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Effect of Farming on Asthma

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Abstract

In this review, we discuss an immunobiology model of farm exposure towards the protective effect of asthma. Unraveling the protective effect of farming exposure could help develop novel strategies to prevent asthma. Asthma is a chronic airway inflammation that causes coughing, wheezing, chest tightness or shortness of breath. The reasons for the increase in the prevalence of asthma worldwide is still unclear but has been hypothesized to be attributable to westernization/urbanization of rural regions thus resulting in the loss of rural farming environmental. In this review we discuss the effect of the environmental factors, specifically farming, on the risk of asthma in children. Here, we will summarize the main findings of 27 studies related to 11 different cohorts. Several studies have shown preventive effect of traditional farming on the prevalence and incidence of asthma in childhood. Furthermore, consumption of unpasteurized cow's milk, exposure to farm animals as well as fodder have been shown to have a protective effect on asthma. The precise mechanism of the protective effect is still unclear. There are assumptions, that maternal/childhood exposures to farm animals result in higher microbial exposures through which the protective effect might be mediated. Also, consumption of unpasteurized milk (when consumed during pregnancy by mother or early childhood by children) can modulate cytokine production patterns which could be responsible for the observed protective effect. **Conclusion.** This review provides evidence of the protective effect of farming environment i.e., exposure to farm animals, their fodder as well as consumption of unpasteurized cow's milk suggesting that novel strategies could be developed to prevent asthma.

Key Words: Asthma Epidemiology ▪ Childhood ▪ Farming ▪ Farm Milk ▪ Stables.

Introduction

Asthma is a chronic airway inflammation that causes coughing, wheezing, chest tightness or shortness of breath. It is a complex disease and is likely to be determined by multiple intrinsic and extrinsic factors. The intrinsic factors comprise gender, race, genetic predisposition and atopy whereas the extrinsic factors involves environmental influences like air pollution, allergens and smoking (1). Globally, asthma is ranked 16th among the leading causes of years lived with disability and 28th among the leading causes of burden of disease (2). The prevalence of asthma varies with global geographical position, with higher prevalence observed in Australasia, Europe and North America, as well as in parts of Latin America with lowest prevalence observed in the Indian subcontinent, Asia-Pacific, Eastern Mediterranean, and Northern and Eastern Europe (2). Based on the survey of the respiratory disease statistics from the European Union (EU)-28, 5.9% of the adult population reported that they suffered from asthma, with higher prevalence reported in women (6.6%) than in men (5.2%) (3). Similarly, according to the Centers for Disease Control and Prevention (CDC) from the United States, 1 out of 13 people have asthma which is more common in children (8.4%) than in adults (7.7%). Furthermore, children aged 5–11 years (9.6%) and 12–17 years (10.5%) were more affected than children aged 0–4 years (3.8%) (4). Moreover, the annual

Table 1. Overview of the Studies Assessing the Effect of Farm Environment on Asthma

Studies*	Size of the study	Exposure	Main findings
Riedler et al. (9).	Cross-sectional study mostly rural area in Austria; N=2001.	Living on farm.	Austrian children living on a farm have less asthma than children from a non-farming environment.
von Ehrenstein et al. (10).	Cross-sectional study in two Bavarian districts with extensive farming activity; N=10163.	Living on farm.	Farmers' children had lower prevalence of asthma. The protective effect was stronger for children whose families were running the farm on a full-time basis as compared with families with part-time farming activity.
Perkin et al. (11).	Cross-sectional study in the rural county of Shropshire, England; N=4767 (Stage 1) and N=879 (Stage 2).	Farm-related exposure and endotoxin.	Farmers' children had significantly less current asthma symptoms compared to nonfarming children. Children drinking unpasteurized milk were producing higher levels of IFN-γ.
Midodzi et al. (12).	Longitudinal study consisting of rural farming, rural non-farming and non-rural environments children; N=13524.	Living on farm.	The 2-year cumulative incidence of asthma was higher in children living in non-rural environment. Children living in a non-rural environment with parental history of asthma had an increased risk of asthma incidence when compared with children living in rural non-farming environment.
Illi et al. (13).	Rural regions of Austria, Germany and Switzerland; N=79888 (Phase I) and N=8419 (Phase II).	Farm-related exposures (contact with animals, stay in animal sheds, contact with animal feed, presence during parental farming activities, stay in barn or fodder storage room, and consumption of cow's milk produced on the farm) and IgE measurements.	Children living on a farm were at significantly reduced risk of asthma compared with nonfarm children. Traditional farming (i.e., with cows and cultivation) was protective against asthma.
Riedler et al. (14).	Cross-sectional study in rural areas of Austria, Germany, and Switzerland; N=812.	Timing, frequency, and intensity of children's exposure to stables and farm and pet animals, mothers' activity on the farm, duration of breastfeeding, timing of consumption of home-grown food and farm milk, vaccinations, avoidance of allergens, dust samples and serum IgE measurements.	Exposure of children younger than 1 year, compared with those aged 1–5 years, to stables and consumption of farm milk was associated with lower frequencies of asthma. Protection against development of asthma was independent from effect on atopic sensitization. Continual long-term exposure to stables until age 5 years was associated with the lowest frequencies of asthma.
Ege et al. (15).	Cross-sectional study from rural areas in 5 European countries; N=8263.	Farm-related exposures, allergen-specific IgE, RNA expression of CD14 and Toll-like receptor genes, and dust from children's mattresses was collected for microbial components.	Protective effect was found for keeping pig, farm milk consumption, frequent stay in animal sheds, child's involvement in haying and usage of silage. Protective factors were related with higher expression levels of genes of the innate immunity. Fungal extracellular polysaccharides was associated with protective effect for asthma.
Brunekreef et al. (16).	The study consist of data from 28 countries; N=194794 (exposure to farm animals) and N=194598 (maternal exposure to farm animals during pregnancy).	Early life exposure to farm animals.	A positive association was found between early exposure to farm animals and the prevalence of symptoms of asthma.

