

Enterovirus infections in early childhood and the risk of atopic disease – a nested case–control study

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Summary

Background Enterovirus infections in childhood have been associated with a reduced risk of atopy in cross-sectional studies.

Objective To study the relation between enterovirus infections in the first 2 years of life and atopic disease with IgE sensitization in a prospective study setting.

Methods This was a nested case–control study among children who had been followed from birth. Neutralizing antibodies against 12 enterovirus serotypes were analysed at the age of 2 years from 71 atopic children and 142 non-atopic control children. Atopy was defined as having an atopic disease and IgE antibodies against at least one aeroallergen by the age of 5 years.

Results Cumulative exposure to different enterovirus serotypes was inversely associated with atopy [odds ratio (OR) 0.73; 95% confidence interval (CI): 0.56–0.96]. The most pronounced protection was seen when echoviruses were analysed as a separate group (OR 0.63; 95%CI: 0.46–0.88).

Conclusions and Clinical Relevance We propose that exposure to several different enteroviruses in early childhood is inversely associated with atopic diseases. Our results support the hypothesis that repeated microbial infections in early life may protect from atopic sensitization and atopic diseases.

Keywords allergy, atopic disease, atopic sensitization, atopy, enterovirus, hygiene hypothesis, neutralizing antibodies

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Introduction

The prevalence of atopic diseases in childhood is high in most Western industrialized countries and the increasing trend is still continuing [1]. Also in Finland, the prevalence of atopic diseases, such as asthma and allergic rhinitis, as well as IgE sensitization has increased manifold in recent decades [2, 3]. According to the “hygiene hypothesis” this increase is related to a reduced microbial load in early childhood [4]. Numerous studies have provided indirect evidence to support the hypothesis, as growing up on a farm [5, 6], having one or more older siblings [4, 5, 7] and early atten-

dance in daycare [8] have been shown to be associated with protection from allergy. It has also been reported that geographically adjacent but socioeconomically contrasting countries differ markedly in the prevalence of allergic sensitization [9–11]. However, direct observations of the role of microbial infections in the development of atopic diseases have been somewhat controversial [12].

Viruses could theoretically be highly effective microbes in preventing atopy, as they induce a strong T helper (Th) 1 type immune response, which might suppress the excessive Th2 immune response often seen in atopic diseases. In fact, certain viral infections, such as

hepatitis A virus, Epstein Barr virus, varicella zoster virus, and measles [13–16], have been associated with lower risk of atopic diseases and allergic sensitization. However, there are also studies reporting conflicting results [17–20].

The possible protective effect conferred by infections may depend on their timing [12]. Early life has been proposed as an especially favourable period for protective microbial contacts [6, 14, 15, 21]. The route of exposure to microbial antigens may also be critical. The gut is believed to be an important organ in the early development of immunological tolerance and accordingly, previous studies suggest that orofecally transmitted infections are especially potent in protecting from allergic sensitization [22–24].

Enteroviruses are small RNA viruses comprising almost 100 serotypes. They are traditionally classified into echoviruses, coxsackie A and B viruses, and polioviruses. Vaccination against polio serotypes has almost eradicated wild-type polioviruses, while other enterovirus serotypes are common world-wide. Enterovirus infections are frequent from early infancy onwards. They are mainly subclinical or cause only mild upper respiratory tract infections, gastroenteritis or non-specific febrile illnesses, although severe manifestations, such as meningitis and myocarditis, also occasionally occur. Enteroviruses are transmitted mainly orofecally and replicate primarily in the gut. They have also been linked to the pathogenesis of type 1 diabetes [25], another disease with a rapidly increasing incidence over recent decades [26]. As the concept of hygiene hypothesis has recently been extended from allergies to other immune-mediated disorders including autoimmune diseases such as type 1 diabetes [27], we hypothesized that enteroviruses might also modulate the risk of atopic diseases. This presumption has recently been reinforced by our own studies showing an association between enterovirus infections and a lower prevalence of atopy in a cross-sectional setting [11, 28].

