve (AUC) can be used, and it provides insight into the interpretation of the derived parameter (Fekedulegn et al., 2007). AUC_i is derived as the area under the curve above the baseline value minus the area below the baseline value (Fekedulegn et al., 2007). Some studies have used the cortisol awakening response, which is the change in cortisol concentration that occurs in the first hour after waking in morning and in some studies cortisol concentrations from the amniotic fluid was used. Another difference was that the type of infant stressor varied. It should also be noted that not all effects of maternal psychological stress on the fetus and child are mediated by cortisol.

2.9 Gene-Environment interaction

Although in a modern era of medical research, the GWAS have become more common because of cost reductions, the identified variants explain only a small proportion of the heritability of many diseases. This unexplained heritability could be partly due to the gene-environment (GxE) interaction (Thomas, 2010). The gene (or genotype)–environment interaction, in its simplest terms, is the phenomenon of two different genotypes responding to environment exposures in different ways. Sometimes, sensitivity to environmental risk factors for a disease are inherited rather than the disease itself and that is why GxE studies can be considered to be related to

fetal programming. Through GxE analysis, it is possible to find subpopulations that are most susceptible to prenatal environmental events and are at risk for morbidity later in life. There have been arguments that GxE studies provide more precise targets to improve public health compared to traditional epidemiological studies (Hutter et al., 2013). There is some evidence that in healthy general populations, the mild elevations of maternal stress and cortisol might be advantageous to the fetus and offspring (DiPietro et al., 2006; Doyle et al., 2015). Negative effects of prenatal stress and cortisol exposures are most consistently observed when high cortisol exposures occur early during early pregnancy but potentially also very low levels of maternal prenatal stress can be harmful to the fetus (Giesbrecht et al., 2017; DiPietro, 2012).

New tools for studies have been the development of biological response markers for assessment of exposure including gene expression, transcriptomic signatures and DNA methylation profiles (McAllister et al., 2017; Thomas, 2010). Publishing negative findings is helpful, because this allows researchers to avoid repeating failed experiments (Hutter et al., 2013). As we learn more about the effects of early environmental exposures on the developing fetus, new opportunities for disease prevention are created. Knowledge about the mechanisms of disease susceptibility will help us to better understand the mechanisms of disease formation. Observations of GxE interactions can lead to important policy implications on environmental health standards and on to the targeting of interventions and treatment (Hutter et al., 2013).

3 Aims

The main aim of this work was to assess the relationship between maternal prenatal psychological distress and the risk for RTIs in children up to two years of age. The first objective was to assess the relationship between maternal prenatal stress and the risk for RRIs in children. We further studied if the exposure to maternal prenatal psychological distress was associated with the infant HPA axis reactivity in response to an acute stressor. Furthermore, we studied the association between genetic variants of innate immunity and the susceptibility to RTIs in early childhood in two independent birth cohorts. We investigated GxE interactions between genetic variants of innate immunity and maternal psychological distress with the clinical outcome of RRIs. Lastly, we aimed to investigate the presence of sexually dimorphic responses in the studied associations. Specifically, the aims were:

1. To assess the relationship between maternal prenatal psychological distress comprising of depression and anxiety symptoms and relationship quality and the risk of RRIs in children up to two years of life.
2. To investigate whether maternal prenatal psychological distress is associated with 10-week-old infants HPA axis reactivity, and whether this association is different in the presence of a subclinical rhinovirus infection that challenges the immune system.
3. To determine the association of IFI44L polymorphisms rs273259 and rs1333969 with rates of RTIs, AOM episodes and antibiotic treatments during the first two years of age. The effect of these IFI44L gene polymorphisms on blood messenger RNA (mRNA) transcriptional profiles were further analyzed in a subset of children.
4. To investigate the association of preselected genetic variants (*IL6, IFI44L, IL10, IFIH1, MBL2, IL17A, TLR4*, *TLR2*, *IL4* and *TNF)* reportedly affecting the function of innate immune system with the clinical outcome of RRIs and to test a GxE hypothesis whether the risk for recurrent respiratory infections is dependent on both the genetic variants and *in utero* exposure to maternal psychological distress.

4 Materials and Methods

4.1 Study design

This work was carried out as part of transgenerational prospective observational birth cohort study: The FinnBrain Birth Cohort Study (www.finnbrain.fi)(Karlsson et al., 2018). The questionnaire data and biological sample collection time points and procedures are presented in Table 2. The research questionnaires were either mailed to the participants or could be filled out online according to each participant's choice. Recruited women gave birth at the Turku University Hospital in the Hospital District of Southwest Finland and after delivery, the umbilical cord was clamped, and blood from the umbilical cord vein was obtained for genetic analyses. The FinnBrain Birth Cohort Study visits at 3 months of age were performed in the research facilities. During the study visit, a pediatric examination was performed, and a blood sample was taken from the children as well as a nasopharynx/nasal swab. Five saliva cortisol samples were collected, at the beginning of visit and 0, 15, 25, and 35 minutes after the stressor.

Table 2. The questionnaire data and biological sample collection time points.

	Hypothesis	Prenatal psychological distress exposure	Sample collection at birth	Study visit, child 3 months	Recorded outcomes, child 12 or 24 months
Study I	Maternal prenatal psychological distress (PD) independently increases the risk of RRIs	Questionnaires EPDS,PRAQR2, SCL,RDAS at gwks 34			Questionnaires: RRIs, respiratory infections, antibiotic treatments
Study II	Maternal PD is related to early infant HPA axis reactivity in the context of subclinical rhinovirus infection	Questionnaires EPDS, PRAQR2, SCL, RDAS at gwks 14,24 and 34 (Focus cohort)		Saliva cortisol samples, NPS for virus PCR, e.g rhinovirus	
Study III	To assess the impact of polymorphisms in type I interferon related gene IFI44L on susceptibility to RTIs and AOM	-	Cord blood	mRNA blood samples, NPS for virus PCR	Questionnaires: respiratory infections, antibiotic treatments
Study IV	The risk for RRIs is elevated in children with innate immune gene variants, and maternal PD might further increase the risk	Questionnaires EPDS, PRAQR2, SCL, RDAS at gwks 34	Cord blood		Questionnaires: RRIs, respiratory infections, antibiotic treatments

The Edinburgh Postnatal Depression Scale (EPDS), Gestational week (GWK), Nasopharynx/nasal sample (NPS), Polymerase chain reaction (PCR), Prenatal psychological distress (PD), Pregnancy-Related Anxiety QuestionnaireRevised2 (PRAQ-R2), Recurrent respiratory tract infection (RRI), the Revised Dyadic Adjustment Scale (RDAS) questionnaires and the Symptom Checklist 90 anxiety subscale (SCL90/Anxiety).

4.2 Study population

Recruitment took place at three maternal welfare clinics of a geographically defined area, which performed pregnancy ultrasound scans at gwk 12 for the women eventually referred to give birth at the Turku University Hospital in the Hospital District of Southwest Finland and the Åland Islands in Finland. The recruitment took place between December 2011 and April 2015 and relied upon personal contact by research nurses, who were placed at the recruitment sites. The cohort consisted of 3808 women, who attended the free-of-charge ultrasounds during early pregnancy, their 3837 babies and 2623 fathers or partners. Mothers and fathers were considered eligible to participate in the study if they had a verified pregnancy and sufficient knowledge of Finnish or Swedish (the official languages of Finland) to fill in the study questionnaires.

The study population regarding children with RRIs (Study I) was selected from among the Cohort participants, who had responded to the 12- and 24- month questionnaires on child health by December 2017 (N=1262). Of the entire RRI group, 91 children were included based on the 12-month questionnaire (6% of cohort children) and the remaining 155 (11% of cohort children) based upon the 24-month questionnaire. There were 40 families (20%) out of the 204 who had responded "yes" to the question on RRIs both at 12 and 24 months. The group of children without RRIs were also selected on the basis of parental responses on the 12-month and 24-month questionnaires. Responding "no" regarding recurrent infections at both time points was the criterion to be in the comparison group (N= 1014). Within these children, genotyping from cord blood was successful with 990 children. Thus, Study IV consisted of 96 children with RRIs and of a comparison group of 894 children.

Within the main Cohort, a nested case-control study, called the Focus Cohort, was established to enable comparisons between subjects exposed to different types of prenatal psychological distress and their non-exposed controls. To define maternal psychological distress, the questionnaires for symptoms of depression (Edinburgh Postnatal Depression Scale [EPDS]), overall anxiety (Symptom Checklist-90, anxiety scale [SCL-90]) and pregnancy-related anxiety symptoms (Pregnancy-Related Anxiety Questionnaire-Revised 2 [PRAQ-R2]) were used at gwks 14, 24, and 34. Exploratory analyses establishing cut-points for the approximately highest and lowest 25th percentiles of maternal psychological distress during pregnancy were performed. The total sum score cut-off points for the psychological distress exposure and non-exposure were as follows: ≥ 12 and ≤ 6 for the EPDS, ≥ 10 and ≤ 4 for the SCL-90 anxiety subscale and ≥ 34 and ≤ 25 points for PRAQ-R, respectively (Karlsson et al., 2018). To become identified as a case required: 1) scoring at least once above the selected threshold on two different questionnaires, or 2) scoring at least twice above the selected threshold on the same instrument at any of the three prenatal time points or 3) prenatal maternal use of serotonin reuptake

inhibitors (SSRIs) should have occurred. The non-exposed controls needed to remain below the thresholds in all assessments (Karlsson et al., 2018).

Due to project logistics (i.e., availability of assisting personnel) and factors not systematically related to any family characteristics, 792 families from the Focus Cohort target population (N = 1219) were attempted to be reached by phone for recruiting the infants for Study II. Out of those families who were reached (N = 586), a total of 418 (71%) agreed to participate in the study, and 168 (21%) declined. Eventually 374 infants attended the stress test, and 38 infants were excluded from the data analyses. Eleven children were excluded from the cortisol analyses, as 4 children had extremely high cortisol concentrations, and mothers of 7 children did not fulfill the criteria for the Focus Cohort. Six children were excluded from the nasal sample (NPS) virologic analyses, two because the mother denied the nasal sampling, there was no consent for the NPS regarding one child and 3 NPS were not successfully studied in the laboratory. 21 children were excluded from the AUC analyses due to less than 3 cortisol measurements Thus, the final analyses in Study II comprised 336 infants with adequate cortisol and virologic samples.

Study III consisted of two cohorts being the Steps to the Healthy Development and Well-being of Children (STEPS) Study and the FinnBrain Birth Cohort. The STEPS Study is a cohort of 1135 children born between years 2008-2010 in the Hospital District of Southwest Finland. Blood samples for genetic analyses were obtained from STEPS study children at the age of two months. Children were followed intensively for respiratory infections from birth to two years of age (Toivonen et al., 2016b). In the FinnBrain Birth Cohort Study, a total of 1443 children were followed for respiratory infections from birth to one year of age. At the age of 3 months children were examined by a study physician in the FinnBrain Birth Cohort Study, and nasal swabs were collected as well as bloods samples for mRNA analyses (N = 71). Cord blood was used for genotyping. The final analysis included children with successful genotyping and information on respiratory infections (N = 971 for rs273259 and N = 972 for rs133969) (Figure 2).

