

Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level

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To cite this article: Joerink M, Oortveld MAW, Stenius F, Rindsjö E, Alm J, Scheynius A. Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02328.x.

Keywords

allergy; farming; gene expression; lifestyle; placenta.

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Accepted for publication 19 December 2009

DOI:10.1111/j.1398-9995.2010.02328.x

Edited by: Hans-Uwe Simon

Abstract

Background: Environmental factors, including the intrauterine environment, can influence the risk of allergy development. In the present study, we investigated whether lifestyle and parental allergen sensitization status are reflected at gene expression level in the intrauterine environment.

Methods: mRNA expression of 17 genes was determined by means of quantitative real-time PCR in term placenta of 36 families participating in the ALADDIN study (Assessment of Lifestyle and Allergic Disease During Infancy). Data were analysed using a linear regression model to estimate the influence of lifestyle and parental allergen sensitization on the relative mRNA expression levels. Immunohistochemistry on placenta biopsies was used to verify protein expression.

Results: Significant differences in mRNA expression levels were detected at the foetal side of the placenta, where CD14 was expressed at higher levels in placentas from families living on a farm compared to not living on a farm, and IL-12(p40) was expressed at lower levels when the father was sensitized compared to nonsensitized. At the maternal side of the placenta, higher expression of STAT4 and lower expression of GATA3 were detected in families with sensitized compared to nonsensitized mothers, and IL-12(p40) was lower expressed when the families were living on a farm compared to not living on a farm. Immunohistochemistry performed for STAT4 and GATA3 showed that protein and mRNA levels correlated well.

Conclusion: Living on a farm and parental allergen sensitization are reflected in the intrauterine environment at the gene expression level.

Allergy often starts early in life, and maternal atopy has been reported to confer a higher risk to the development of asthma and eczema in children than paternal atopy (1–3). Therefore, it has been suggested that priming for allergic diseases can already occur *in utero* (4, 5). Multiple intrauterine exposures such as exposure to maternal smoking, diet, infections and stress have been indicated to influence the developing foetus and the risk of allergy development (6, 7).

Abbreviations

CI, confidence interval; GR, glucocorticoid receptor; qPCR, quantitative real-time polymerase chain reaction; R, relative expression levels; RT, reverse transcriptase.

Protective effects in relation to allergy development can also be transferred from mother to child, as demonstrated by lower cord blood IgE levels in children from mothers exposed to animal sheds and hay during pregnancy (8). Although the usefulness of cord blood IgE as a predictor for later allergy development has been questioned (1, 9), cord blood is one of a few samples suitable to estimate influences of exposures during pregnancy. In addition, the childhood environment can influence the risk of early development of allergic diseases. In this respect, both growing up on a farm (being exposed to stables and farm milk) and having an anthroposophic lifestyle have been demonstrated to decrease the risk of allergic diseases in children (10–15). An

anthroposophic lifestyle is characterized by, for example, a diet containing biodynamic products and a restricted use of antibiotics, antipyretics and vaccinations (10). To investigate the protective factors with regard to allergy development in the anthroposophic lifestyle, the ALADDIN study (Assessment of Lifestyle and Allergic Disease During Infancy) started in 2004. The lifestyle of so far 330 recruited families has been assessed by means of a questionnaire, and different biological samples from mother, father and child have been and will be collected.