In this study, we wanted to investigate the relation between enteroviruses and atopic diseases in a nested case-control setting based on the prospective Diabetes Prediction and Prevention (DIPP) Study. Furthermore, we set out to study the importance of different enterovirus serotypes in atopic diseases.

Methods

Study population

The study population was derived from the DIPP Study, initiated in Finland in 1994. The basic design of the DIPP study has been described in detail elsewhere [29]. Briefly, newborn infants at the University Hospitals in Turku, Oulu and Tampere were screened at birth with

parental consent for type 1 diabetes associated HLA-DQB1 alleles [30]. The designated HLA-DQB1 risk genotypes are present in about 15% of the Finnish population [31]. Infants with these designated HLA-DQB1 risk alleles were invited to enter the DIPP study follow-up with regular visits to the DIPP study clinic with an interval of 3–6 months for the first 2 years of life and thereafter with an interval of 6–12 months. At each visit, the children underwent a comprehensive interview and a clinical examination, and a venous blood sample was taken. Serum samples were stored at -70°C in the DIPP study biobank. Data about diagnosed atopic diseases, parental education, attendance in daycare, pets at home, and siblings were asked regularly and recorded in the DIPP database.

For this study, a cohort of children was selected as follows. In spring 2009, we screened all DIPP children born in Tampere University Hospital between January 1, 1998 and December 31, 2003 for the physician-set diagnosis of bronchial asthma, atopic dermatitis, or allergic rhinitis. Age of the children at the time of the screening ranged from 5 to 11 years. The diagnoses had been made according to national guidelines and they had been recorded in the DIPP database. Only children with serum samples taken during the DIPP follow-up at the ages of 1, 2, and 5 years were eligible for the study. Children with type 1 diabetes associated autoantibodies and/or type 1 diabetes were excluded. Eligible children were then screened for IgE antibodies against a mixture of aeroallergens from the serum samples taken at the age of 5 years. The serum samples for IgE analyses were obtained from the DIPP study biobank. Children who had both an atopic disease and IgE antibodies comprised the group of case children. For each case child, two control children having neither a diagnosis of an atopic disease or IgE antibodies against aeroallergens were chosen. The case and control children were matched for the time of birth, gender, and HLA-DQB1 alleles.

Information about parental education, pets at home, number of older siblings, and child's age at the beginning of daycare was obtained from the DIPP database. Data about maternal smoking during pregnancy were received from the Finnish birth register.

This study was carried out within the DIPP cohort born in Tampere University Hospital. The DIPP study protocol has been approved by the ethical committee of the Tampere University Hospital (Tampere University Hospital approval number ETL 97193M).

IgE antibodies

IgE measurements were made from serum samples taken at the age of 5 years. IgE antibodies against a mixture of common airborne allergens [a multi-allergen

test (Phadiatop[®], Thermo Fisher Scientific, Uppsala, Sweden) containing allergens of birch, timothy, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*] were measured using the ImmunoCAP[®] enzyme immunoassay (Thermo Fisher Scientific, Uppsala, Sweden) according to manufacturer's protocol. Values of 0.35 kU/L or more against the allergen mixture were considered positive.

Virus strains

The ATCC (American Type Culture Collection) reference strains of enteroviruses [coxsackievirus B2 (CBV2), coxsackievirus B6 (CBV6), echovirus 9 (EV9), echovirus 11 (EV11), echovirus 30 (EV30)] as well as wild-type enterovirus isolates from Finland [coxsackievirus A9 (CAV9), coxsackievirus B1 (CBV1), coxsackievirus B3 (CBV3), echovirus 3 (EV3), echovirus 5 (EV5), echovirus 21 (EV21), echovirus 26 (EV26)] were used in this study.

Cell lines

A continuous cell line from African green monkey kidneys (GMK cells) was used for virus propagation. Cells were grown in minimal essential medium supplemented with foetal bovine serum (10% for cell growth, 1% for virus propagation), 15 mM Hepes, pH 7.4 and 15 mM MgCl₂. A549 cell line from human alveolar adenocarcinoma was used for EV30 virus propagation.