Table 1 (continued)

Studies*	Size of the study	Exposure	Main findings
Dong et al. (17).	Cross-sectional study in 3 cities in Liaoning province, China; N=16789.	Exposures to common indoor allergens.	Positive association was found between exposure to pets and farm animals with asthma.
Hugg et al. (18).	Cross-sectional study in towns of Finnish-Russian border; N=1093.	Exposures to pet and farm animals.	Increased risk of asthma in the urban children exposed to farm animals during early life.
Waser et al. (19).	Cross-sectional study from rural areas in 5 European countries; N=14893.	Dietary component and allergen-specific IgE.	Farm milk consumption ever in life showed an inverse association with asthma. Protective effect of other farm products except vegetables or fruits on asthma was found.
Brick et al. (20).	Birth cohort from the rural areas of Austria, Germany, Switzerland, Finland and France; nested case-control study of 35 asthmatic and 49 nonasthmatic children.	Fatty acid (FA) composition of unprocessed farm milk and industrially processed milk.	Consumption of unprocessed farm milk compared with shop milk was associated with protective effect of asthma. Part of the effect was explained by the higher levels of omega-3 polyunsaturated FAs.
Pfefferle et al. (21)*.	Birth cohort from the rural areas of Austria, Germany, Switzerland, Finland and France; N=625.	Maternal exposure to farming activities and farm dairy products during pregnancy. Outcome: Cytokine production in cord blood of children.	Maternal exposure to farming activities and farm dairy products during pregnancy modulated cytokine production patterns of offspring at birth.
Douwes et al. (22).	Rural cross-section study from New Zealand; N=1899.	Current, early and prenatal farm-related exposures.	Farmers' children had a lower incidence of asthma symptoms. Maternal exposure during pregnancy to farm animals and grain and/or hay reduced the risk of asthma symptoms.
Schaub et al. (23)*.	Birth cohort in rural southern Germany; N=82.	Maternal farming exposures. Outcome: Treg cells in cord blood stimulated with microbial stimulus and cytokines.	Farm exposures during pregnancy increase the number and function of cord blood Treg cells associated with lower TH2 cytokine secretion and lymphocyte proliferation on innate exposure.
Braun-Fahrländer et al. (24)*.	Rural areas of Germany, Austria or Switzerland; N=812.	Endotoxins levels in the bedding of children. Outcome: Asthma and cytokines production.	Endotoxin levels from the child's mattress were inversely related to the asthma. Cytokine production by leukocytes was inversely related to the endotoxin level in the bedding.
Schram et al. (25)*.	Cross-sectional study from rural areas in 5 European countries; N=478.	Living on farm. Outcome: To assess the levels of bacterial endotoxin, mould beta(1, 3)-glucans and fungal extracellular polysaccharides in house dust of children.	Farm children are not only consistently exposed to higher levels of endotoxin, but also to higher levels of mould components.
Stein et al. (26).	60 Amish and Hutterite children.	Levels of allergens, endotoxins and assessing the microbiome composition of indoor dust samples.	The prevalence of asthma was low in Amish children. Median endotoxin levels in Amish house dust was high. There was differences between the proportions, phenotypes, and functions of innate immune cells between both the two groups of children.
Ege et al. (27)*.	Cross-sectional studies; N=6843 (Parsifal) and N=9668 (Gabriela).	Living on farm and microbial exposure. Outcome: Asthma, screening for bacterial DNA and assess the levels and bacterial and fungal taxa in house dust of children.	In both studies, children who lived on farms had lower prevalence of asthma. The diversity of microbial exposure was inversely related to the risk of asthma.
Karvonen et al. (28).	Birth cohort in Finland; N=410.	Environmental microbial from house dust samples.	A score for the total quantity of microbial exposure was significantly (inverted-U shape) associated with asthma incidence.