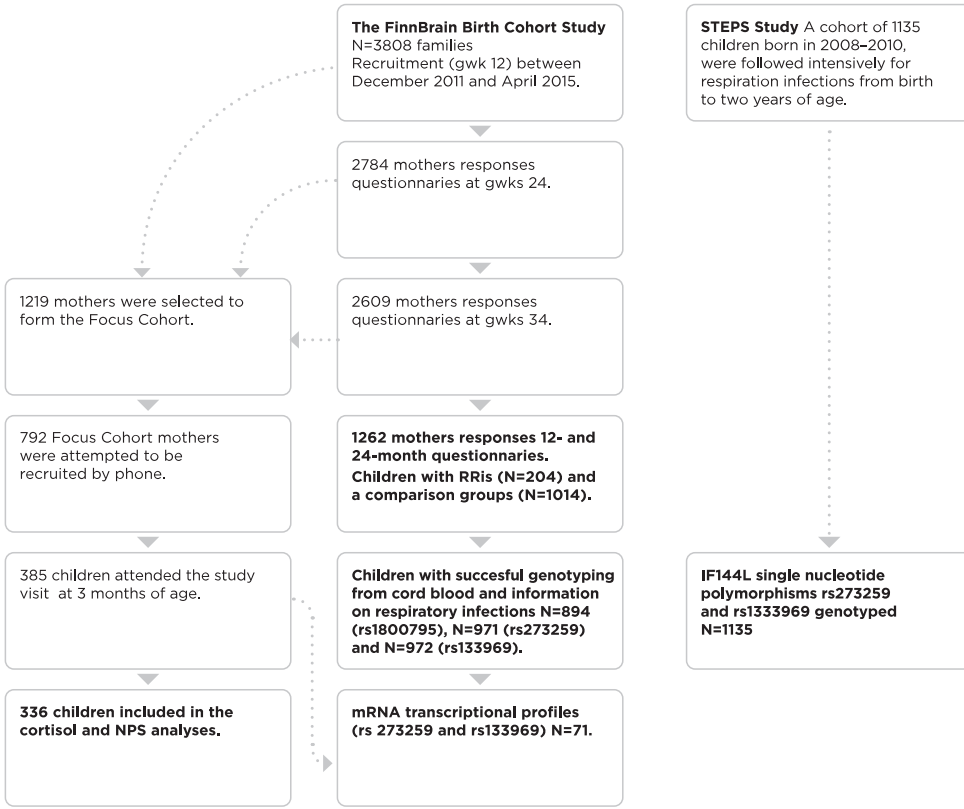

Figure 2. Flow chart of study participants in Studies I, II, III and IV. Interferon-induced protein 44-like (IFI44L), Nasopharynx/nasal sample (NPS), Steps to the Healthy Development and Well-being of Children (STEPS).

4.3 Self-report questionnaires

The parents answered the questionnaires at gwks 14, 24 and 34 as well as at child ages of 3, 6, 12, and 24 months. The data on background factors were collected from maternal questionnaires. Pregnancy and infant birth characteristics were obtained from the Finnish Medical Birth Register kept by the National Institute for Health and Welfare of Finland (www.thl.fi). In this work, the source of prenatal stress data were questionnaires of maternal symptoms of depression, anxiety and pregnancy specific anxiety (i.e., psychological distress).

4.3.1 Psychological distress: symptoms of depression and anxiety and relationship satisfaction/quality

Parental depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS). The EPDS is a widely used and sensitive meter of both

postnatal and prenatal depressive symptoms with 10 items each rated from 0 to 3 (higher scores indicate more depressive symptoms)(Cox et al., 1987). Parental symptoms of anxiety were assessed using the Symptom Checklist 90 (SCL-90) anxiety subscale, which is a reliable and valid symptom measure consisting of 10 items each rated from 0 to 5 (Derogatis et al., 1983). Again, higher scores indicate more pronounced symptoms.

Pregnancy-specific anxiety was assessed using The Pregnancy-Related Anxiety Questionnaire–Revised (PRAQ-R2), a 10-item shortened version of the PRAQ. The items of the PRAQ-R2 can be ordered into three subscales (Huizink et al., 2016). The first subscale called, "Fear of giving birth," consists of three items such as "I am worried about the pain of contractions and the pain during delivery." The second subscale called, "Worries about bearing a physically or mentally handicapped child," consists of four items, including "I sometimes think that our child will be in poor health or will be prone to illnesses." The third subscale called, "Concern about own appearance," consists of three items, such as "I am worried about my enormous weight gain." Scores on each item ranged from 1 to 5.

Relationship satisfaction was assessed using the Revised Dyadic Adjustment Scale (RDAS), a 14-item version of the DAS24, measuring couple/partner adjustment in three domains (Busby, D. M., Christensen, C., Crane, D. R., & Larson, 1995). Factor 1 Consensus consists of items called, "Career decisions," and "Religious matters" (with answers being always agree, almost always agree, occasionally agree, frequently disagree, almost always disagree and always disagree). Factor 2 being, "Satisfaction," consists of items called, "How often do you discuss or have you considered divorce, separation, or terminating your relationship?" and "How often do you and your partner quarrel?" (with answers being all the time, most of the time, more often than not, occasionally, rarely and never). Factor 3 called, "Cohesion," consists of items such as, "Work together on a project" and "Calmly discuss something" (with answers of: never, less than once a month, once or twice a month, once or twice a week, once a day and more often). Each item is scaled from 1 to 6, the total scores thus ranging from 14 to 84. Higher scores represent lower levels of relationship satisfaction.

Continuous sum scores of the EPDS, SCL-90/anxiety PRAQ-R2 and RDAS subscale were used in Study I, as this general population-based sample was likely to yield a very low number of subjects scoring above the clinical thresholds. In Study IV, a cut point of 10 points in The Edinburgh Postnatal Depression Scale was used as a marker for clinical depression and the upper quartile of Pregnancy-Related Anxiety Questionnaire–Revised2 (≥ 26 points) was the indicator for high stress.

4.4 Respiratory virus PCR

The nasal swab specimen for respiratory virus assessment was taken from front nostril and stored at -80°C before the analysis. 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. Polymerase chain reaction (PCR) for adenovirus, bocavirus, coronaviruses, enteroviruses, metapneumovirus, influenza A and B viruses, respiratory syncytial virus A and B, rhinovirus and parainfluenza virus types 1-4 was performed using a commercial multiplex test kit (Anyplex RV16, Seegene, Soul, Korea)

4.5 Genotyping of innate immunity genes and mRNA analyses

In the FinnBrain study, DNA samples were extracted according to standard procedures at the National Institute for Health and Welfare. DNA samples were genotyped with Illumina Infinium PsychArray BeadChip comprising 603132 SNPs (single nucleotide polymorphism) at the Estonian Genome Centre, and quality control (QC) was performed with PLINK (Chang et al., 2015). Markers were removed for missingness (> 5%) and the Hardy-Weinberg equilibrium (P-value $< 1x10^{-6}$). Individuals were checked for missing genotypes (> 5%), relatedness (identical by descent calculation, PI_HAT> 0.2) and population stratification (multidimensional scaling). In Study IV, genotyped data was inputted with IMPUTE2 using the 1000 Genomes project phase 3 haplotypes and a haplotype set of 1941 whole genome sequenced Finnish individuals as reference panels. Individuals were checked for missing genotypes (> 5%), relatedness (identical by descent calculation, PI_HAT> 0.2) and population stratification (multidimensional scaling). Genotyped data was inputted with IMPUTE2 using the 1000 Genomes project phase 3 haplotypes and a haplotype set of 1941 whole genome sequenced Finnish individuals as reference panels (Howie et al., 2009).

In the STEPS Study, DNA samples were extracted according to standard procedures in Immunogenetics Laboratory, University of Turku. For genetic analysis, IFI44L single nucleotide polymorphisms rs273259 and rs1333969 were analysed using the Sequenom (San Diego, California, USA) platform (Genome Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland).

For mRNA transcriptional profiles whole 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, USA) and stored at -20 °C. RNA was extracted and hybridized to Illumina HT-12 V4 beadchips (Illumina, San Diego, CA, USA). After hybridization, beadchips were scanned on the Illumina Beadstation 500

and Illumina GenomeStudio software (Illumina, San Diego, CA, USA) 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.

4.6 Outcomes

Study I's outcomes were recurrent respiratory infections at the child age of 12 and/or 24 months. Children with RRIs were identified from maternal reports with a question: "Has your child had recurrent infections" (with an answer choice of yes/no). This single question was applied as a method to determine the RRI group, as no absolute consensus exists on the number of infections per year that would define recurrent infections. Information on the number and timing (i.e., the child's age) of infections and antibiotic treatments as well as the number of physician visits at ages 3, 6, 12 and 24 months, and any physician-assessed pediatric diagnoses were inquired. The number of RTIs was then categorized in four groups: 0, 1-4, 5-10 and > 10, and the number of antibiotic treatments into groups: 0, 1-4, and 5-10 and > 10. The categorization was made because mothers had responded inaccurately to questionnaires on the number of infections or antibiotic treatments, for example, 3 to 6 or "too many." As the number of infections and antibiotic treatments had also been requested monthly before one year of age, the number of infections/antibiotic treatments was partially revised.

Study II's outcome was the infant's HPA axis reactivity. The stressor included a standardized pediatric examination with a venipuncture and a nasal swab with each being a source of mild physical discomfort. The infant HPA axis reactivity to the stressor was assessed measuring five saliva cortisol samples: at baseline, 0, 15, 25 and 35 minutes after a stressor. The saliva cortisol samples were collected using Salimetrics infant swabs (Stratech, Suffolk, UK) by a research nurse or a researcher. The polymer swab was held in an infant's mouth for two minutes. Saliva was collected by centrifuging tubes (15 minutes, 1800 g, 4°C) and immediately frozen at -70°C. The research nurse filled the protocol record form to keep track of timing, infant feeding and possible deviations from the study protocol. The cortisol concentrations were measured with a Cortisol Saliva Luminescence Immunoassay (IBL International, Hamburg, Germany).

Study III's outcomes were the association of IFI44L-gene polymorphisms rs273259 and rs1333969 with the frequency of RTIs, the number of rhinovirus infections, number of episodes of AOM and number of days with respiratory disease symptoms during the first two years of age in the Steps to the Healthy Development and Well-being of Children (STEPS) study. The findings were validated in the FinnBrain Birth Cohort study conducted geographically from the same area. In the

FinnBrain Birth Cohort study, the number of RTIs was then categorized in four groups: 0, 1-4, 5-10 and > 10, and the number of antibiotic treatments in the three groups with 0, 1-4 and 5 treatments or more. Each separate episode in a one-month period and any episode reported to continue over the turn of the month, were defined as a separate event. The effect of these gene polymorphisms on blood messenger RNA (mRNA) transcriptional profiles were analyzed in a subset of study subjects in the FinnBrain study.

Study IV's first outcome was the association of preselected genetic variants affecting the function of innate immune system (*IL6, IFI44L, IL10, IFIH1, MBL2, IL17A, TLR4*, *TLR2*, *IL4* and *TNF*) with the clinical outcome of recurrent respiratory infections after adjusting for sex. A second outcome was to analyze children with genetic variants of innate immunity and *in utero* exposure of maternal psychological distress for a higher risk score regarding RRIs defined as the interactions among the *IL-6* Rs1800795 polymorphism and the *IFI44L* gene rs273259 and rs1333969 polymorphisms and the maternal prenatal symptoms of depression and pregnancy specific anxiety.

4.7 Covariates and potential confounders

In Studies III and IV, genetic analyses were adjusted for sex. In Studies II and IV, analyses were also done separately for girls and boys. Maternal age at the birth of child (Study I, II and IV) and number of siblings (Study I and III) were included as potential confounders. Maternal educational level was included in Study I, II and IV. Maternal educational level was categorized into low (up to 12 years of education), medium (13-15 years of education) and high (over 15 years of education). Study I's covariates included gestational age (weeks), maternal smoking during the postnatal period (with an answer of yes/no), duration of breastfeeding (months), daycare attendance at the child age of 12 months (with an answer of yes/no) and the number of daycare days per week. Study II's covariates included: gestational age (weeks), birth weight (g) / length (cm), head circumference (cm), umbilical artery blood pH, maternal pre-pregnancy body-mass index (kg/m^2), maternal use of inhaled or oral corticosteroids (with an answer of yes/no), maternal smoking during the postnatal period (with an answer of yes/no) and maternal use of SSRIs (with an answer of yes/no). Study IV's covariates included: maternal smoking during the postnatal period (with an answer of yes/no), gestational age (weeks) and an Apgar score at 1 minute of age.