Plaque neutralization assay

Serum samples taken at 1 and 2 years of age were available from each case and control child to be used for the classical plaque neutralization assay [32]. This method measures antibodies in a serotype-specific manner against the enterovirus serotypes included in the assay. Neutralizing antibodies remain elevated for several years or decades reflecting the past infection history of the child. The serum samples taken at 2 years of age were analysed for the presence of neutralizing antibodies against each of the 12 enterovirus serotypes studied. The 1-year samples were analysed for echovirus serotypes 3, 5, 9, 11, and 30. In the assay, a four-fold dilution of serum was mixed with an equal volume of pretitrated virus (~100 plaque-forming units in 6 µL) and allowed to react for 1 h at 36°C followed by an overnight incubation at room temperature. After addition of the virus propagation medium, the sample was placed on monolayers of GMK cells or A549 cells on six well plates, density of $\sim 0.8 \times 10^6$ cells per well. After 30 min incubation at 36°C, 2 mL of plaquing overlay (cellulose in the virus propagation medium) was added. The amount of infectious virus was detected by counting the plaques after 2 days of incubation at

36°C. The serum was considered antibody positive if it blocked more than 80% of the virus infectivity.

Statistical methods

Conditional logistic regression analysis was used to determine the association between enterovirus infections and atopic diseases in the matched data. The results are presented as odds ratios (OR) and 95% confidence intervals (CI) for atopic diseases. Conditional logistic regression analysis was applied when comparing the association for each serotype individually, for having antibodies against at least one enterovirus serotype, for the cumulative prevalence of enterovirus antibodies and for confounding factors. Categorized variables were used for confounding factors: pets at home (no pets vs. at least one indoor pet of any kind), maternal education (no professional training or lower secondary training vs. higher secondary or tertiary training), paternal education (no professional training or lower secondary training vs. higher secondary or tertiary training), maternal tobacco smoking during pregnancy (no vs. yes), and having older siblings (no older siblings vs. at least one older sibling). A continuous variable was used for the attendance in daycare (number of months in daycare before the diagnosis of an atopic disease was recorded in the DIPP database). Conditional logistic regression analysis was applied when determining the association between individual confounders and atopic diseases. All analyses were performed using STATA 8.2 (Statacorp, College Station, TX, USA).

Results

A total of 2942 infants born in Tampere between January 1, 1998 and December 31, 2003 entered the DIPP study and 1852 (63%) stayed in the follow-up for at least until the age of 5 years. One hundred eighty-three children had a diagnosis code for an atopic disease and blood samples taken at the ages of 1, 2, and 5 years. 71 (39%) of them were IgE positive for aeroallergens at the age of 5 years. The case children having both a diagnosed atopic disease and IgE sensitization ($n = 71$) included 28 (39%) girls and 43 (61%) boys. Thirty-seven (52%) of the case children had atopic dermatitis, 29 (41%) bronchial asthma, and 5 (7%) allergic rhinitis. The median age when the atopic disease was recorded in the DIPP database was 22 months (range 2–102 month). There were 142 control children matched for age, gender, and HLA-DQB1 genotype. The median age difference between cases and controls was 9 days (range 0–64 days) and 14% of them had the HLA-DQB1 *02/*0302 genotype and 86% had the *0302/x genotype (x referring to other alleles than *02, *0301 or *0602).

Data about pets were available from 209 (98%), maternal tobacco smoking from 204 (96%), maternal education from 206 (97%), paternal education from 194 (91%), older siblings from 209 (98%), and attendance in daycare from 194 (91%) children. There were no statistically significant associations between the confounders analysed and atopy.

Neutralizing antibodies against 12 different enterovirus serotypes were measured from the serum samples taken at the age of 2 years. The prevalence of neutralizing antibodies against different enterovirus serotypes varied greatly (Fig. 1). Sixty-one (85.9%) case children and 119 (83.8%) control children had neutralizing antibodies against one or more enterovirus serotypes ($p = \text{ns}$). However, the control children were seropositive against a higher number of different enterovirus serotypes as compared with the cases (OR: 0.73; 95%CI: 0.56–0.96; $p = 0.025$) (Fig. 2a).