We hypothesized that lifestyle (conventional/anthroposophic and living on a farm) and parental allergen sensitization status can influence the intrauterine immunological environment at the level of gene expression of immune-related genes. Changes in gene expression could affect the placental cytokine production, which has been suggested to reflect alterations in the development of the foetal immune system and therefore could have implications for foetal programming of allergic diseases in children (16). The placenta, being the interface between the mother and the foetus, is an essential part of the intrauterine environment. Therefore, we determined the mRNA expression levels in both the maternal and the foetal side of the placenta of 17 genes in 36 placentas. Because of the relatively small sample size, not more than four main factors based on lifestyle and parental sensitization status were analysed. The studied genes were selected to represent the innate, stress related and adaptive immune responses. Selected genes were previously shown to be expressed in placenta, or, are key transcription factors or signature cytokines involved in the adaptive immune response. For the innate immune system: CD14, TLR2 and TLR4, for stress: GR α and GR β , for Th1: Tbet, STAT4, IL-12(p40) and IFN γ , for Th2: STAT6, GATA3, SOCS3 and IL-4, for immune modulation: FOXP3, IL-10 and TGF β and for Th17 responses: RORc were selected. This study reveals that lifestyle and parental sensitization are reflected at gene expression level, thus contributing to a better understanding of the intrauterine environment and its influence on the early development of allergic diseases.

Materials and methods

Subjects

Pregnant women ($n = 330$) were recruited to the ALADDIN study from the anthroposophic maternity welfare centre at the Vidar clinic and the conventional maternity welfare centre in Järna, south of Stockholm, Sweden. The following three questions from a questionnaire based on previous studies (10, 13, 15) and filled out by the parents 2 months after the birth of the child were used to determine the lifestyle of the families: 1. 'What kind of preschool/school will your newborn child probably go to?', 2. 'Has any of the parents, no matter which type of school you have planned for your child, an anthroposophic view of life?' and 3. 'Is the family's everyday life influenced by an anthroposophic view of life?'. Families answering 'anthroposophic school' to question 1 and 'yes' to

question 2 and 3 were considered to have an anthroposophic lifestyle, and families answering 'conventional type of school' to question 1 and 'no' to question 2 and 3 were considered to have a conventional lifestyle. The question 'Are you living on a farm?' was used to define families living on a farm or not living on a farm. This study was approved by the local Ethics Committee, and all parents gave their written informed consent.

Blood samples and IgE analysis

Parental blood samples were collected during the third trimester of pregnancy, and plasma was stored at -20°C until analysis. Sensitization was defined as having a positive allergen-specific plasma IgE measurement ($\geq 0.35 \text{ KU}_A/\text{l}$) to a mix of 11 common airborne allergens (PhadiatopTM, Phadia AB, Uppsala, Sweden). Nonsensitized was defined as being Phadiatop negative ($< 0.35 \text{ KU}_A/\text{l}$).

Placenta samples

A total number of 36 term placentas were selected based on questionnaire answers regarding lifestyle and parental sensitization, see Table 1 for demographic data on the study population. Placenta biopsies from both maternal and foetal side of the placenta were taken by midwives immediately after birth and placed in 2-ml RNAlater (Ambion, Applied Biosystems, Foster City, CA, USA). After at least 24 h at room temperature, the RNAlater was removed, and samples were stored at -80°C . Remaining placentas were transported on ice to the laboratory, and two additional placenta biopsies,

Table 1 Demographic data on the study population

	Nonfarm* ($n = 29$)	Farm* ($n = 7$)
Lifestyle conventional/anthroposophic [†]	15/14	3/4
Sensitized mother, yes/no [‡]	15/14	3/4
Sensitized father, yes/no [‡]	8/21	3/4
Mother contact with animals during pregnancy, yes/no	19/10	5/2
Age mothers in years, median (range)	32 (21–43)	34 (20–42)
Age fathers in years, median (range)	34 (24–56)	27 (21–72)
Vaginal delivery/caesarean section	21/8	6/1
Delivery at home/hospital	6/23	3/4
Gestational age placenta in weeks, median (range)	40 (36–41)	39 (38–41)
Boys/girls	17/12	3/4
Older siblings, yes/no	16/13	5/2

*Nonfarm are families not living on a farm, Farm are families living on a farm.

[†]Conventional/anthroposophic lifestyle was based on three questions (see Materials and methods).

[‡]Sensitization was defined as having a positive PhadiatopTM (Phadia AB).