Table 1 (continued)

Studies*	Size of the study	Exposure	Main findings
Birzele et al. (29)*.	Cross-sectional; N=86.	Bacterial community composition in mattress dust and nasal samples. Outcome: Asthma and farm exposure.	Farm exposure was positively associated with bacterial diversity in mattress dust samples. Asthma was inversely associated with bacterial diversity in mattress dust.
Kirjavainen et al. (30).	Birth cohorts (farm and non-farm); N=415.	House dust microbiota.	In the children grown up in non-farm homes, the risk of asthma decreases as the similarity of their home bacterial microbiota composition to that of farm homes increases.
Lluis et al. (45)*.	Munich Metropolitan area, Germany; N=200.	Genotypes of ten single nucleotide polymorphisms (SNP) covering the 17q21 locus. Outcome: Gene expression in 17q21 region and T-cell subsets in cord blood as well as gene expression of ORMDL3 in early and adult life.	The results suggest an association of 17q21 SNPs with ORMDL3, GSDMA expression and IL-17 secretion early in life.
Loss et al. (46)*.	Rural regions of Austria, Finland, France, Germany and Switzerland; N=983.	Genotyped SNPs in ORMDL3 and GSDMB genes at 17q21 and farming environmental exposure. Outcome: Asthma and wheeze.	17q21 locus relates to episodes of acute airway obstruction common to both transient wheeze and asthma.
Eder et al. (47).	Cross-sectional study in rural areas of Austria and Germany; N=609.	Genotyped SNPs in the TLR2 and TLR4 genes.	Genetic variation in TLR2 gene is a major determinant of the susceptibility to asthma in children of farmers.
Loss et al. (48)*.	Rural regions of Austria, Finland, France, Germany and Switzerland; N=938 (blood samples at birth) and 752 (year 1).	Framing environmental and nutritional exposure as well as polymorphisms in innate receptor genes. Outcome: mRNA expression of the Toll-like receptor (TLR) 1 through TLR9 and CD14.	Gene expression of innate immunity receptors in cord blood was higher in neonates of farmers. Unpasteurized farm milk consumption during the first year of life showed the strongest association with mRNA expression at year 1. Modification of the association between farm milk consumption and CD14 gene expression by the SNP CD14/C-1721T was not found.
Ege et al. (49)*.	Cross-sectional study from rural areas in 5 European countries; N=8263 and subsample (N=322) used for gene expression.	Maternal and child's exposure to microbial and farming environment. Outcome: Asthma and gene expression of TLR2, TLR4 and CD14.	Gene expression of receptors of innate immunity was strongly determined by maternal exposure to stable during pregnancy. Each additional farm animal species increased the gene expression of TLR2, TLR4 and CD14.

*If outcome other than asthma was used for the discussion of the findings in the study.

economic cost of asthma (from 2008-2013) in the United States, which includes medical costs as well as loss of work and school days, was reported more than $81.9 billion in US (5).

The reasons for the increase in the prevalence of asthma worldwide is still unclear but has been hypothesized to be attributable to westernization/urbanization of rural regions thus resulting in the loss of rural farming environment. Several epidemiological as well as intervention studies have been conducted to understand the effect of the environmental factors on the risk of asthma (6-8). In this review we discuss the effect of the environmental factors, specifically farming environment, on the risk of asthma in children. We would like to state that there are many more farm studies that have been performed. Since this review is not a systematic review we have selected those studies which describes the important aspects of farm upbringing. Table 1 summarizes the main findings of 27 studies related to 11 different cohorts which are discussed in this review.

The aim of this review is to summarize the effect of the farming environment on the risk of asthma in children.

Farming Environment

Epidemiological studies have shown protective effect of traditional farming on the prevalence and incidence of asthma in childhood. There are several studies that have looked at the effect of farming (exposures) with the risk of asthma (8). The study from Riedler et al. conducted in rural area in Austria (with a high proportion of farmers) observed a low prevalence in the farm children compared to the nonfarm children (1.1% vs. 3.9%) (9). In the study from von Ehrenstein et al. conducted in two rural Bavarian districts in Germany showed similar low prevalence of the doctor diagnosed asthma in the farm children (3.4%) compared to nonfarm children (6.4%) (10). Perkin and Strachan compared rural nonfarming and farmers' children and showed that farmer's children when compared to the rural nonfarm children had significantly less current asthma symptoms (adjusted odds ratio (aOR) 95% confidence interval (95% CI): 0.67 (0.49-0.91)) (11). Not only higher prevalence but also higher incidence of asthma was determined in a longitudinal study from Canada. The study by Midodzi et al. consisting of 13524 asthma-free children at baseline (aged 0-11) were surveyed and the 2-year cumulative incidence of asthma was reported to be 2.3% among children living in farm, 5.3% rural nonfarm and 5.7% non-rural environments (12). The study further showed that the children living in the farm environment had a reduced risk of asthma compared with children from rural nonfarm environment (OR (95% CI): 0.22 (0.07-0.74)).