For subgroup analyses, the enteroviruses were divided into echovirus and coxsackievirus groups. Fifty-three (74.7%) case children and 111 (78.2%) control children had neutralizing antibodies against one or more echovirus serotypes ($p = \text{ns}$). The control children were seropositive against a higher number of different echovirus serotypes as compared with cases (OR: 0.63; 95%CI: 0.46–0.88; $p = 0.006$) (Fig. 2b). In the coxsackievirus group, 25 (35.2%) and 45 (31.7%) of case and control children, respectively, had antibodies against at least one serotype ($p = \text{ns}$). The number of neutralizing antibodies against different coxsackievirus serotypes did not differ between cases and controls.

To study the early encounters with enteroviruses, additional measurements of neutralizing antibodies against five echovirus serotypes (EV3, 5, 9, 11 and 30) were carried out from serum samples obtained at the age of 1 year. For most of these serotypes, antibody

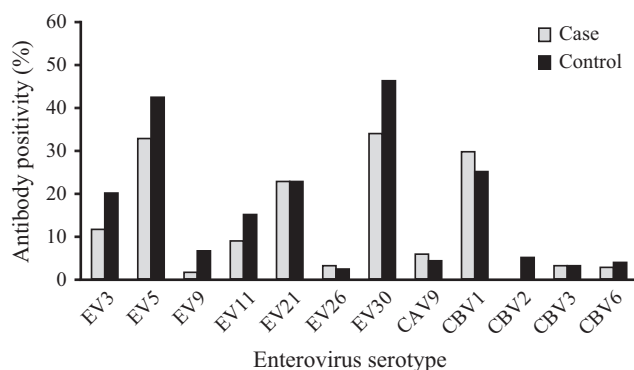


Fig. 1. The prevalence of neutralizing antibodies against different enterovirus serotypes. The percentage of case children ($n = 71$) and control children ($n = 142$) having neutralizing antibodies against individual enterovirus serotypes at the age of 2 years is shown.

positivity was slightly more frequent among the control children, but no statistically significant difference was seen in any individual serotype (Fig. 3a). When the number of neutralizing antibodies in each child was analysed at the age of 1 year, we did not observe any association between the accumulation of infections by different echovirus serotypes and protection from atopy (OR: 0.77; 95%CI: 0.54–1.10; $p = \text{ns}$), although this protective effect was seen at 2 years of age (Fig. 2b). However, 98 (69.0%) control children but only 39 (54.9%) case children had neutralizing antibodies against any echovirus serotype (OR: 0.52; 95%CI: 0.28–0.96; $p = 0.038$) (Fig. 3b).

Discussion

In this study, we found an inverse association between the cumulative exposure to enteroviruses and atopic diseases with IgE sensitization. More specifically, this finding seemed to be related to echoviruses only. Already at the age of 1 year, case children had a lower prevalence of echovirus antibodies and by the age of 2 years they were seropositive against a lower number of echovirus serotypes. This suggests that exposure to a