4.8 Statistical analyses

Study I

All statistical analyses were conducted using IBM SPSS v.24.0 (SPSS Inc, Chicago, IL, USA). Sociodemographic and other background data as well as questionnaire data were compared between the RRI group and comparison group by using a χ^2 or a Mann-Whitney U test.

Binary logistic regression was used to study the association between maternal prenatal psychological distress and a child's RRI status. The dependent variable was RRIs (0 = no, 1 = yes), and independent variables were duration of gestation, duration of breastfeeding, number of siblings, maternal smoking at the child age of 3 months (yes/no), maternal level of education, maternal depressive (EPDS) and anxiety (SCL-90/anxiety) symptoms at the child age of 2 years. These covariates were included, as they are previously identified and are well-established risk factors for RRIs. Postnatal symptoms of depression or anxiety were included to control for possible maternal report bias. As all the children were not born full-term, gestational age was also included as a potential covariate in the analyses. Due to multicollinearity, separate analyses were performed for each questionnaire (EPDS, SCL-90/anxiety, PRAQ-R2 and RDAS total sum scores). Analyses with prenatal EPDS and RDAS total sum scores were adjusted by maternal EPDS score at the child age of 2 years, and the analyses with prenatal SCL-90/anxiety and PRAQ-R2 scores were adjusted by maternal SCL-90/anxiety score at the child's age of 2 years. The effect of a rise on the EPDS score on the RRI risk as an OR was calculated by using a 3-point rise, i.e., minimum versus maximum points per question (max. sum = 30 points), SCL score OR by using a 5-point rise (max. sum = 50), PRAQ-R2 total sum score OR by using a 5-point rise (max. sum = 50) and RDAS total sum score OR was estimated by using a 6-point rise (max. sum = 84).

Study II

As the measure for the HPA axis reactivity, the area under the curve above/below the baseline (AUCi) was used (Figure 3). Log-transformed (natural logarithm) cortisol values were used in the calculations to avoid severe outlying AUCi values. Only the infants with a baseline cortisol value and ≥ 2 measured/available cortisol values after the acute stressor were included in the analyses.

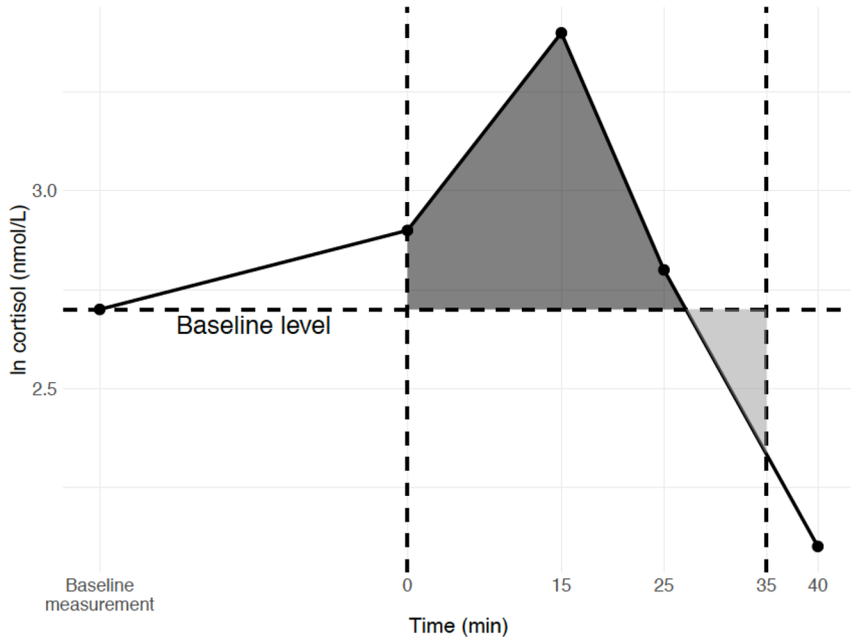

Figure 3. Illustration of AUCi: Area under the curve above/below baseline with respect to baseline (AUCi).

The missing cortisol values for the infants included in the analyses were inputted using multiple inputation. The predictive mean matching multiple inputation technique, by constructing 50 complete datasets, was used to input the missing cortisol values such that all included subjects had all four of the cortisol value measuring timepoints of 0, 15, 25 and 35 minutes cortisol values (Little, 1988). The missing values were predicted using the other cortisol values, their measurement time points, after the acute stressor, and the cortisol measurement kit as the predictors, with one kit/antibody for each sample was used to analyze the cortisol samples. After input, the area under the cortisol curve, between 0 and 35 minutes, above/below baseline (AUC_i), was calculated for each subject, separately in each 50 completed datasets. To be more precise, AUC_i was defined as:

$$AUC_i = \int_0^{35} [f_{PW}(t) - cort_{BL}]dt$$

where fPW(t) is the piecewise linear approximation of the cortisol curve and $cort_{BL}$ is the baseline cortisol value (Figure 3). Finally, standard linear regression techniques were used to analyze the differences in AUC_i. To test our main hypothesis, the model, AUC = psychological distress exposure + rhinovirus + psychological distress exposure × rhinovirus + sex, was used with such coding for

psychological distress exposure and rhinovirus that we were able to test the null hypothesis H0: μ psychological distress + = (μ psychological distress - + μcontrol+ + μcontrol-) / 3 where μxx is the mean AUC_i in group xx. The analyses were first made separately for each inputed dataset. The final results were then obtained by pooling the results using the Rubin's rules (Rubin, 1987). The R package "mice" was used for multiple inputation (Stef van Buuren, 2011) and the package ggplot2 for the results shown in Figures 4 and 5 (Wickham, 2009). The analyses of how AUCi associated with the psychological distress exposure, being psychological distress vs. control, rhinovirus status (+ vs. -) and infant sex were done using the t-tests. The main hypothesis being that the HPA axis reactivity is altered in the psychological distress /rhinovirus+ group was tested with an appropriate linear regression model, where infant sex was also controlled. Covariates for the adjusted model were selected based on statistical and clinical relevance. All statistical analyses were performed in R v.3.5.1 (R Core Team 2018).

Study III

In the STEPS Study, the association between the heterozygous or homozygous IFI44L polymorphisms rs273259 and rs1333969 and the incidence rate of RTIs, rhinovirus infections, days with RTI symptoms, AOM episodes and antibiotic treatments for RTIs during the ages of 0-2 years were analyzed using negative binomial regression analysis with a natural logarithm of the follow-up time as an offset adjusting for sex and the presence of sibling(s) at birth (using R version v.3.5.3). In the FinnBrain Birth Cohort Study, the association between the polymorphisms rs273259 and rs1333969 and RTIs and also antibiotic treatments were first tested with linear regression analysis implemented with PLINK (Purcell). The number of RTIs from birth to 1 year of age were then categorized into four groups 0, 1-4, 5-10, and > 10 infections. The number of antibiotic treatments from birth to 1 year of age were categorized into three groups being those with 0, 1-4, and 5 or more antibiotic treatments. The 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. Regression analyses were performed using R v.3.5.3 with the polar function in the MASS package.

For IFI44L expression, HT-12 V4 beadchips contained two probes targeting IFI44L (ILMN_1723912 and ILMN_1835092). The expression values of these two probes were highly correlated (Spearman $r = 0.906$, $P < 0.0001$) 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 a Mann-

Whitney test. Analysis was performed using GraphPad Prism software v. 8.0.0 Graphpad, San Diego, CA, USA.

Study IV

Sociodemographic, other background and questionnaire data were compared between the RRI and the comparison group by using a t-test, χ^2 or Mann-Whitney U test where appropriate. The association between preselected genetic variants of the innate immune system and RRIs was tested with the logistic regression analysis implemented with PLINK (Purcell) and adjusted for sex. The alleles were classified according to allele frequency so that the associations depended additively on the minor allele (Additive model), which is children having two minor alleles where labelled as (2), for having a minor/major genotype as (1) and a major/major genotype as (0).

Interactions between genotype and maternal prenatal psychological distress were analyzed using logistic regression models of the form:

$$\text{RRI} = \text{Distress} + \text{Genotype} + \text{Distress} \times \text{Genotype}$$

where "RRI" was a binary variable (0 = No, 1 = Yes), "Distress" was either categorized EPDS (< 10 points = Low, High ≥ 10 points) or categorized PRAQ-R2 (< 26 points = Low, High ≥ 26 points) and "Genotype" was a continuous variable labeled as above. The additive models were used to analyze the associations between genotype and RRIs separately in the children with or without *in utero* exposure of maternal psychological distress. EPDS and PRAQ-R2 were selected based on statistical relevance as group comparison between the RRI-group and the comparison group showed significant difference in the EPDS and PRAQ-R2 questionnaire total scores at gwks 34. All statistical analyses were conducted using IBM SPSS v.24.0 (SPSS Inc, Chicago, IL, USA) and R v.3.6.1. (R Core Team, 2019).

4.9 Ethics

The STEPS Study and the Finnbrain 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 own and on their child's behalf. The participants were informed that they could discontinue at any time without having to give an explanation. The Finnish legal regulation protocols were followed in this study including collection and storage of biological samples.

5 Results

5.1 Study population

The FinnBrain Birth Cohort Study consisted of 3837 children (including 29 twin pairs) and their parents. Of these families, 310 discontinued the study by delivery and 57 by three months postpartum, including 24 miscarriages, 8 stillbirths and 3 neonatal deaths. Pregnancy and infant birth characteristics from the Finnish Medical Birth Register were kept by the National Institute for Health and Welfare and were available for 3667 families (96.3%)(Karlsson et al., 2018). During the pregnancy of the 3808 mothers, 3095 (81.3%) answered the questionnaires at gwk 14, 2784 (73.1%) at gwk 24 and 2609 (68.5%) at gwk 34. When the child was one year old, 1693 (44%) mothers responded to the 12-month questionnaires, and when the child was two years old, 1443 (38%) mothers responded to the 24- month questionnaires.

The FinnBrain Birth Cohort Study population resembles the general population with the possible exceptions of a lower prevalence of younger, multiparous and smoking mothers. Also the prevalence of preterm births was lower in the cohort than among all the deliveries at the Turku University Hospital (Karlsson et al., 2018). Karlsson et al. noted that mothers, who responded also to the gwk 34 questionnaires, were older, were more often primiparous, had higher socioeconomic status, smoked less frequently and reported lower depressive symptom scores at gwk 14 than those who did not return the third trimester questionnaires (Karlsson et al., 2018). Children in the STEPS Study were also more often first-borns and were from families with a higher maternal educational level (Lagström et al., 2013).

In Study I and IV, with a partially overlapping population, there were no differences between the RRI-group and comparison group regarding mother's age and smoking during the postnatal period. In Study I, not all children in the population were born full-term, and this resulted in a significant difference between the RRI-group and comparison group. The duration of breastfeeding was shorter in the RRI-group, and there were more often siblings in the family. In Study II, there were no differences regarding infant characteristics at birth between the prenatal psychological stress-exposed children and their controls. Maternal educational level was significantly lower in the prenatal psychological stress -exposed group as well as maternal smoking during pregnancy was more common.

Table 3. Study populations.

	Population (N)	Excluded (N)	Study population (N)
Study I	1262 families responded 12- and 24-month questionnaires	44 (lack of data)	1218
Study II	374 families attended the stress test	38 (lack of data)	336
Study III	1693 families responded 12-month questionnaires	721 (No information of genotype)	971 *IFI44L*(rs273259) 972 *IFI44L*(rs133969)
Study IV	1262 families responded 12- and 24-month questionnaires	272 (No information of genotype)	990

5.2 Prenatal maternal psychological distress and risk for RRIs. (Study I)

In Study I, children were followed from birth to two years of age. Of the entire RRI group, 91 children were included based on the 12-month questionnaire (6% of cohort children) and the remaining 155 (11% of cohort children) were based on the 24-month questionnaire. There were 40 families (20%) out of the 204 who had responded "yes" to the question on RRIs both at 12 and 24 months. By one year of age, 30% of children in the RRI-group had five or greater number of RTIs, while only 5% of children in the comparison group had five or greater number of RTIs. Corresponding percentages for more than five antibiotic treatments were 20% versus 0.06% at one year of age. The majority (77%) of children in the RRI group had more than five respiratory infections before two years of age, while 16% of the comparison group reported more than five RTIs. 57% of children in the RRI-group had more than five antibiotic treatments, while 5% of children in comparison group had 5-10 antibiotic treatments. Half of the children in the RRI group had tympanostomy tubes inserted, whereas in the comparison group, only 4% of the children had tubes inserted.