The relationship between being raised or living on a farm and its protective effect on developing asthma has been investigated intensively. However, the possible causal mechanism between this associations are still not understood. Several studies are carried out to find which aspect of farm characteristics i.e., animal barns, exposure to straw and consumption of farm milk, can explain the protective effect of farming on the risk of asthma. The study from von Ehrenstein et al. showed a protective effect of full-time and part-time farming activity for asthma (part-time: OR (95% CI): 0.80 (0.37-0.83) and full-time: 0.45 (0.26-0.78)) suggesting a dose response effect (10). The study further showed the consumption of whole milk was higher among the farmer's offspring than among other children. Furthermore, the study by Illi et al. using the data of the GABRIEL Advanced Studies showed similar low prevalence of asthma in the farm children (11.4% Phase I and 14.1% Phase II) compared to the nonfarm children, with the exposed nonfarm children (i.e.; children not living on a farm but regularly exposed to stables, barns, or cow's milk produced on a farm) having intermediate prevalence (15.8% Phase I and 20.0 Phase II) (13). In the analysis adjusted for study centers, and potential confounders (family atopy, >2 siblings, sex, maternal smoking in pregnancy, and parental education), a protective effect for asthma was observed (aOR (95% CI): 0.68 (0.59-0.78)). This study further stratified the analysis based on types of farms and the exposure of a child to specific farm characteristics. The authors first identified 3 types of farms based on a latent class analysis. A protective effect of the third type of farming (comprised of dairy cows and breed cattle combined with cultivation mostly of grain and corn) compared to the first type of farming (comprised of pigs, poultry or horses combined with cultivation of grain and feeding of grain shed) within the farm children was observed for asthma (aOR (95% CI): 0.79 (0.65-0.95)). Furthermore, exploring the child's exposure to farm characteristics showed protective effect of i) having contact with cow (aOR (95% CI): 0.74 (0.62-0.89)), ii) staying with cow (aOR (95% CI): 0.79 (0.65-0.95)), iii) contact with straw (aOR (95% CI): 0.79 (0.66-0.95)), iv) present with parents during manuring (aOR (95% CI): 0.65 (0.47-0.90)) and v) consumption of farm milk (aOR (95% CI): 0.77 (0.66-0.90)) (13). The study by Riedler et al. showed similar protective effect of farming on the risk of asthma (aOR (95% CI): 0.30 (0.15-0.61)) and further looked at the exposures to several farming environmental factors (14). A substantial protection against developing asthma was seen only in the children exposed to stables and farm in the 1st year of life (aOR (95% CI): 0.14 (0.04-0.48)). Protection was also related to the continuing exposure after the first year of life to the stable compared to children who had no exposure to the stables in their first 5 years of life (aOR (95% CI): 0.09 (0.01-0.75)) (14). Ege et al. likewise looked at the association between several farming exposures and the risk of asthma showing protective effect of i) keeping pig (OR (95% CI): 0.57 (0.38-0.86)), ii) consumption of farm milk (OR (95% CI): 0.77 (0.60-0.99)), iii) frequent stay in the animal sheds (OR (95% CI): 0.71 (0.54-0.95)) and iv) child's involvement in haying (OR (95% CI): 0.56 (0.38-0.81)) (15).

In the study by Brunekreef et al. i.e., the Phase Three of the International Study of Asthma and Allergies in Childhood (ISAAC), which was carried out in 6- to 7-year-old children in urban populations across the world does not confirm the protective effects of farming environment on asthma. Further, stratifying by gross national income, the association between farm animal exposure in the first year of life with asthma was much stronger in the non-affluent (1.27 (1.12-1.44)) than in the affluent countries (0.96 (0.86–1.08)). Similar effect was observed in the analyses using exposure to farm animals during pregnancy. The reason for this could be that the children enrolled in this study were from urban or semi-urban areas rather than from rural areas having occasional rather than frequent or continuous exposure to farm animals in pregnancy and the first year of life (16). Similarly, the study by Dong et al. found positive association between exposure to cats, dogs, and farm animals with asthma. This study was carried out in 3 cities in Liaoning province, China (17). Another study by Hugg et al. conducted in the towns of Imatra in Finland and Svetogorsk in Russia also showed increased risk of asthma in the urban children exposed to farm animals during early life (18).

Looking at the relationship between farm-produced products and asthma, the study by Waser et al. similar to others showed protective effect of farm milk consumption with asthma (aOR (95% CI): 0.74 (0.61-0.88)) and other farm products except vegetables or fruits (19). To further asses the protective effect of unpasteurized cow's milk consumption on asthma Brick et al. used the data of a birth cohort to determine whether the differences in the fatty acid (FA) composition of the unpasteurized farm milk and the industrially processed milk contributed to this effect. The study showed that the consumption of unpasteurized farm milk compared to the shop milk was associated with a protective effect on asthma (aOR for consumption at the age of 4 years (95% CI): 0.26 (0.10-0.67)). The author further showed that the part of the effect could be explained by the higher fat content of the farm milk, especially the higher levels of ω-3 polyunsaturated FAs (aOR (95% CI): 0.29 (0.11-0.81)) (20). The study by Perking and Strachan investigated the relationship between farming environment and developing allergic problem and suggests that one of the possible mechanism for this observed protective effect may be through greater consumption of farm or unpasteurized milk. However, to note that the effect of frequent consumption of farm or unpasteurized milk on the risk of asthma did not show statistical significant association (OR (95% CI): 0.73 (0.53-1.02)) in the study (11). This non statistical significant association could be due to confounding factors in the farming environment that could be correlated with the farm-milk consumption. Pfefferle et al. investigated the associations between maternal farm exposures and cytokine levels in cord blood using the data of the Protection Against Allergy: Study in Rural Environments (PASTURE) birth cohort concluding that the maternal exposure to farming activities and farm dairy products during pregnancy modulate cytokine production patterns of offspring (21).