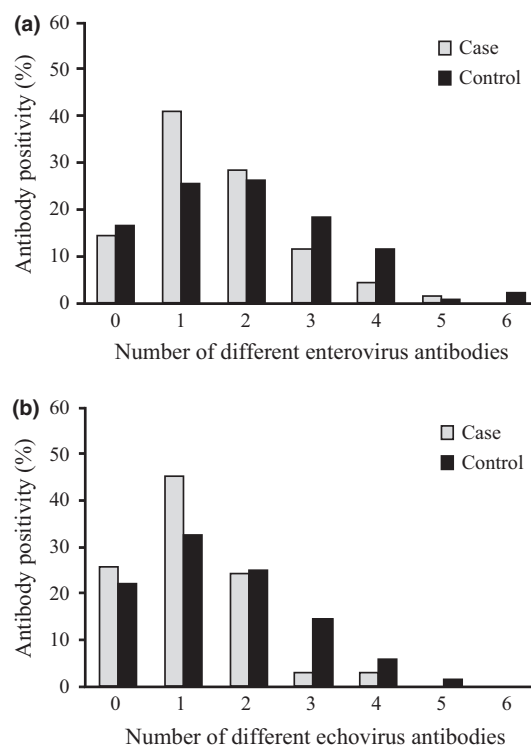


Fig. 2. The number of positive antibody test results against different enterovirus serotypes at the age of 2 years. The proportion of case ($n = 71$) and control ($n = 142$) children falling into each category (antibodies against 0–6 different serotypes) is presented as a percentage. Altogether 12 serotypes were analysed. Panel (a) shows the results for all enterovirus serotypes (echoviruses and coxsackieviruses) and panel (b) for the seven echovirus serotypes.

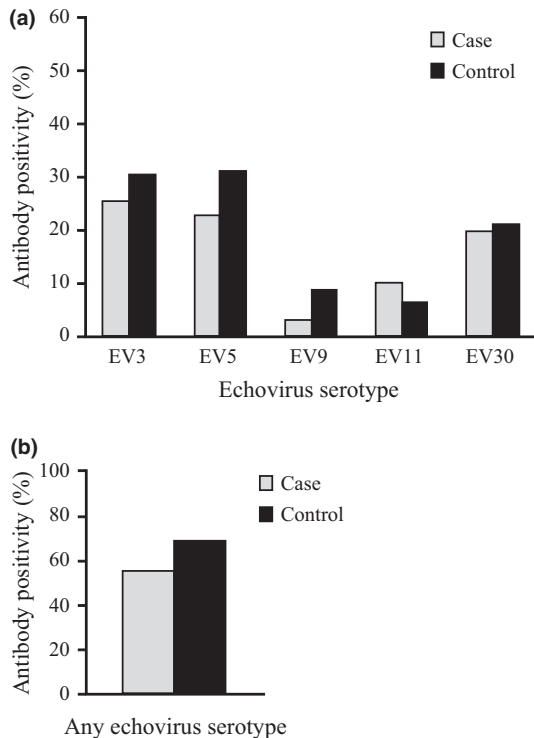


Fig. 3. The prevalence of neutralizing antibodies against echoviruses at the age of 1 year. The percentage of case ($n = 71$) and control children ($n = 142$) having neutralizing antibodies against individual echovirus serotypes is shown in the panel (a). Panel (b) shows the proportion of case and control children having neutralizing antibodies against one or more echovirus serotypes.

high number of different echoviruses during the first years of life might protect from atopic diseases.

There are only a few previous reports on the role of enteroviruses in atopy [11, 28, 33, 34], and to our knowledge, no earlier studies have been carried out in a prospective setting. In a cross-sectional study, seropositivity against enteroviruses protected from atopic sensitization in Russian Karelia but not in Finland [11]. However, in that study enterovirus antibodies were measured from older children using an EIA assay, a method known to lack serotype specificity. In a more recent study from the Russian Karelia, the presence of neutralizing antibodies against echoviruses was associated with a reduced risk of IgE sensitization in school-children [28]. This is in line with our results from this study carried out among very young children, suggesting that enterovirus infections are associated with lower risk of atopy and that enterovirus serotypes differ in terms of their effect on atopic diseases. These findings further emphasize the advantages of serotype-specific assays in studies evaluating the possible role of enteroviruses in atopic sensitization and atopic diseases.