Group comparisons showed significant differences in all maternal prenatal psychological distress questionnaires, as RRI group mothers reported significantly higher scores on EPDS-, PRAQ-R2-, SCL-90/anxiety- and RDAS-questionnaires. Mothers of the RRI-group had elevated levels of symptoms of depression ($P < 0.001$), pregnancy-specific anxiety ($P = 0.001$), anxiety ($P = 0.04$) and lower levels of parental relationship satisfaction ($P = 0.008$) when compared with the comparison group (Table 4). Postnatally, the RRI group mothers reported more symptoms of depression, anxiety and lower levels of relationship satisfaction, when the child was two years old ($P = 0.001$, $P = 0.03$, $P = 0.003$, respectively) (Table 4).

Table 4. Mean (SD) values of total scores of the Pregnancy-Related Anxiety Questionnaire–Revised2 (PRAQ-R2), the Symptom Checklist 90 anxiety subscale (SCL90/Anxiety), the Edinburgh Postnatal Depression Scale (EPDS) and the Revised Dyadic Adjustment Scale (RDAS) questionnaires in recurrent respiratory tract infections (RRI) and comparison groups.

		Mothers		
		RRI-group[1]	**Comparison group[2]**	**P value**
PRAQ-R2	gwks 34	24.2(6.9)	22.6 (6.5)	0.001
SCL90/Anxiety	gwks 34	3.8 (4.5)	3.0 (3.8)	0.04
	child 24 mo	3.5 (4.3)	2.5 (3.8)	0.03
EPDS	gwks 34	5.6 (4.2)	4.4 (4.0)	<0.001
	child 24 mo	5.4 (4.1)	4.3 (4.1)	0.001
RDAS	gwks 34	32.1 (8.0)	30.4 (6.0)	0.008
	child 24 mo	34.0 (8.6)	31.7 (7.4)	0.003

Data available N at gestational weeks (gwks) 34 /at child 24 months (mo): 1) PRAQ 188/-, SCL 191/177, EPDS 192/177, RDAS 189/169; 2) PRAQ 983/-, SCL/Anxiety 982/1006, EPDS 986/1006, RDAS 967/967. P values are based on Mann-Whitney U tests.

Logistic regression analyses were performed to see if the associations between maternal prenatal psychological distress and the child's RRIs remained after adjusting for the selected covariates. Analyses with prenatal EPDS and RDAS total sum scores resulted in an OR of 1.24 (95% CI 1.08-1.44) and an OR of 1.32 (95% CI 1.01-1.58), respectively, for a child´s RRIs. Regarding the prenatal maternal anxiety symptom questionnaires, ORs for a child's RRIs were 1.40 (95% CI 1.01-1.76, SCL/90 anxiety scale) and 1.28 (95% CI 1.11-1.47, PRAQ-R2). To conclude, when taking into account the number of siblings and the duration of breastfeeding and post-natal symptoms of depression and anxiety, psychological stress during the pre-natal period remained an independent RRI risk factor in our analyzes of the first 24 months of postnatal life (Table 5).

Table 5. Results of binary logistic regression analyses on maternal psychological distress at gestational weeks 34 predicting child recurrent respiratory tract infections (RRIs) by the age of two years (N = 1218) separately for each questionnaire (EPDS, SCL90/Anxiety, PRAQ-R2, RDAS).

MODEL 1	OR	95% CI	P value
EPDS at gwks 34	1.24	1.08–1.44	0.003
The duration of breastfeeding	0.90	0.82–0.98	0.02
N siblings	1.32	1.08–1.62	0.008
The duration of gestation	0.96	0.86–.08	0.53
Maternal level of education (ref. High)			0.06
Low	0.73	0.45–1.20	0.21
Middle	1.34	0.89–2.05	0.17
Maternal smoking	1.15	0.57–2.34	0.70
EPDS at the child age of 2 years	1.01	0.97–1.06	0.60
MODEL 2			
SCL90/Anxiety at gwks 34	1.40	1.01–1.76	0.006
The duration of breastfeeding	0.9	0.83–0.99	0.02
N siblings	1.36	1.11–1.67	0.003
The duration of gestation	0.95	0.85–1.07	0.42
Maternal level of education (ref. High)			0.045
Low	0.74	0.45–1.20	0.22
Middle	1.38	0.90–2.11	0.14
Maternal smoking	1.2	0.60–1.66	0.60
SCL90/Anxiety at the child age of 2 years	1.00	0.96–1.1	0.74
MODEL 3			
PRAQ-R2 at gwks 34	1.28	1.11–1.47	0.001
The duration of breastfeeding	0.90	0.83–1.00	0.02
N siblings	1.46	1.18–1.80	<0.001
The duration of gestation	0.9	0.83–0.99	0.58
Maternal level of education (ref. High)			0.048
Low	0.73	0.45–1.20	0.21
Middle	1.37	0.89–2.10	0.15
Maternal smoking	1.14	0.55–2.38	0.72
SCL90/Anxiety at the child age of 2 years	1.02	0.98–1.08	0.38
MODEL 4			
RDAS at gwks 34	1.32	1.01–1.58	0.003
The duration of breastfeeding	0.90	0.82–0.98	0.02
N siblings	1.32	1.08–1.62	0.007
The duration of gestation	0.95	0.85–1.07	0.42
Maternal level of education (ref. High)			0.07
Low	0.75	0.46–1.23	0.25
Middle	1.35	0.88–2.08	0.17
Maternal smoking	1.14	0.56–2.32	0.72
EPDS at the child age of 2 years	1.04	1.00–1.08	0.08

Independent variables: EPDS, SCL90/Anxiety, PRAQ-R2 total sum, RDAS total sum, at gestational weeks 34, duration of breastfeeding, number of siblings, duration of gestation, maternal smoking and level of education. Mothers' EPDS or SCL90/Anxiety score at the child age of 2 years. RDAS: The Revised Dyadic Adjustment Scale; EPDS: The Edinburgh Postnatal Depression; SCL90/Anxiety: Symptom Checklist 90 (SCL-90)/anxiety subscale; The Pregnancy-Related Anxiety Questionnaire–Revised (PRAQ-R2)

5.3 Prenatal maternal distress associates with blunted HPA axis function in rhinovirus-positive infants. (Study II)

Within the FinnBrain Birth Cohort, a nested case-control study, the Focus Cohort, was established to enable comparisons between subjects exposed to different types of prenatal psychological distress with their non-exposed controls (see: Methods section, pages 30-31). Study II includes a subset of children from Focus Cohort, that is, 336 10-week-old infants who participated in study visits and stress tests. Of these infants, 177 (53%) were boys, 148 (44%) were exposed to psychological distress and 76 (23%) were rhinovirus-positive (Table 6). Virus positive children did not have any signs of acute infection during study visit. Other viruses than the rhinovirus presented with only single positive findings in this general population-based study population.

Table 6. Study characteristics. P values are based on χ^2 and Mann-Whitney U tests for categorical and continuous variables, respectively. Bold values signify significance. PD: prenatal psychological stress; SD: standard deviation.

	PD EXPOSED (N = 148)	CONTROLS (N = 188)	P VALUE
Infant characteristics at birth			
Gestational age, weeks (SD)	39.4 (1.4)	40.0 (1.6)	0.30
Birth weight, g (SD) / length, cm (SD)	3595 (440)/ 51 (2)	3581 (487) / 51 (2)	0.69/0.89
Head circumference, cm (SD)	35 (1.3)	35 (1.5)	0.90
Umbilical artery, pH (SD)	7.27(0.8)	7.28 (0.8)	0.13
Male sex, nr (%)	80 (54)	97 (52)	0.65
Maternal characteristics during pregnancy			
Maternal age at birth, years (SD)	30 (4.5)	31 (4.3)	0.05
Maternal pre-pregnancy body-mass index,kg/m^2 (SD)	25.1 (5.1)	24 (4.0)	0.11
Maternal education, nr (%)			0.004
low (up to 12 years)	63 (44)	48 (25)	
middle (13-15 years)	36 (24)	56 (30)	
high (over 15 years)	47 (32)	84 (45)	
Maternal smoking, nr (%)	24 (16)	6 (3)	<0.001
Maternal use of inhaled or oral corticosteroids, nr (%)	3 (2)	7 (4)	0.37
Rhinovirus-positive infants at stress test, nr (%)	31(21)	45 (24)	0.50
Infant age at stress test, weeks (SD)	10.8 (2.0)	10.6 (2.0)	0.21

The infant HPA axis reactivity to the stressor was assessed measuring five saliva cortisol samples: baseline, 0, 15, 25 and 35 minutes after a stressor. The stressor included during study visit a standardized pediatric examination and blood sampling along with a nasal swab collection in the laboratory during the study visits. The infant HPA axis reactivity was not independently associated with the prenatal

psychological distressexposure (P = 0.30) or rhinovirus status (P = 0.09), but girls had higher AUCi than boys (P = 0.003) (Figure 4).

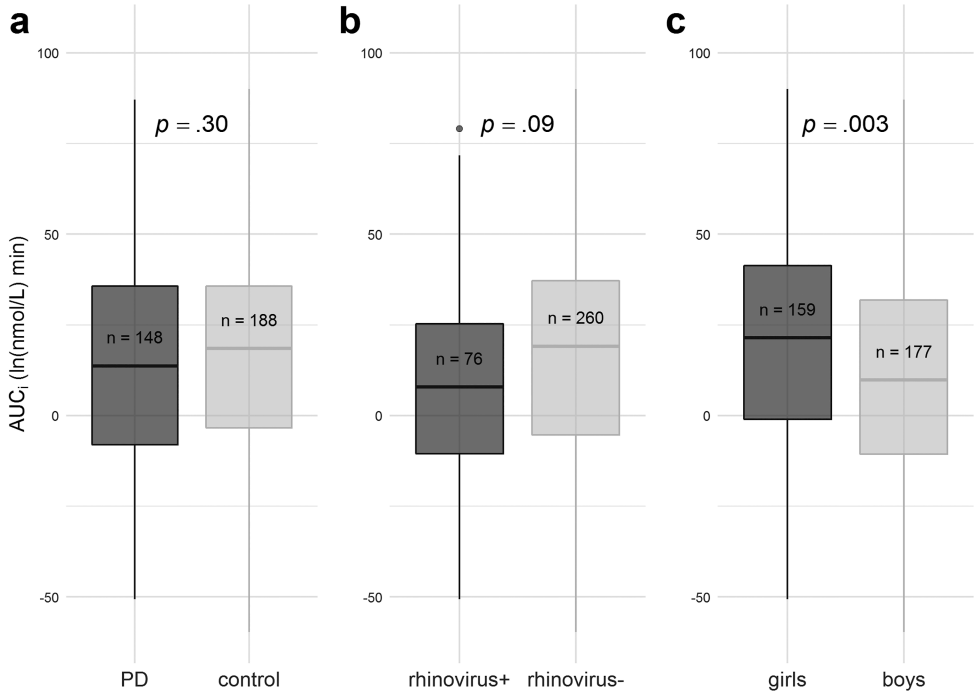

Figure 4. During the stress test, AUC_i was compared between: a) PD and control, b) rhinovirus- and rhinovirus+ or between c) girls and boys. The standard boxplots demonstrate the AUC_i in the groups. *AUC_i:* area under the curve above/below baseline; *PD:* exposure to prenatal psychological distress.