The study by Douwes et al. further looked at the mother's farm exposure toward the protective effect of children's asthma (22). The study showed that farmer's children had lower incidence of asthma symptoms compared to the nonfarm children. Current and maternal exposure during pregnancy to animals and/or grain and hay reduced the risk of asthma symptoms. A combination of prenatal and current exposure was strongly associated with asthma medication (OR (95% CI): 0.50 (0.30–0.82)) and asthma ever (OR (95% CI): 0.50 (0.33–0.76)) in the study. The study concluded that prenatal exposure may contribute to the low prevalence of asthma, hay fever and eczema in farmers' children, but continued exposure may be required to maintain optimal protection.

Immunological Studies of Farm Exposures

Schaub et al. showed that the farm exposures during pregnancy increase the number and function of cord blood Treg cells associated with lower T_H2 cytokine secretion and lymphocyte proliferation on innate exposure speculating that maternal farm exposure might reflect a natural model of immunotherapy (23). It has been suggested that the enhanced exposure to endotoxin (bacterial lipopolysaccharide (LPS)) is an important protective factor of farm environments. Braun-Fahrländer et al. assessed the levels of endotoxin in the bedding used by the farming and nonfarming children and examined it's relation to asthma. The results of the study showed that the endotoxin levels in samples of dust from the child's mattress were inversely related to the occurrence of asthma (24). Schram et al. evaluated the levels and determinants of bacterial endotoxin, mould beta (1, 3)-glucans and fungal extracellular polysaccharides (EPS) in the house dust of farm children, Steiner school children and reference children (25). The authors concluded that the farm children are not only consistently exposed to higher levels of endotoxin, but also to higher levels of mould components.

The above studies show that children who are grown up in the traditional farm environments are protected from developing asthma. This "farm effect" can be mainly hypothesized by the child's early life interaction with farm animals, in particular cows, and their milk products and microbes. The study from Stein et al. further demonstrated that this protective effect is mediated through innate immune pathways (26). The study used the data from the Amish and Hutterite school children living on farms in the United States. The prevalence of asthma in the Amish farm children was lower (5.2%) compared to the Hutterite farm children (21.3%). The authors further showed that microbial burden and composition differ between the Amish and Hutterite home environments. Although, there is a remarkable genetic similarity between Amish and Hutterite children, the opposite effects of their house dust on the airway responses and inflammation as observed in the mouse models, suggest that environmental exposures confer strong protection from asthma among the Amish by engaging innate immune responses, whereas the lack of such exposures and/or the presence of unidentified risk exposures promotes asthma risk among the Hutterites. Ege et al. used the data of the PARSIFAL and GABRIELA studies and concluded that children living on farms were exposed to a wider range of microbes than were the children from the reference group and the range of microbial exposure was inversely associated with asthma (27). Furthermore, a study by Karvonen et al. looked at the microbial exposures as a predictor of asthma using a birth cohort (28). The study showed that the associations of single microbial markers with risk of asthma was nonsignificant. However, the total quantity of microbial exposure (sum of indicators for fungi, Gram-positive bacteria, and Gram-negative bacteria) showed significant inverted-U-shaped association with incidence of asthma (28). The highest risk was found at medium levels and the lowest risk at the highest level. Birzele et al. showed that the farm exposure was positively associated with bacterial diversity found in the mattress dust samples (as determined by richness and Shannon index) of 86 school age children (29). In this study, asthma was inversely associated with richness and Shannon index in mattress dust. A recent study by Kirjavainen et al. modeled differences in house dust microbiota composition between farm and non-farm homes of Finnish birth cohorts, LUKAS1 and LUKAS2, showing that in children who grow up in non-farm homes, asthma risk decreases as the similarity of their home bacterial microbiota composition to that of farm homes increases. This effect was replicated in the GABRIELA Study. The authors conclude that the indoor dust microbiota composition appears to be a potential modifiable target for asthma prevention (30).

Genetic Factors Related to Asthma

In this section we will only focus on gene-environment interaction i.e., how few genes predispose towards an effect of farm-environment on asthma.