Enteroviruses are transmitted principally orofecally, a feature suggested to be an important determinant of the

protection provided by certain microbes against atopic diseases. In adults, seropositivity to orofecal microbes has been shown to be associated with a reduced prevalence of atopic diseases and IgE sensitization [13, 22, 23]. Matricardi et al. reported that Italian atopic adults had a lower prevalence of hepatitis A virus antibodies than non-atopic controls, and that the risk of atopy decreased with a gradient of exposure to foodborne microbes but not with cumulative exposure to viruses transmitted via other routes [23]. However, in a nested case-control study from Scotland, seropositivity to hepatitis A was unrelated to the atopic status in adults [17]. In young children, it has been shown in a longitudinal study that children with salmonellosis had a lower prevalence of allergic rhinoconjunctivitis and asthma than children with enteritis due to other microbes [24]. It was also recently reported from a prospective birth cohort study, that seropositivity to rota- or norovirus did not protect from IgE sensitization [20].

In our study, the risk of childhood atopy decreased along with the gradient of exposure to different enterovirus serotypes, especially to echoviruses. This is in line with the results from some previous reports showing the importance of cumulative exposure to microbes [11, 23, 28]. In addition, atopic diseases have quite consistently been observed to be less frequent in children having several siblings, especially older siblings, most likely reflecting the importance of repeated early microbial contacts [4, 7, 35]. It has also been shown for enteroviruses that infants with one or more siblings had stronger T cell responses to coxsackievirus B4 and polioviruses than single infants [36].

As the immunological events resulting in the Th2-skewed immune response characteristic for atopic diseases are thought to begin already in neonates or even *in utero*, microbial contacts very early in life can be assumed to be of special importance. In a longitudinal birth cohort study, children with recurrent episodes of runny nose before the age of 1 year were less likely to develop asthma and IgE sensitization than children experiencing viral infections later in childhood [21]. The protective effect of early viral infections has been reported from other studies as well [14, 15] but there is also contradictory data suggesting that early viral infections might in fact increase the risk for atopy [18, 37, 38]. These conflicting data might partly be due to the heterogeneous nature of atopic diseases, perhaps modulated by different environmental factors [39, 40].

Previous studies have often relied on parental reported or physician diagnosed infections, thus detecting only those microbial contacts resulting in a clinical disease. However, viruses frequently cause subclinical infections and there are some reports implying a poor correlation between parental reported infections and virus serology [7]. It is likely that focusing on clinically

manifested infections misses a substantial proportion of microbial contacts. Serological assays, such as the plaque neutralization assay, have the advantage of detecting both subclinical and clinically overt infections and therefore provide a more comprehensive picture of microbial contacts. We were also able to measure neutralizing antibodies at two distinct time points, thus providing reliable information about the accumulation of antibodies to different enterovirus serotypes over time. In addition, atopy was defined as having both IgE antibodies against common aeroallergens and a clinically relevant atopic disease, a definition often used also in clinical settings. This strict definition reduces heterogeneity within the case group, thus facilitating a more reliable evaluation of risk factors. However, this also limits our conclusions to IgE positive atopic patients.

The genetic background of atopic diseases is complex and a significant number of susceptibility genes may be involved [41]. Our study population was selected from the cohort of DIPP study children carrying designated HLA-DQB1 alleles. Sporadic studies have reported some association between HLA-DQB1 alleles and atopic diseases or IgE sensitization [42, 43] but in other reports, no association has been observed [44, 45]. A recent Finnish study found no association between type 1 diabetes associated HLA genotypes and atopic sensitization [46]. Therefore, we do not suspect any bias due to the genetic selection of the population in this study. Most importantly, to further rule out the possibility of bias, the case and control children were matched for their HLA-DQB1 genotypes on study inclusion. They were also matched on study inclusion for age and gender. Other confounding factors analysed and possibly influencing the infectious environment of a child showed no independent association with atopic diseases.

The precise age of the case child at the time of the diagnosis of the atopic disease or IgE sensitization was

not available and therefore any conclusions about causality should be made with caution. However, we did show a dose-dependent like effect with exposure to different enterovirus serotypes, thus supporting true causality. Further studies are needed to find out whether this effect is specific for enteroviruses or if they are merely surrogate markers for the total burden of infections or some other environmental factors.