The hypothesis that HPA axis activity is different in psychological distress/ rhinovirus + recombinant phenotype was tested by comparing psychological distress /rhinovirus+ group with the average of AUC_i control/rhinovirus+, psychological distress /rhinovirus- and control/rhinovirus- groups, while controlling for infant sex. The subclinical rhinovirus constituted a natural stressor. The AUC_i was lower in psychological distress /rhinovirus+ than in the other groups (difference: 14.7 ln [nmol/L] × min; 95% (CI) 3.8-25.6; P = 0.008) (Fig. 5). The same comparison was separately tested for boys and girls, and the AUC_i difference was significant in boys (P = 0.04) but not in girls (P = 0.09) (Figure 5).

Controlling for maternal education level and smoking did not affect the results (15.3 ln [nmol/L]×min; 95%CI 4.1-26.4; P = 0.007). Finally, we tested the role of the rhinovirus status within the prenatal psychological distress exposure group. The AUC_i was lower in psychological distress /rhinovirus+ than in psychological distress

/rhinovirus- group (difference: 14.9 ln [nmol/L] × min; 95%CI 3.3-27.3; P = 0.01). Sensitivity analyses excluding the mothers using SSRIs or corticosteroids were performed to estimate the potential confounding effect of maternal medication. As the exclusion of these two groups did not alter the results, they were retained in the final analyses to maintain normal population representativity.

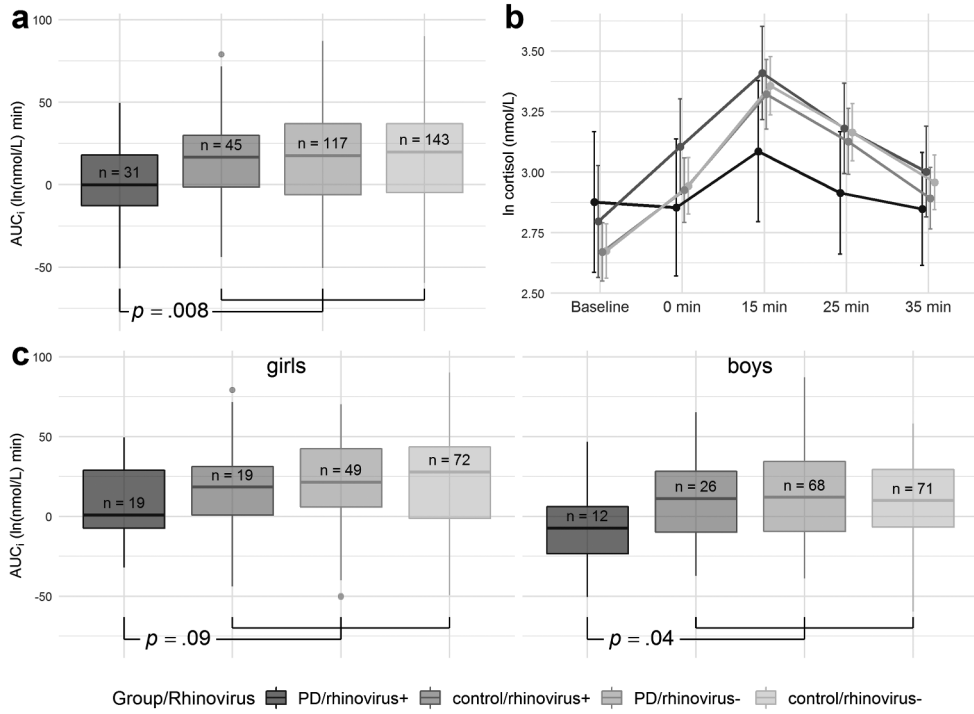

Figure 5. During the stress test: a) AUCi was compared between the PD/rhinovirus+ group and the average of control/rhinovirus+, PD/rhinovirus-, and control/rhinovirus- groups; b) mean cortisol values at five time points: baseline, 0 min, 15 min, 25 min and 35 min after stressor; and c) AUCi was compared between the groups separately in girls and boys. The standard boxplots demonstrate the AUCi in the groups. AUCi: area under the curve above/below baseline; PD: exposure to prenatal psychological distress.

5.4 The relationship of IFI44L gene polymorphisms rs1333969 and rs273259 to respiratory infections. (Study III)

In the STEPS Study, the final analysis included 1135 children that had genotypes and RTIs data including 738 with data on the 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 7 and RTI-related outcomes in Table 8 and 9.

Table 7. Background characteristics and allelic distribution of IFI44L polymorphisms in children in the STEPS Study and in the FinnBrain Birth Cohort Study.

	STEPS Study, No. (%) (N = 1135)	**FinnBrain Cohort, No. (%) (N = 971)**
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

5.4.1 IFI44L gene polymorphisms and respiratory tract infections in the STEPS Study cohort

In the STEPS cohort, slightly decreased rates of days with RTI symptoms were observed for children with a CT (major/minor) genotype of rs1333969 compared to those with CC (major/major) genotype. The minor G allele of the rs273259 polymorphism was associated with a decreased rate of AOM episodes (Table 7). Children with a homozygous GG (minor/minor) genotype had a lower rate of AOM episodes compared to children with the AA (major/major) genotype. Similarly, the minor T allele of the rs1333969 polymorphism was associated with a decreased rate of AOM episodes. The GG (minor/minor) genotype of the rs273259 and the TT (minor/minor) genotype of the rs1333969 were associated with lower rates of antibiotic treatments for RTIs compared to children with the respective major/major genotypes (Table 8).

Table 8. Association between IFI44L polymorphisms and rates of respiratory tract infections (RTIs) and related outcomes during the age of 0–2 years in the STEPS Study children (N = 1135).

	IFI44L gene polymorphism	Genotype (No.)	Incidence rate per child-year (95% CI)[a]	Incidence rate ratio (95% CI)[a]	P value
RTIs[a]	rs273259	AA (491)	6.0 (5.7–6.3)	reference	
		AG (497)	5.8 (5.6–6.1)	0.98 (0.92–1.04)	0.46
		GG (147)	5.7 (5.3–6.2)	0.97 (0.89–1.06)	0.56
	rs1333969	CC (641)	6.0 (5.7–6.2)	reference	
		CT (414)	5.7 (5.4–6.0)	0.96 (0.90–1.01)	0.14
		TT (80)	6.0 (5.4–6.7)	1.04 (0.93–1.16)	0.46
Rhinovirus-positive RTIs[a]	rs273259	AA (319)	2.1 (1.9–2.2)	reference	
		AG (321)	1.9 (1.7–2.0)	0.92 (0.83–1.03)	0.16
		GG (96)	2.1 (1.8–2.5)	1.05 (0.89–1.24)	0.54
	rs1333969	CC (409)	2.0 (1.9–2.2)	reference	
		CT (269)	1.9 (1.8–2.1)	0.95 (0.85–1.06)	0.35
		TT (58)	2.2 (1.8–2.6)	1.12 (0.93–1.36)	0.24
Days with RTI symptoms[a]	rs273259	AA (445)	52.2 (48.7–55.9)	reference	
		AG (457)	50.0 (46.8–53.6)	0.96 (0.87–1.06)	0.41
		GG (134)	45.6 (40.3–51.8)	0.90 (0.78–1.04)	0.13
	rs1333969	CC (579)	52.7 (49.6–56.0)	reference	
		CT (384)	47.2 (43.9–50.9)	0.89 (0.81–0.98)	0.02
		TT (73)	48.8 (41.3–58.1)	0.96 (0.81–1.15)	0.67
Acute otitis media episodes[a]	rs273259	AA (491)	1.1 (1.0–1.2)	reference	
		AG (497)	0.9 (0.8–1.0)	0.87 (0.75–1.01)	0.07
		GG (147)	0.8 (0.6–1.0)	0.77 (0.61–0.96)	0.02
	rs1333969	CC (641)	1.0 (1.0–1.2)	reference	
		CT (414)	0.9 (0.8–1.0)	0.87 (0.75–1.01)	0.06
		TT (80)	0.7 (0.6–1.0)	0.74 (0.55–0.99)	0.04
Antibiotic treatments for RTIs[a]	rs273259	AA (491)	1.4 (1.3–1.6)	reference	
		AG (497)	1.3 (1.1–1.4)	0.89 (0.78–1.03)	0.13
		GG (147)	1.0 (0.9–1.3)	0.76 (0.62–0.95)	0.02
	rs1333969	CC (641)	1.4 (1.3–1.5)	reference	
		CT (414)	1.3 (1.1–1.4)	0.91 (0.79–1.05)	0.18
		TT (80)	1.0 (0.7–1.3)	0.73 (0.56–0.97)	0.03

CI, Confidence Interval; RTI, Respiratory Tract Infection.

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

5.4.2 IFI44L gene polymorphisms and respiratory tract 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 a lower number of RTIs during the first year of life in the FinnBrain Study Cohort. Children with the minor/minor genotype GG had an OR of 0.64 (95% CI, 0.42-0.97; P = 0.04), and children with the major/minor AG genotype an OR of 0.65 (95% CI, 0.48-0.86; P = 0.003) for RTI frequency when compared to the major/major genotype (Table 9). The rs1333969 minor allele T was similarly associated with a decreased RTI frequency (Table 9).

The heterozygous genotypes rs273259 AG and rs1333969 CT were significantly associated with decreased rates of antibiotic treatments for RTIs from birth to 1 year of age compared to the major/major genotypes (P = 0.02 and P = 0.04, respectively). The rates of antibiotic treatments were also lower in children with minor/minor compared to major/major genotypes of these polymorphisms, but the differences were not statistically significant. SNPs rs273259 and rs1333969 show mild linkage disequilibrium (r^2=0.58) among the Finnish population (1000 Genomes Project Consortium et al., 2015).

Table 9. 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)[B]	P value
RTIS[A]	IFI44L rs273259	AA (396)	reference	
		AG (441)	0.65 (0.48–0.86)	0.003
		GG (133)	0.64 (0.42–0.97)	0.04
	IFI44L rs1333969	CC (535)	reference	
		CT (357)	0.70 (0.53–0.94)	0.02
		TT (79)	0.67 (0.40–1.09)	0.11
Antibiotic treatments for RTIs[c]	IFI44L rs273259	AA (396)	reference	
		AG (441)	0.68 (0.50–0.93)	0.02
		GG (133)	0.76 (0.48–1.18)	0.23
	IFI44L rs1333969	CC (535)	reference	
		CT (357)	0.72 (0.52–0.98)	0.04
		TT (79)	0.92 (0.53–1.53)	0.75

CI, Confidence Interval; OR, Odds Ratio; RTI, Respiratory Tract Infection.
[a]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.
[b]Ordinal logistic regression adjusted for gender and presence of sibling(s) at birth.
[c]Antibiotic treatments from birth to 1 year of age were categorized in three groups: 0, 1-4, and 5 or more antibiotic treatments.

5.4.3 Effects of IFI44L gene variants on peripheral blood transcription patterns

Blood mRNA transcriptional profiles were analyzed in 71 children. These children had participated the FinnBrain birth cohort study visit at the age of 10 weeks and provided blood samples for mRNA analyses and NPS for respiratory tract virus detection. At least one respiratory virus, with rhinovirus being the most frequent, was detected in 25 (35%) of the children.

First, we compared IFI44L expression in all children according to rs273259 and rs1333969 genotypes and found no differences (Figure 6, Panels A and B). Next, we compared the IFI44L expression in a subset of children (N = 25), who were positive for at least one respiratory virus. In these virus-positive children, the rs1333969 genotype CC (major/major) was associated with higher IFI44L expression (P = 0.0036). A similar finding was observed with rs273259 (P = 0.048) (Figure 6, Panels C and D).