Asthma is not only influenced by environmental factors but also has genetic determinants associated with it. Twin studies have estimated the heritability of asthma to range between 35 to 95% (31, 32). Largescale genome-wide association studies have identified almost 30 loci that are associated with asthma (32-41). The analyses of the UK Biobank data consisting of 380503 study participants indicated that the asthma associated risk variants collectively explains 2.5% of the variation in disease (42). Many of these loci map to the genes that are involved in immune responses or transcription factors that mediate the immune responses. Single nucleotide polymorphisms (SNP) on chromosome 17q21 have been most robustly associated with childhood asthma and asthma in children exposed to environmental tobacco smoke. Two genes ORMDL3 and GSDM have emerged as the most likely candidate genes for asthma (43, 44). Lluis et al. investigated the relationship between the polymorphisms and the mRNA expression of 17q21 locus genes and their influence on the T-cell subsets in the cord blood of the children from the rural areas showing an association of 17q21 polymorphisms with ORMDL3 and GSDMA expression, as well as the secretion of IL-17 early in life. These results imply a functional role of the 17q21 locus affecting T-cell development during immune maturation (45). Further to test the environmental determinants of infections and wheeze in the first year of life, potential modifications of these associations by SNPs at ORMDL3 (rs8076131) and GSDMB (rs7216389, rs2290400) genes at 17q21, and the implications for different trajectories of wheeze using the data of the PASTURE birth cohort was conducted (46). The findings of the study suggest that the chromosome17q21 locus relates to episodes of acute airway obstruction which is common to both transient wheeze and asthma. The authors further suggests that the asthma risk alleles are the ones susceptible to the environmental influences. This gene–environment interaction revealed that the same genotype constitutes genetic risk and also allows for environmental protection, thereby providing options for prospective prevention strategies (46). Eder et al. used the data of the ALEX study to access if the polymorphisms in genes encoding TLRs might modulate the protective effects observed in farming populations. The carrier of a T allele in TLR2/−16934 among farmers' children compared to the children with AA genotype were significantly less likely to have a diagnosis of asthma (3% vs 13%, P=0.012) and current asthma symptoms (3% vs 16%, P=0.004), suggesting that the genetic variation in TLR2 gene is a major determinant of the susceptibility to asthma and allergies in children of farmers (47). In another study by Loss et al. the authors sought to determine the environmental and nutritional exposures associated with the gene expression of innate immunity receptors during pregnancy and the first year of a child's life using the data of the PASTURE birth cohort (48). Gene expression of innate immunity receptors in cord blood was overall higher in neonates of farmers (TLR7 and TLR8). Moreover, the unpasteurized farm milk consumption during the first year of life showed the strongest association with mRNA expression at year 1 (TLR4 and TLR6). Ege et al. sought to investigate the role of maternal exposures to environments rich in microbes for development of asthma in the innate immune system in offspring. The gene expression of receptors of innate immunity was strongly determined by maternal exposure to stables during pregnancy. With each additional farm animal species increased the expression of TLR2, TLR4 and CD14 (49). Thus farming and farming-related exposures, such as contact with animals and/or consumption of unpasteurized farm milk, that were previously reported to decrease the risk for allergic outcomes were associated with a change in gene expression of innate immunity receptors in early life. These observations support the possibility that the 17q21 locus as well as innate immunity receptors indirectly impact the risk of childhood onset asthma through its direct effect on early life wheezing illnesses or risk of allergic outcomes through gene-environment interaction.

Mechanisms of the Protective Effect of Farming on Asthma

The studies discussed above facilitated to identify a working model of the immunobiology of farm exposure as described in two important reviews (8, 50). This model suggests the contact with multiple animal species such as cows, pigs, poultry, horses, sheep and goats along with consumption of (unpasteurized) farm milk results in strong microbial exposure of women who carry out farming duties during pregnancy. This model emphasizes on the timing of the exposure i.e., during pregnancy and early life. This time exposure represents a biological window of opportunity for shaping subsequent immune reactivity. The results of the study by Pfefferle et al. (mentioned above) showed that the maternal exposure to farm animals is related to an increased production of interferon γ (INFγ) and TNFα from stimulated but unfractionated cord blood nuclear cells (21). Additional, maternal exposure to number of farm animals substantially enhanced the expression of Treg cell marker glucocorticoid-induced TNF receptor and secretion of IFNγ (Der p 1 and Ppg) by cord blood cells in response to the allergen and peptidoglycan (23). Likewise, the results from the PARSIFAL study regarding maternal exposure to number of farm animals with increase in gene expression of TLR2, TLR4 and CD14 in offspring additionally support this model (49). Finally, as shown in the study by Ege et al. the presence of many different farm animal species may increase the dose and diversity of the related microbial species which results in the protective effect (27).

Further, in the study by Pfefferle et al. the consumption of farm-produced butter during pregnancy has been shown to enhance the production of INFγ and TNFα from the unfractionated cord blood mononuclear cells. An additive effect was observed for the combined consumption of butter and unskimmed farm milk on INFγ and TNFα. These results suggest that consumption of dairy products originating from the unpasteurized cow's milk during pregnancy modulates cytokine production pattern in the newborn babies (21). Schaub et al. showed that the maternal intake of unprocessed farm milk was related to the epigenetic changes in the cord blood (23). The amount of Treg cell-specific demethylated region was higher in newborns of mothers consuming unprocessed cow's milk during pregnancy. Unprocessed cow's milk contains natural amount of milk fat and polyunsaturated- as well as conjugated fatty acids (CLA) (21). A randomized controlled trial in young healthy volunteers found decreased plasma immunoglobulin E levels and increased interleukin (IL)-10 levels after CLA supplementation. These results suggest an antiallergic potential of CLA in immune function (51), thus proposing the role of FA composition of the unprocessed cow's milk on the protective effect of asthma. Although the current evidence is weak to suggest a major role for microbial constituents of raw cow's milk, however, one cannot exclude the possibility that specific probiotics might be detected in future.

Conclusion

The evidence of the protective effect of farming environment i.e., exposure to farm animals, their fodder as well as consumption of unpasteurized cow's milk advocates that novel strategies to prevent asthma could be developed.