In conclusion, this study shows that cumulative exposure to enterovirus infections in early childhood is associated with a reduced risk of atopic diseases. As the factors influencing the atopic outcome are still largely unknown, measures aimed at eliciting the role of different environmental factors are urgently needed. Enteroviruses are suspected to play a role in the pathogenesis of type 1 diabetes and our research brings new insight into their possible role in atopic diseases as well. However, larger prospective studies are needed to further define their role in the pathogenesis of atopic diseases.

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Conflict of interest

Professor Hyöty and Professor Knip are minor shareholders of Vactech Ltd, which develops vaccines against picornaviruses. Other authors declare no conflict of interest.

References

- 1 Asher MI, Montefort S, Björkstén B *et al*. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; **368**:733–43.
- 2 Kosunen TU, Hökk-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002; **32**:373–8.
- 3 Latvala J, von Hertzen L, Lindholm H, Haahtela T. Trends in prevalence of asthma and allergy in Finnish young men: nationwide study, 1966–2003. *BMJ* 2005; **330**:1186–7.
- 4 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**:1259–60.
- 5 Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; **30**:201–8.
- 6 Riedler J, Braun-Fahrlander C, Eder W *et al*. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**:1129–33.
- 7 Nilsson C, Linde A, Montgomery SM *et al*. Does early EBV infection protect against IgE sensitization? *J Allergy Clin Immunol* 2005; **116**:438–44.
- 8 Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. *Lancet* 1999; **353**:450–4.
- 9 von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; **149**:358–64.