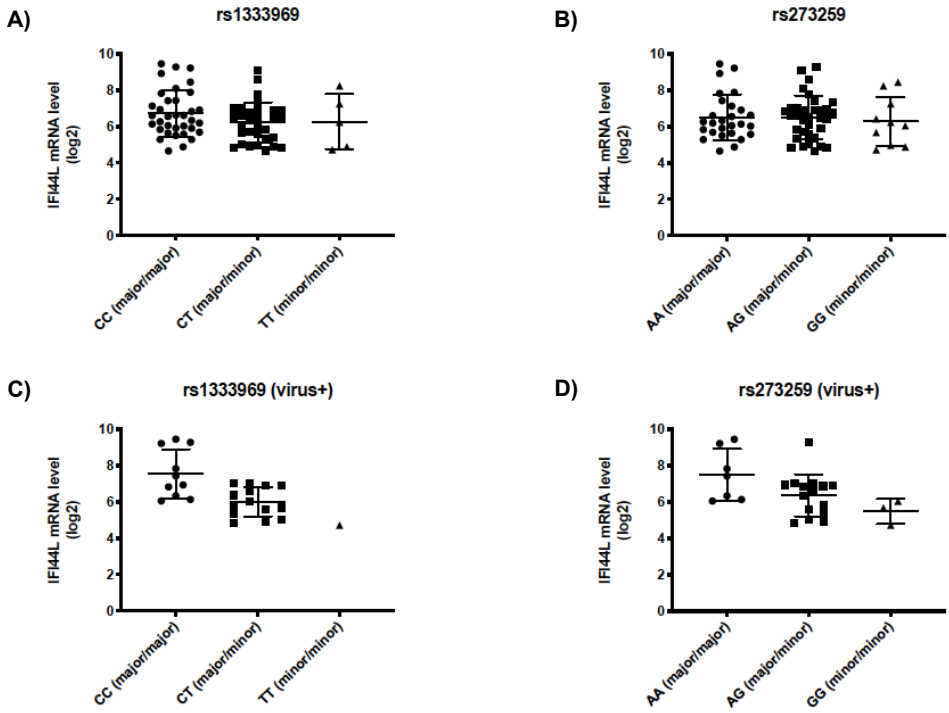

Figure 6. Expression values of IFI44L according to IFI44L polymorphisms and virus detections. In all 71 children with transcriptome data available, there were no differences in the IFI44L expression according to rs1333969 (Panel A) or rs273259 (Panel B) genotypes. However, in a subgroup of children with respiratory virus detection at the time of mRNA sampling, IFI44L expression level differed according to genotype (Panels C and D). In virus-positive children, the rs1333969 major/major genotype was associated with higher IFI44L expression levels compared to other genotypes (major/minor and minor/minor genotypes combined) (Panel C; Mann-Whitney P =0.0036). A similar finding was observed with rs273259 (Panel D; P = 0.048).

To explore if different IFI44L genotypes were associated with differences in the expression of other genes, we first performed differentially expressed genes (DEGs) analysis among all children. No DEGs were detected when comparing rs273259 and rs1333969 (major/major vs. other genotypes). However, when we included only children with virus detections (N = 25) and compared rs1333969 CC (major/major) genotype to other genotypes, we detected 116 DEGs. Of these 116 DEGs, 105 (91%) were overexpressed, and 11 (9%) were under expressed in the CC (major/major) genotype. These DEGs were strongly associated with activation of the interferon pathway and immune responses against viruses. Gene set enrichment analysis showed that the most significant GO biological process was the "type I interferon signaling pathway" with 17/66 overlapping transcripts ($P = 3.3 \times 10^{-21}$). Interferon signaling was also the most significant pathway in the IPA (Ingenuity Pathway Analysis software, QIAGEN, Redwood City, CA, USA, 12/36 genes; $P = 1.4 \times 10^{-18}$).

In a similar analysis comparing rs273259 genotypes among children with virus detections, we detected 23 DEGs. Of these 23 DEGs, 19 (82%) were overexpressed and 4 (18%) were under-expressed in the AA (major/major) genotype (Figure 7). These DEGs included immune response related genes such as granulysin (GNLY) and granzyme A (GZMA). However, no statistically significant GO biological processes or IPA pathways were identified. When comparing DEGs associated with rs1333969 (N=116) and rs273259 (N = 23) genotypes, we found that 16 DEGs were identified in both comparisons (Figure 8).

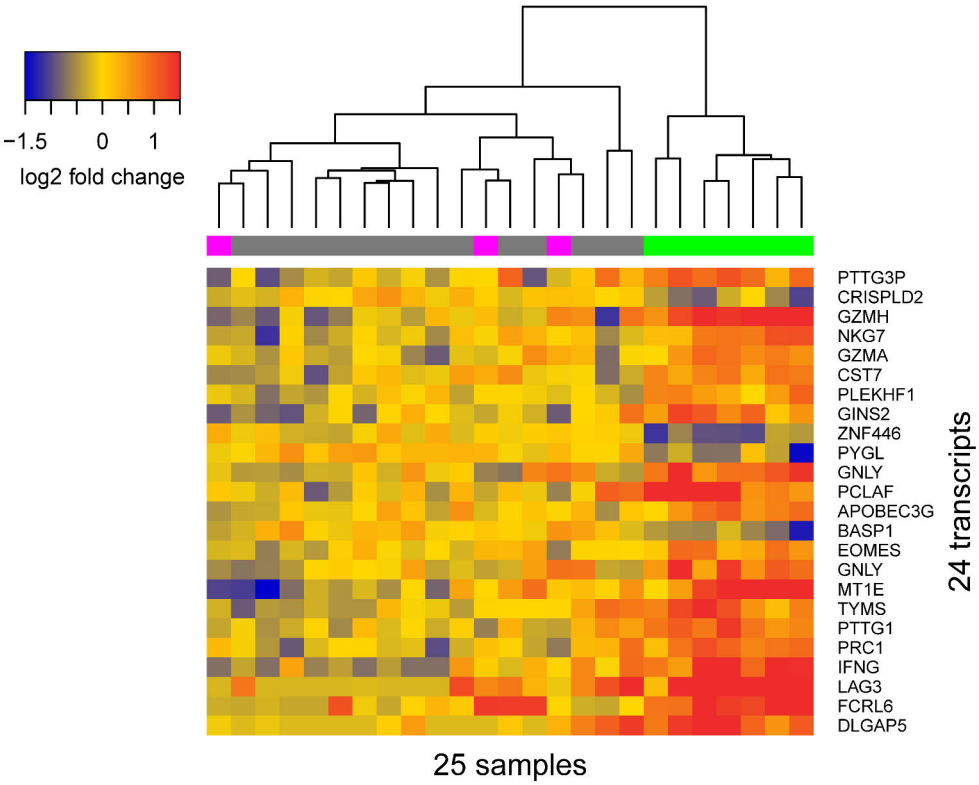

Figure 7. Heatmap visualizing the expression levels of 24 probes targeting 23 differentially expressed genes (DEGs) between rs273259 AA (major/major) and other genotypes in children with viral detections (N=25). False discovery rate corrected P values of 0.05- and 1.25- fold change were used as cut-offs to detect DEGs. 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

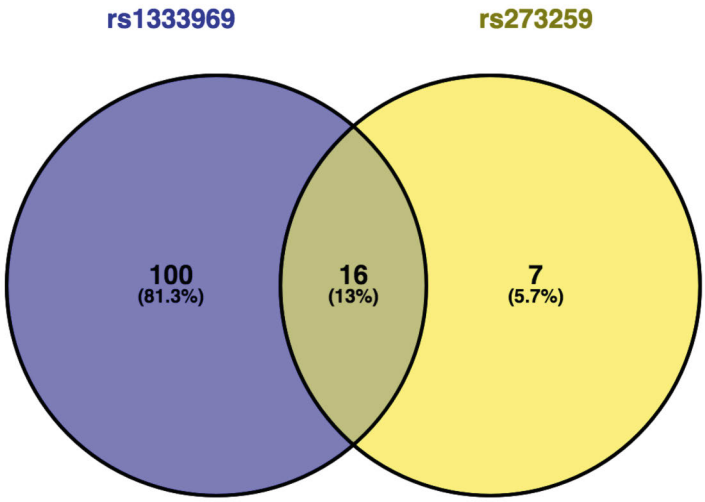

Figure 8. Venn diagram presenting the number of differentially expressed and overlapping genes in children with different genotypes of rs1333969 and rs273259 polymorphisms. Differential expression analysis was performed comparing major/major genotype to other genotypes (major/minor and minor/minor). False discovery rate adjusted P values .05- and 1.25- fold change were used as cut-offs for differentially expressed genes.

5.5 Gene variants of innate immunity and RRIs

5.5.1 The association between genetic variants of the innate immune system and RRIs

Of the 1262 children, whose mothers had responded to the questionnaires on child health at the child's ages of 12 and 24 months, 990 subjects (78.4%) provided a cord blood sample with successful genotyping. Children with RRIs (N = 96; 9.7% of the 990 subjects) were identified by maternal answers to a question, "Has your child had recurrent infections" (yes/no). Of the RRI group, 27/96 (28.1%) responded yes to the 12-month questionnaire, 56/96 (58.4%) responded yes to the 24-month questionnaire and 13/96 (13.5%) responded yes to both questionnaires. The remaining study population with successful genotyping from cord blood (N = 894/90.3%) was regarded as the comparison group. In contrast with the general population comparison group, the children with reported RRIs had more than five respiratory infections before one year of age (31% *vs.* 4%), more than five antibiotic treatments before two years of age (69% *vs.* 12%), and more often had tympanostomy tubes inserted (42% *vs.* 1%). As expected, based on Study I, from the partially overlapping population, RRI status was related to prenatal symptoms of depression (EPDS total sum score, P = 0.009) and pregnancy-specific anxiety (PRAQ-R2 total sum score, P = 0.028) but not to general anxiety symptoms.

The *IL6* (rs1800795) variant was associated with an elevated risk for RRIs (aOR 1.55; 95% CI, 1.14-2.12; P = 0.006, adjusted for sex) (Table 10). Other studied SNPs in innate immune genes were not significantly associated with increased or decreased risk for RRIs. Two polymorphisms in the interferon pathway participating gene *IFI44L*, rs1333969 (aOR 0.71; 95% CI, 0.50-1.01; P = 0.058) and rs273259 (aOR 0.74; 95%CI, 0.53-1.02; P = 0.070) appeared as protective of RRIs. For the variant forms of MBL, the adjusted OR for RRIs was 1.28 (95% CI, 0.83-1.97). In *MBL2* gene, the most common coding allele was labelled as "A," and the three variants were collectively labelled as "O." Variants selected for this study were rs1333969 and rs273259 at *IFI44L*; rs1990760, rs35667974, and rs3747517 at *IFIH1*; rs2243250 at *IL4*, rs1800795 at *IL6*; rs1800896 at *IL10*; rs2275913 at *IL17A*; rs5030737, rs1800450, and rs1800451 at *MBL2*; rs4986790 at *TLR4*; rs5743708 at *TLR2;* and rs361525 and rs1800629 at *TNF* (Table 10).

Table 10. The association between single nucleotide polymorphism of innate immune genes and risk for recurrent respiratory infections.

Gene	Reference SNP	MAF	N	FUNCTIONAL CONSEQUENCE	OR (95%CI)	P value
IFI44L	rs1333969	0.26	990	intron variant	0.71 (0.06–0.50)	0.058
IFI44L	rs273259	0.36	982	missense variant	0.74 (0.53–1.02)	0.070
IFIH1 (MDA5)	rs1990760	0.41	990	missense variant	1.03 (0.77–1.40	0.83
IFIH1 (MDA5)	rs35667974	0.02	990	missense variant	1.01 (0.31– 3.27)	0.99
IFIH1 (MDA5)	rs3747517	0.31	990	missense variant	0.82 (0.59–1.14)	0.24
IL4	rs2243250	0.32	980	upstream gene variant	0.92 (0.67–1.27)	0.62
IL6	rs1800795	0.46	976	intron variant	1.55 (1.14–2.12)	0.006
IL10	rs1800896	0.45	990	upstream gene variant	1.00 (0.75–1.35)	0.98
IL17A	rs2275913	0.44	898	upstream gene variant	0.98 (0.72–1.33)	0.88
MBL	rs5030737	0.06	990	missense variant		
MBL	rs1800450	0.13	990	missense variant	1.28 (0.83–1.97)	0.26
MBL	rs1800451	0.005	990	missense variant		
TLR4	rs4986790	0.10	990	missense variant	0.83 (0.50–1.41)	0.49
TLR2	rs5743708	0.03	957	missense variant	1.18 (0.49–2.84)	0.72
TNF	rs361525	0.02	990	upstream gene variant	1.18 (0.45–3.08)	0.73
TNF	rs1800629	0.15	990	upstream gene variant	0.75 (0.47–1.18)	0.21

Tested with the logistic regression analysis implemented with PLINK (Purcell) and adjusted for sex. N= number of genotyped children. Mainor allele frequency (MAF), Odds Ratio (OR) for recurrent respiratory infections, single nucleotide polymorphism (SNP).