Conflict of Interest: During the past five years Dr von Mutius received honoraria from Massachusetts Medical Society and from The American Academy of Allergy Asthma & Immunology for acting as Editorial Board member; fees for consulting from European Commission, Tampereen Yliopisto, University of Edinburgh, Nestec S.A., University of Veterinary Medicine, Vienna, Chinese University of Hongkong, Research Center Borstel - Leibniz Lung Center, OM Pharma S. A., Pharmaventures Ltd., Peptinnovate Ltd., Turun Yliopisto, Helsingin Yliopisto, Chinese University of Hongkong, Imperial College London, Universiteit Utrecht, Universität Salzburg, Österreichische Gesellschaft f. Allergologie u. Immunologie, HiPP GmbH & Co KG; fees for speaking from The American Academy of Allergy Asthma & Immunology, British Society for Immunology, Medical University of Vienna, Schweizerisches Institiut für Allergie- und Asthmaforschung, Howard Hughes Medical Institute, University Hospital Erlangen, Margaux Orange, Deutsche Akademie der Naturforscher Leopoldina e.V., Hannover Medical School, American Thoracic Society, Inc., European Academy of Allergy and Clinical Immunology, Mundipharma Deutschland GmbH & Co. KG, DOC Congress

SRL, ITÄ-Suomen Yliopisto, Interplan - Congress, Meeting & Event Management AG, INC, Ökosoziales Forum Oberösterreich, Imperial College London, WMA Kongress GmbH, University Hospital rechts der Isar, European Respiratory Society, HAL Allergie GmbH, PersonalGenomes.org, Nestlé Deutschland AG, Universitätsklinikum Aachen, SIAF - Swiss Institute of Allergy and Asthma Research, Deutsche Pharmazeutische Gesellschaft e. V.; Verein zur Förderung der Pneumologie am Krankenhaus Großhansdorf e.V., Pneumologie Developpement, Mondial Congress & Events GmbH & Co. KG,, Volkswagen Stiftung, Böhringer Ingelheim International GmbH, Hanson Wade Ltd., DSI Dansk Borneastma Center; author honoraria from Elsevier Ltd., Springer-Verlag GmbH, Schattauer GmbH, Georg Thieme Verlag, Springer Medizin Verlag GmbH; In addition, Dr. von Mutius has a patent Application number LU101064, Barn dust extract for the prevention and treatment of diseases pending, a patent Publication number EP2361632: Specific environmental bacteria for the protection from and/or the treatment of allergic, chronic inflammatory and/or autoimmune disorders with royalties paid to Protectimmun GmbH, a patent Publication number EP 1411977: Composition containing bacterial antigens used for the prophylaxis and the treatment of allergic diseases. licensed to Protectimmun GmbH, a patent Publication number EP1637147: Stable dust extract for allergy protection licensed to Protectimmun GmbH, and a patent Publication number EP 1964570: Pharmaceutical compound to protect against allergies and inflammatory diseases licensed to Protectimmun GmbH.

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References

1. von Mutius E, Radon K. Living on a farm: impact on asthma induction and clinical course. Immunol Allergy Clin North Am. 2008;28(3):631-47, ix-x.
2. Global Asthma Network. The Global Asthma Report 2018. Auckland, New Zealand; Global Asthma Network; 2018.
3. Respiratory diseases statistics [Internet]. November 2019 [cited 2020 Feb 20]. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Respiratory_diseases_statistics.
4. 2017 National Health Interview Survey (NHIS) Data [Internet]. 2017 [cited 2020 Feb 20]. Available from: https://www.cdc.gov/asthma/most_recent_national_asthma_data.htm.
5. Nurmagambetov T, Kuwahara R, Garbe P. The Economic Burden of Asthma in the United States, 2008-2013. Ann Am Thorac Soc. 2018;15(3):348-56.
6. Custovic A, Simpson BM, Simpson A, Hallam C, Craven M, Brutsche M, et al. Manchester Asthma and Allergy Study: low-allergen environment can be achieved and maintained during pregnancy and in early life. J Allergy Clin Immunol. 2000;105(2 Pt 1):252-8.
7. von Mutius E, Fritzsch C, Weiland SK, Roll G, Magnussen H. Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. BMJ. 1992;305(6866):1395-9.
8. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol. 2010;10(12):861-8.
9. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy. 2000;30(2):194-200.
10. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy. 2000;30(2):187-93.
11. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol. 2006;117(6):1374-81.
12. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. Respirology. 2007;12(5):692-9.
13. Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, et al. Protection from childhood asthma and allergy in Alpine farm environments-the GABRIEL Advanced Studies. J Allergy Clin Immunol. 2012;129(6):1470-7.e6.
14. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet. 2001;358(9288):1129-33.
15. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol. 2007;119(5):1140-7.
16. Brunekreef B, Von Mutius E, Wong GK, Odhiambo JA, Clayton TO, Group IPTS. Early life exposure to farm animals and symptoms of asthma, rhinoconjunctivitis and eczema: an ISAAC Phase Three Study. Int J Epidemiol. 2012;41(3):753-61.
17. Dong GH, Ding HL, Ma YN, Jin J, Cao Y, Zhao YD, et al. Asthma and asthma-related symptoms in 16 789 Chinese children in relation to pet keeping and parental atopy. J Investig Allergol Clin Immunol. 2008;18(3):207-13.
18. Hugg TT, Jaakkola MS, Ruotsalainen R, Pushkarev V, Jaakkola JJ. Exposure to animals and the risk of allergic asthma: a population-based cross-sectional study in Finnish and Russian children. Environ Health. 2008;7:28.
19. Waser M, Michels KB, Bieli C, Floistrup H, Pershagen G, von Mutius E, et al. Inverse association of farm milk consumption with asthma and allergy in rural and sub-

urban populations across Europe. Clin Exp Allergy. 2007;37(5):661-70.