- 10 Vartiainen E, Petays T, Haahtela T, Jousilahti P, Pekkanen J. Allergic diseases, skin prick test responses, and IgE levels in North Karelia, Finland, and the Republic of Karelia, Russia. *J Allergy Clin Immunol* 2002; **109**: 643–8.
- 11 Seiskari T, Kondrashova A, Viskari H *et al.* Allergic sensitization and microbial load – a comparison between Finland and Russian Karelia. *Clin Exp Immunol* 2007; **148**:47–52.
- 12 von Hertzen LC, Haahtela T. Asthma and atopy – the price of affluence? *Allergy* 2004; **59**:124–37.
- 13 Linneberg A, Ostergaard C, Tvede M *et al.* IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study. *J Allergy Clin Immunol* 2003; **111**: 847–53.
- 14 Calvani M, Alessandri C, Paolone G, Rosengard L, Di Caro A, De Franco D. Correlation between Epstein Barr virus antibodies, serum IgE and atopic disease. *Pediatr Allergy Immunol* 1997; **8**:91–6.
- 15 Silverberg JI, Norowitz KB, Kleiman E *et al.* Association between varicella zoster virus infection and atopic dermatitis in early and late childhood: A case-control study. *J Allergy Clin Immunol* 2010; **126**:300–5.
- 16 Shaheen SO, Aaby P, Hall AJ *et al.* Measles and atopy in Guinea-Bissau. *Lancet* 1996; **347**:1792–6.
- 17 Bodner C, Anderson WJ, Reid TS, Godden DJ. Childhood exposure to infection and risk of adult onset wheeze and atopy. *Thorax* 2000; **55**: 383–7.
- 18 Paunio M, Heinonen OP, Virtanen M, Leinikki P, Patja A, Peltola H. Measles history and atopic diseases: a population-based cross-sectional study. *JAMA* 2000; **283**:343–6.
- 19 Sidorchuk A, Wickman M, Pershagen G, Lagarde F, Linde A. Cytomegalovirus infection and development of allergic diseases in early childhood: interaction with EBV infection? *J Allergy Clin Immunol* 2004; **114**:1434–40.
- 20 Reimerink J, Stelma F, Rockx B *et al.* Early-life rotavirus and norovirus infections in relation to development of atopic manifestation in infants. *Clin Exp Allergy* 2009; **39**:254–60.
- 21 Illi S, von Mutius E, Lau S *et al.* Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; **322**:390–5.
- 22 Matricardi PM, Rosmini F, Ferrigno L *et al.* Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997; **314**:999–1003.
- 23 Matricardi PM, Rosmini F, Riondino S *et al.* Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000; **320**:412–7.
- 24 Pelosi U, Porcedda G, Tiddia F *et al.* The inverse association of salmonellosis in infancy with allergic rhinoconjunctivitis and asthma at school-age: a longitudinal study. *Allergy* 2005; **60**:626–30.
- 25 Tauriainen S, Oikarinen S, Oikarinen M, Hyoty H. Enteroviruses in the pathogenesis of type 1 diabetes. *Semin Immunopathol* 2011; **33**:45–55.
- 26 Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet* 2009; **373**:2027–33.
- 27 Rook GAW. Review series on helminths, immune modulation and the hygiene hypothesis: The broader implications of the hygiene hypothesis. *Immunology* 2009; **126**:3–11.
- 28 Seiskari T, Kondrashova A, Tauriainen S *et al.* Role of enterovirus infections in IgE sensitization. *J Med Virol* 2012; **84**:268–71.
- 29 Salminen K, Sadeharju K, Lonnrot M *et al.* Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. *J Med Virol* 2003; **69**: 91–8.
- 30 Ilonen J, Reijonen H, Herva E *et al.* Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. The Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care* 1996; **19**:795–800.
- 31 Kupila A, Muona P, Simell T *et al.* Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia* 2001; **44**:290–7.
- 32 Roivainen M, Knip M, Hyoty H *et al.* Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. Childhood Diabetes in Finland (DiMe) Study Group. *J Med Virol* 1998; **56**:74–8.
- 33 Copenhaver CC, Gern JE, Li Z *et al.* Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004; **170**:175–80.
- 34 Jartti T, Kuusipalo H, Vuorinen T *et al.* Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatr Allergy Immunol* 2010; **21**: 1008–14.
- 35 Matricardi PM, Franzinelli F, Franco A *et al.* Sibship size, birth order, and atopy in 11,371 Italian young men. *J Allergy Clin Immunol* 1998; **101**:439–44.
- 36 Juhela S, Hyoty H, Lonnrot M, Roivainen M, Simell O, Ilonen J. Enterovirus infections and enterovirus specific T-cell responses in infancy. *J Med Virol* 1998; **54**:226–32.
- 37 Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. The Aberdeen WHEASE Group. *Thorax* 1998; **53**:28–32.
- 38 Benn CS, Melbye M, Wohlfahrt J, Bjorksten B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. *BMJ* 2004; **328**:1223–6.
- 39 Sly PD, Kusel M, Holt PG. Do early-life viral infections cause asthma? *J Allergy Clin Immunol* 2010; **125**:1202–5.
- 40 Flohr C, Pascoe D, Williams HC. Atopic dermatitis and the 'hygiene hypothesis': too clean to be true? *Br J Dermatol* 2005; **152**:202–16.
- 41 Grammatikos AP. The genetic and environmental basis of atopic diseases. *Ann Med* 2008; **40**:482–95.
- 42 Gao J, Lin Y, Qiu C, Liu Y, Ma Y, Liu Y. Association between HLA-DQA1, -DQB1 gene polymorphisms and susceptibility to asthma in northern Chinese subjects. *Chin Med J (Engl)* 2003; **116**:1078–82.
- 43 Parapanissiou E, Papastavrou T, Deligiannidis A *et al.* HLA antigens in Greek children with allergic bronchial

- asthma. *Tissue Antigens* 2005; **65**: 481–4.
- 44 Holloway JW, Doull I, Begishvili B, Beasley R, Holgate ST, Howell WM. Lack of evidence of a significant association between HLA-DR, DQ and DP genotypes and atopy in families with HDM allergy. *Clin Exp Allergy* 1996; **26**:1142–9.
- 45 Aron Y, Desmazes-Dufeu N, Matran R *et al*. Evidence of a strong, positive association between atopy and the HLA class II alleles DR4 and DR7. *Clin Exp Allergy* 1996; **26**:821–8.
- 46 Seiskari T, Viskari H, Kondrashova A *et al*. Co-occurrence of allergic sensitization and type 1 diabetes. *Ann Med* 2010; **42**:352–9.