5.5.2 IL6 and IFI44L genetic variants x prenatal maternal stress interaction

As expected based on our earlier study from the partially overlapping population (Study I), RRI status was related to maternal total symptom scores of the EPDS and PRAQ-R2 questionnaires at gwks 34. For the GxE interaction analyses, *IL6* and *IFI44L* polymorphisms were selected, because the association between *IL6* genetic variants and RRIs was significant and between *IFI44L* variants and RRIs nearly significant. The population was categorized into minor/minor, major/minor and major/major genotypes of rs1800795, rs1333969 and rs273259 (Table 11). No interactions were identified, when continuous sum scores of the EPDS and PRAQ-R2 were applied or when the children were categorized into two groups: 1) been born to a mother with clinically relevant scores on a depression/ pregnancy-specific anxiety questionnaire or 2) been born to a mother with low scores on a depression/ pregnancy-specific anxiety questionnaire (Table 11).

Table 11. Exposure to prenatal maternal psychological distress and genotype interaction logistic regression models. Odds Ratios, 95% Confidence Intervals and P values for the interaction terms.

EPDS/PRAQ-R2 scores at gwks 34[a]-genotype[b]	N	OR	95% CI	P
EPDS scores high - *IL6* (rs1800795)	893	1.24	0.56–2.73	0.60
PRAQR2 scores high - *IL6* (rs1800795)	894	1.15	0.61–2.16	0.67
EPDS scores high - *IFI44L* (rs1333969)	905	1.15	0.43–3.11	0.78
PRAQR2 scores high - *IFI44L* (rs1333969)	906	1.62	0.77–3.42	0.20
EPDS scores high - *IFI44L* (rs273259)	904	0.62	0.24–1.61	0.32
PRAQR2 scores high - *IFI44L* (rs273259)	905	1.31	0.67–2.54	0.43

a) The stress scores were categorized. The Edinburgh Postnatal Depression Scale (EPDS): <10 points = Low (the reference category); ≥ 10 points = High. Pregnancy-Related Anxiety Questionnaire–Revised2 (PRAQ-R2): < 26 points = Low (the reference category); ≥ 26 points = High. b) Additive Model: major/major allele = 0, minor/major allele = 1 and minor/minor allele = 2.

Finally, we addressed sex differences and performed *post hoc* analyses in children having *in utero* exposure of maternal psychological distress and children without *in utero* exposure psychological distress, separately for boys and girls, including the *IL6* (Rs1800795) polymorphism. These analyses showed a significant association for the risk for recurrent infections (OR 1.96; 95% CI, 1.04-3.67, P = 0.04) in boys carrying the G-allele and born to mothers with high scores in the dichotomized pregnancy-specific anxiety questionnaire. In a small group of N = 75 mothers with clinical depression during late pregnancy, boys had increased risk for RRIs (OR 1.95; 95% CI, 0.65-5.81, P = 0.23), but this observation lacked statistical power. The

risk for RRIs was greater among girls born to mothers with low scores in the pregnancy-specific anxiety questionnaire and to mothers with clinical depression, but the findings were not significant (Table 12).

Table 12. How the IL-6 genotype was associated with the risk for RRIs after exposure to prenatal maternal psychological distress. Studied separately for boys and girls.

***IL6* (rs1800795)[a]-EPDS/PRAQR2 scores[b]**	**N**	**OR**	**95%CI**	**P value**
Boys				
PRAQR2 scores low	317	1.17	0.68–2.01	0.58
PRAQR2 scores high	152	1.96	1.04–3.67	0.04
EPDS scores low	394	1.40	0.90–2.16	0.13
EPDS scores high	75	1.95	0.65–5.81	0.23
Girls				
PRAQR2 scores low	310	1.83	0.97–3.44	0.06
PRAQR2 scores high	114	1.23	0.58–2.61	0.59
EPDS scores low	354	1.57	0.90–2.75	0.11
EPDS scores high	69	1.69	0.66–4.34	0.28

a) Additive Model: major/major allele = 0, minor/major allele = 1 and minor/minor allele =2. b) The maternal prenatal psychological distress scores were categorized. The Edinburgh Postnatal Depression Scale (EPDS): <10 points = Low (the reference category); ≥ 10 points = High. Pregnancy-Related Anxiety Questionnaire–Revised2 (PRAQ-R2): < 26 points = Low (the reference category); ≥ 26 points = High. Odds Ratio (OR) for recurrent respiratory infections.

6 Discussion

6.1 Novel risk factors for respiratory infections: Prenatal maternal psychological distress and IFI44L and IL6 gene polymorphisms

In Study I, the symptoms of maternal depression and anxiety and declined marital satisfaction at the end of pregnancy were associated with RRIs in children. It has been established before that families are burdened with the child's RRIs and their parents' experience psychological distress (Louhi-Pirkanniemi et al., 2004). A novelty in our study was that maternal psychological distress was measured during pregnancy, before the child was born. The fact that maternal psychological distress was an independent risk factor for RRIs after adjusting for other important environmental risk factors place our results in the context of fetal programming supporting maternal prenatal stress effects on the development of the child's immune system. Mothers of the future RRI-group children reported during late pregnancy more symptoms of depression, pregnancy-specific anxiety, anxiety and lower levels of parental relationship satisfaction than mothers in the comparison group. This can be considered as notable source of psychological distress. A greater number of siblings and shorter duration of breastfeeding were also risk factors for child's RRIs, which is in line with previous studies (Nokso-Koivisto et al., 2002; Toivonen et al., 2016a). Children in the Study I were, to some extent, more often first-borns as 57% of the mothers were primiparous.

Previous work regarding maternal prenatal stress and alterations of child's immune system suggests that different forms of prenatal stress may have different outcomes. Sources of prenatal maternal stress according to studies have been major life events, for example, death of a close relative, natural catastrophes, daily hassles, poor relationship satisfaction, symptoms of depression, anxiety or pregnancy-specific anxiety. In order to standardize prenatal stress exposure, validated questionnaires on poor relationship satisfaction, symptoms of depression, anxiety or pregnancy-specific anxiety would help to compare results and enable repeated studies. The timing of the prenatal exposure should be taken into account, as previous studies suggest windows of vulnerability in fetal programming. Very early stages of development have been considered to be the most vulnerable time frame, but in our

Studies I and IV the association between maternal psychological distress and a child's RRIs were found during the third trimester.

In addition to environmental risk factors, the child's own characteristics affect the susceptibility to RRIs. Part of this variance can be explained by genetic variants, essentially through single nucleotide polymorphisms. In two prospective birth cohort studies (the STEPS study and the FinnBrain Birth Cohort Study), we analyzed the effect of two common single nucleotide polymorphisms in the type I interferon pathway gene IFI44L on children's susceptibility to RTIs. The function of several interferon stimulated genes with potential antiviral action is unclear. In the FinnBrain Birth Cohort Study, the minor alleles of rs273259 and rs1333969 were associated with a decreased RTIs and antibiotic treatments frequency during the first year of life. In the STEPS study cohort, the 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 decrease in the rate of AOM before the age of two years. The FinnBrain Cohort was followed less intensively as only RTIs for which a visit to a physician was needed were recorded. In the STEPs study, only 41% of RTIs necessitated a physician visit. This partly explains the different relationships observed between respiratory tract infection frequency and *IFI44L* gene variants in the two Cohorts. The association of minor alleles of *IFI44L* with a lower rate of antibiotic use suggests that these polymorphisms protect against more severe RTIs. These findings suggest that proper levels of IFI44L, being not too high or low, are important. This regulation may have an effect on later complications leading to, for example, acute otitis media. In mRNA analyses, notably, differences were seen only in children who were positive for a respiratory virus. Compared to children with major/major genotype, children with major/minor or minor/minor genotypes had weaker transcriptional activity of *IFI44L* and, also, in expression of other IFNs signaling pathway genes.

Although genetic variants of innate immunity and risk for RTIs have been studied before, there are only a few studies on genetic variants and RRIs (Westra et al., 2014; Toivonen et al., 2017; Nokso-Koivisto et al., 2014). In Study IV, we show an association between *IL6* polymorphisms and risk for RRIs during the first two years of life in a partly overlapping population of Study I. The *IL6* gene is located at the chromosome 7p21–24 locus with a 303bp promoter (Fishman et al., 1998). The polymorphism is located within the promoter region of the gene and influences transcriptional regulation and plasma IL-6 levels. It has been established with larger samples that the C allele is associated with significantly lower levels of plasma IL-6 (Fishman et al., 1998). The association between psychological distress and RTIs could be mediated by IL-6. In adults, psychological distress predicts elevated IL-6 levels in response to an upper respiratory infection with greater severity of symptoms (Cohen et al., 1999). IL-6 release is suggested to be partly mediated by

glucocorticoids, as almost all immune cells have glucocorticoid receptors. In addition, IL-6 has been shown to stimulate the adrenocortical axis showing bi-directional communication between the HPA axis and immune system (Dobbs et al., 1996; Bailey et al., 2003). IFNs are also sensitive to glucocorticoid regulation (Shodell and Siegal, 2001). The associations between *IFI44L* polymorphisms (rs1333969 and rs273259) and a child's RRIs were close to the significance level in Study IV. The *IL6* -174 CC genotype has been found to be a protective factor against childhood asthma (Li et al., 2015). A defective interferon response has been previously shown to be in relation to asthma (Durrani et al., 2012). More chronic proinflammation of the lung and airway may predispose to RRIs and the development of asthma. The susceptibility to RRIs and asthma may share common immunological pathways.

6.2 HPA axis as a mediating mechanism between maternal psychological distress and respiratory infections

Development of the HPA axis of the fetus and offspring plays a very important role for the child's subsequent susceptibility to infection. Lymphoid organs, and particularly the thymus, display a markedly elevated expression of glucocorticoid receptors during extra-uterine life (Merlot et al., 2008). In transgenic mice with impaired glucocorticoid function, an important role of glucocorticoids in the ontogeny of the immune system has been demonstrated (Sacedón et al., 1999). Maternal psychological distress and the associated high levels of cortisol convey information to the fetus about future environmental demands. According to the fetal programming theory, the fetus or newborn responds to adverse conditions by making developmental changes that are likely to increase survival and resilience in the expected environment (Bateson et al., 2004). If the environment matches the fetal programming, there are few effects on the future health of the child. However, a mismatch between actual environment and experience with fetal programming would increase the risk of illness (Avitsur et al., 2015). Study II was the first human study to investigate associations between prenatal psychological distress exposure and early infant HPA axis reactivity, when a concurrent rhinovirus infection that challenged the immune system was present. Interestingly, we found that after the preceding prenatal psychological distress exposure, the rhinovirus-positive infants had a blunted cortisol response in the stress test in comparison with their non-psychological distress exposed and psychological distress exposed counterparts. This association was more clearly seen in boys than in girls in analyses stratified by sex. Virus infection activates the systemic stress response. With infectious stressors, immune activation precedes, and glucocorticoids start to shape and restrain the

immune response. Studies have shown that normal glucocorticoid levels help mediate immune activation, whereas stress-induced glucocorticoid elevations begin to suppress the same activation later on in life (Dhabhar et al., 2012;McEwen, 2018). If the HPA axis has impaired function, then alterations in anti-viral immune responses may take place (Bailey et al., 2003; Webster and Sternberg, 2004). We can hypothesize that in our study, rhino-positive male infants were more prone to prolonged inflammation, as they failed to have an accurate HPA axis response to virus challenge. Both maternal prenatal stress and early rhinovirus wheezing illness have been linked to childhood asthma. The consensus has been that rhinovirus does not cause asthma in a straight-forward manner, but rather brings up a child's underlying vulnerability trait of an altered immune response (Lukkarinen et al., 2017).