20. Brick T, Schober Y, Bocking C, Pekkanen J, Genuneit J, Loss G, et al. omega-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk. J Allergy Clin Immunol. 2016;137(6):1699-706.e13.
21. Pfefferle PI, Buchele G, Blumer N, Roponen M, Ege MJ, Krauss-Etschmann S, et al. Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study. J Allergy Clin Immunol. 2010;125(1):108-15.e1-3.
22. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J. 2008;32(3):603-11.
23. Schaub B, Liu J, Hoppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol. 2009;123(4):774-82.e5.
24. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med. 2002;347(12):869-77.
25. Schram D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children--the PARSIFAL Study. Allergy. 2005;60(5):611-8.
26. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. N Engl J Med. 2016;375(5):411-21.
27. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364(8):701-9.
28. Karvonen AM, Hyvarinen A, Rintala H, Korppi M, Taubel M, Doekes G, et al. Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study. Allergy. 2014;69(8):1092-101.
29. Birzele LT, Depner M, Ege MJ, Engel M, Kublik S, Bernau C, et al. Environmental and mucosal microbiota and their role in childhood asthma. Allergy. 2017;72(1):109-19.
30. Kirjavainen PV, Karvonen AM, Adams RI, Taubel M, Roponen M, Tuoresmaki P, et al. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat Med. 2019;25(7):1089-95.
31. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev. 2011;242(1):10-30.
32. Hernandez-Pacheco N, Pino-Yanes M, Flores C. Genomic Predictors of Asthma Phenotypes and Treatment Response. Front Pediatr. 2019;7:6.
33. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363(13):1211-21.
34. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet. 2011;43(9):887-92.
35. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet. 2014;46(1):51-5.
36. Yan Q, Brehm J, Pino-Yanes M, Forno E, Lin J, Oh SS, et al. A meta-analysis of genome-wide association studies of asthma in Puerto Ricans. Eur Respir J. 2017;49(5):1601505.
37. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al. Variants of DENND1B associated with asthma in children. N Engl J Med. 2010;362(1):36-44.
38. Wan YI, Shrine NR, Soler Artigas M, Wain LV, Blakey JD, Moffatt MF, et al. Genome-wide association study to identify genetic determinants of severe asthma. Thorax. 2012;67(9):762-8.
39. Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le Souef P, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. Lancet. 2011;378(9795):1006-14.
40. Noguchi E, Sakamoto H, Hirota T, Ochiai K, Imoto Y, Sakashita M, et al. Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations. PLoS Genet. 2011;7(7):e1002170.
41. Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Doi S, et al. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. Nat Genet. 2011;43(9):893-6.
42. Vicente CT, Revez JA, Ferreira MAR. Lessons from ten years of genome-wide association studies of asthma. Clin Transl Immunology. 2017;6(12):e165.
43. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature. 2007;448(7152):470-3.
44. Acevedo N, Reinius LE, Greco D, Gref A, Orsmark-Pietras C, Persson H, et al. Risk of childhood asthma is associated with CpG-site polymorphisms, regional DNA methylation and mRNA levels at the GSDMB/ORMDL3 locus. Hum Mol Genet. 2015;24(3):875-90.
45. Lluis A, Schedel M, Liu J, Illi S, Depner M, von Mutius E, et al. Asthma-associated polymorphisms in 17q21 influence cord blood ORMDL3 and GSDMA gene expression and IL-17 secretion. J Allergy Clin Immunol. 2011;127(6):1587-94.e6.

46. Loss GJ, Depner M, Hose AJ, Genuneit J, Karvonen AM, Hyvarinen A, et al. The Early Development of Wheeze. Environmental Determinants and Genetic Susceptibility at 17q21. Am J Respir Crit Care Med. 2016;193(8):889-97.
47. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol. 2004;113(3):482-8.
48. Loss G, Bitter S, Wohlgensinger J, Frei R, Roduit C, Genuneit J, et al. Prenatal and early-life exposures alter expression of innate immunity genes: the PASTURE cohort study. J Allergy Clin Immunol. 2012;130(2):523-30.e9.
49. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol. 2006;117(4):817-23.
50. von Mutius E. Maternal farm exposure/ingestion of unpasteurized cow's milk and allergic disease. Curr Opin Gastroenterol. 2012;28(6):570-6.
51. Song HJ, Grant I, Rotondo D, Mohede I, Sattar N, Heys SD, et al. Effect of CLA supplementation on immune function in young healthy volunteers. Eur J Clin Nutr. 2005;59(4):508-17.