In response to viral infection, activation of innate and adaptive immunity is required to limit the virus shedding and to stop viral replication. Pro-inflammatory cytokines induced during viral infection activate the HPA axis. For example, IL6 and IFNs are sensitive to glucocorticoid regulation. In addition to the traditional view of glucocorticoids as immunosuppressant hormones, an acute stress-induced rise of cortisol and adrenalin actually helps the immune system to respond to a pathogen (Dhabhar et al., 2012). HPA axis function and glucocorticoids do have immunomodulatory capacity that can both stimulate as well as suppress immune function (McEwen, 2018). Children in the RRI-group had exposure to maternal prenatal psychological distress and significantly more respiratory infections and antibiotic treatments before two years of age compared to the general population/comparison group. These findings suggest that children in the RRI-group can have altered immune responses to viral infections and susceptibility to later complications, such as AOMs. Animal and human studies suggest that alterations in HPA axis function affect the function or/and development of child immune system towards proinflammatory state. Moreover, high acute-phase cytokine producers from various cytokine polymorphisms may be susceptible to recurrent otitis media infections by different mechanisms (Revai et al., 2009). Children in the RRI group took significantly more antibiotics even before one year of age compared to the comparison group in both Studies I and IV. The strongest evidence of recurrent ear infections in the RRI-group in the Studies was the number of children that had tympanostomy tubes inserted as demonstrated in Study I (RRI group = 59% *vs.* the comparison group = 4%) and Study IV (RRI group = 42% *vs.* the comparison group = 1%).

6.3 Gene-environment interaction: Prenatal maternal psychological distress and the IL6 gene polymorphism

Mothers of children in the RRI group in Study IV had more symptoms of depression and pregnancy-specific anxiety during pregnancy, but the interaction models exploring the effect of prenatal maternal psychological distress to risk for recurrent infections in children with IL6 and IFI44L polymorphisms did not show increased risk for RRIs. This is in line with previous work of Andersson et al. suggesting that the prenatal maternal psychological distress influences immune development independently of genetic predisposition (Andersson et al., 2016).

Pregnancy-specific anxiety can be considered as a specific component of prenatal psychosocial stress and has been found to be a powerful predictor of child health outcomes (Beijers et al., 2010; Beijers et al., 2014). Our observation that the analyses conducted separately by child's sex yielded different results for boys and girls is suggestive of potential gender differences in GxE vulnerability for RRIs. As shown in earlier GxE studies, sensitivity to environmental risk factors for a disease can be inherited instead of the disease itself. It has been shown before that the effects of *in utero* psychological distress exposure on HPA axis function is different for male and female offspring and that these effects depend on the timing of *in utero* exposure to maternal distress at least for female offspring (Giesbrecht et al., 2017). In our study, maternal pregnancy-specific anxiety was associated with an increased the risk for RRIs in male offspring carrying the rs1800795 G-allele in a subgroup of high maternal prenatal psychological distress exposure that was in the highest 25th percentiles of PRAQ-R2 scores. In a small group of N = 75 mothers with clinical depression during late pregnancy, boys had a high risk for RRIs in association with *IL6* G allele, whereas the risk was low in boys of mothers without depression. Regarding girls, such findings were not seen.

6.4 Strengths and Limitations

The data on child health and identification of the recurrent RTIs group were based on maternal reports. Studies have shown that mothers experiencing elevated levels of stress are more likely to seek medical treatment for minor symptoms that could be treated at home (Horwitz et al., 1993; Louhi-Pirkanniemi et al., 2004). A recent study suggested that particularly prenatal maternal anxiety — both general anxiety and pregnancy-specific anxiety — were important predictors of almost all aspects of parenting stress later in life (Huizink et al., 2017). In the STEPS study, regarding the children with RRIs, 60% were diagnosed with at least 3 episodes of AOMs and 73% of children with RRIs received at least 3 antibiotic treatments before the age of two years (Toivonen et al., 2016a). In our RRI group, the majority of children had more

than 5 antibiotic treatments before the age of two years. This gives some validation to our findings, because the fact that our RRI group clearly differed from the control group in antibiotic treatments and insertion of tympanostomy tubes indicates that the maternal report actually depicts children with exceptionally high rates of RTIs. Also, the maternal report reflects importance of child's RRIs for the family.

In Study II, the number of infants in the subgroups was low due to recruitment challenges. As for generalizability of the results, it is noteworthy that the relationship between prenatal psychological distress and HPA axis responses may depend on infant age, sex and/or the nature of the stressor (Giesbrecht et al., 2017; Tollenaar et al., 2011). In our study, during the research visit, there were three types of stressors, namely a standardized pediatric examination was performed by a venipuncture and a nasal swab with each being a source of mild physical discomfort. While again, the concurrent rhinovirus infection can be considered as a natural stressor challenging the HPA axis functioning.

In Study III, the clinical outcomes were partly different between the two cohorts, and in the FinnBrain Cohort the follow-up was not as detailed as in the STEPS Study. However, the number of the antibiotic treatments and RTIs are similar in both cohorts and the associations between *IFI44L* polymorphisms and antibiotic use as well as frequency of RTIs support the findings in both cohorts (Korhonen et al., 2019; Toivonen et al., 2016a). Finnish people are a homogeneous genetic population of Caucasian origin, and as in all genetic association studies, corresponding data from other populations would be informative.

In Study IV, the outcomes of RRI group were based on maternal report, which resulted in some inaccuracy in characterization of the phenotype. Another limitation was that the findings on differences between sexes were based on *post hoc* analyses.

The main strengths of this work are the prospective design and relatively large sample size without any major exclusion criteria. This general population-based study allowed us to test of independent samples for the variances of the normal distribution. As for measurement of maternal prenatal psychological distress, we used longitudinal measurements during pre- and postnatal periods with validated questionnaires. For example, in the Norwegian Mother and Child Cohort Study 2015, they used a self-constructed measure for declined marital satisfaction (Henriksen and Thuen, 2015). Also, our questionnaires covered both depression and anxiety symptoms prenatally at three time points. Compared to previous studies, research on this topic has not focused on recurrent respiratory infections but on a broader scale of infections in children, including, for example, gastroenteritis or serious infections that required hospitalization (M Tegethoff et al., 2011; Beijers et al., 2010; Henriksen and Thuen, 2015; Nielsen et al., 2011).

We had high quality of our biological samples including the saliva cortisol, virus PCR, cord blood for genotyping and whole blood samples for mRNA transcriptional

profiles. We were able to search for functional effects of IFI44L polymorphisms by global transcriptome analysis in children with or without a virus infection in Study III. In Studies II and IV, we were not able to measure the inflammatory response to the infectious challenge, which is a limitation.

There is novelty in our finding in all of these Studies I, II, III and IV, as similar hypotheses have not been studied previously. Only some published papers have taken into account child sex, while studying biological susceptibility to infections, although a child's sex contributes to physiological and anatomical differences that influence innate and adaptive immune function (Klein and Flanagan, 2016). The genetic basis of risk for diseases differs between males and females, that is, that disease phenotypes have sex-specific genetic architectures (Morrow and Connallon, 2013). In our Studies II and IV, analyses were done separately for boys and girls and in Study III, analyses were adjusted by sex.

6.5 Future perspectives

Improving the understanding of individual and environmental risk factors for recurrent respiratory infections may lead to new prevention strategies. Such strategies would be needed to lower morbidity in children under the age of two and to reduce the use of antibiotics and heath care services including tympanostomy tubes insertions. Upper- and lower respiratory tract infections, AOMs, RRIs, bronchiolitis, wheezing and childhood asthma constitute a significant burden of disease for children, families and society.

Previously established risk factors for RTIs and AOMs, namely a shorter duration of breastfeeding and parental smoking, coexist with maternal psychological well-being. In our studies, potentially novel modifiable risk factors were maternal symptoms of depression and anxiety as well as relationship satisfaction. Pediatricians should seek to address families with psychosocial problems during the pre- and postnatal periods. We should create interventions that would support the mother's psychological well-being, and thus we could improve the health of the children.

Social and psychological factors should be considered already during pregnancy and across the early postnatal period. Epigenetic changes following prenatal stress are associated with changes in the *in utero* environment related to high levels of maternal stress hormones or stress-related alterations in placenta function (Monk et al., 2016). These changes may in turn affect the development of immune organs and host responses to infection later in life (Merlot et al., 2008). Although it is thought that the epigenetic changes that occur during pregnancy are permanent, there is also evidence of their resolution. Parental care during the neonatal period was found to be particularly important to the development of the HPA axis in both humans and

animals (Avitsur et al., 2015). In rats, higher quality maternal care, as quantified by more maternal licking and grooming, alter stable epigenetic states through effects on specific intracellular signaling pathways (Weaver et al., 2014). Mechanisms behind prenatal maternal psychological distress and the development of immune-related disorders deserve further research. For example, could the suppression of HPA axis functions (observed in Study II) associate with later disease development, such as atopic disorders and wheezing or asthma? What are the time points for windows of vulnerability during the pre- and postnatal period and therefore best times for intervention?

The host response to RTIs is unique, as some children have more severe and prolonged symptoms as well as more complications such as AOM and need more antibiotic treatments. Genetic variation of the innate immunity explains part of variance in the host response. When we better understand the pathogenesis of diseases and gather more information about genomes and phenotypes, in the future, there will be possibilities to have more targeted immunomodulatory treatments. We will be able to identify increased genotype-specific risk groups. Genes interact with the environment and in preventing diseases, GxE research is important for finding increased environment-specific risk for individuals with a particular genotype. Mainly animal studies, but also human studies have shown that there is resilience in immunity (Dantzer et al., 2018). Resilient individuals have a different immune phenotype than individuals that are vulnerable to stress. It could be possible to turn stress-susceptible individuals resilient and vice versa by changing their inflammatory phenotype. The resilient immune phenotype also influences the ability to recover from inflammation-induced symptoms. Bidirectional relationships between resilience and immunity by the gut microbiota open the possibility to influence them through probiotics and prebiotics (Dantzer et al., 2018).

This thesis provides epidemiological support for creating new hypotheses for both intervention studies as well as experimental studies on the biological mechanisms behind the associations between maternal psychological distress and a child's recurrent respiratory tract infections and other immune related disorders such as wheezing and asthma. Our work suggests that possible sex differences and an individual's genetic traits should be considered when focusing on environmental risk factors and prenatal programming.

7 Conclusions

This work suggests that maternal prenatal psychological distress potentially influences a child's susceptibility to RTIs. The HPA axis and its end-product cortisol are most likely the mediating mechanisms behind this association.

1. Maternal symptoms of depression, anxiety, pregnancy-specific anxiety and declined relationship quality, after controlling for essential confounding factors, associated with recurrent respiratory infections in children up to two years of life.
2. We observed a blunted cortisol response to a natural stressor, which was a concurrent subclinical rhinovirus infection, in children, whose mothers experienced psychological distress during pregnancy.
3. IFI44L gene polymorphisms showed to be protective against RTIs and AOMs. Minor alleles associated with a weaker interferon response. Part of the unique host response to viral infections could be explained by genetic trait and single nucleotide variations.
4. Prenatal maternal psychological distress influences on fetal programming can potentially explain a part of the non-genetic variability in immune-related disorders. Our gene-environment study suggested that prenatal maternal psychological distress does play an independent role as a risk factor for RRIs. Among the studied genetic variants of innate immunity, polymorphisms in the *IL6* gene could be a risk factor for RRIs.

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