A two-tailed t-test or Fisher's exact test indicated nonsignificant differences between the groups.

about 0.5 cm thick and spanning the whole thickness of the placenta, were fixed in formalin and paraffin-embedded.

RNA isolation and cDNA synthesis

Total RNA was isolated using RNeasy® Fibrous Tissue Minikit (Qiagen, Hilden, Germany). The pieces were homogenized using Tissue tearor (Biospec products, Inc, Bartlesville, OK, USA). RNA concentrations and purity were measured using NanoDrop® spectrophotometer ND-1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA). RNA was stored at -80°C . First-strand cDNA was generated from 1.0 or 1.5 μg total RNA, depending on RNA yield, using Omniscript® reverse transcriptase (RT) Kit (Qiagen). The manufacturer's protocol was used except for the primers; 1.8 μl Random primer 100 μM (Invitrogen, Paisley, UK) and 1 μl Oligo dT primer 0.02 $\mu\text{g}/\text{ml}$ (Invitrogen). A control sample without the reverse transcriptase (–RT) was synthesized for every sample. cDNA was diluted 15 times with nuclease-free water and stored at -20°C .

Quantitative real-time PCR (qPCR)

All qPCRs were performed in duplicate in a volume of 25 μl : 12.5 μl iQTM SYBR Green Supermix (Biorad, Hercules, CA, USA), 5 μl cDNA, 1.5 μl forward and 1.5 μl reverse primer (5 μM each) and 4.5 μl nuclease-free water. The reaction was performed in an iCycler iQTM Multicolor Real-Time Detection System (Biorad). The following PCR programme was used: 95°C for 2 min, followed by 40 cycles of 15 s at 95°C , 40 s at $58/60/62^{\circ}\text{C}$ (depending on the primer pair) and 20 s at 60°C . Nuclease-free water and –RT controls were used as negative controls. Primers were confirmed to have a specific melting curve and temperature, only one band of the expected size on a 1.5% agarose gel, and the expected sequence was confirmed by sequencing. To calculate primer pair specific threshold and efficiency, a standard curve was run on a purified PCR product. Detailed information about RNA, primers and PCR products is available upon request.

Protein verification

Six micrometre sections were cut from formalin-fixed paraffin-embedded placenta biopsies, deparaffinized, and antigen retrieval was performed in a pressure cooker with Antigen Decloaker (Histolab Products AB, Sweden). Immunohistochemical staining was performed with mouse monoclonal antibodies against STAT4 (IgG₁, clone PL68 Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA.) and GATA3 (IgG₁, clone HG3-31 Santa Cruz Biotechnology Inc) diluted in REALTM Antibody Diluent (Dako, Glostrup, Denmark) at 1 : 50. A biotinylated horse-anti-mouse was used as secondary antibody (1 : 400, Vector Laboratories, Burlingame, CA, USA). The ABC-ELITE method and diaminobenzidine substrate (DAB, Vector Laboratories) were used to visualize bound antibodies. Slides were incubated with normal horse serum and 3% H₂O₂ in methanol

prior to antibody incubations to reduce background staining. An irrelevant isotype-matched mouse IgG₁ (Dako) was used as negative control and gave no staining (data not shown). Pictures from the maternal side of the placenta were taken using a light microscope. Staining intensities of positive cells were evaluated by eye by two independent investigators and ranked from low to high. Samples were coded to prevent bias during the analysis.

Statistical analysis

The relative gene expression was calculated for the different samples using the mean of the duplicate measurements and the Pfaffl method (17). Similar results were observed for both reference genes (GAPDH and EF1A), and all results are shown relative to EF1A. The D'Agostino and Pearson omnibus normality test was used to test for Gaussian distribution. The Spearman's rank correlation test and Pearson correlation coefficient test were used for the not normally and normally distributed data, respectively. A two-tailed *t*-test and Fisher's exact test were used to test for differences between groups. $P < 0.05$ was accepted as statistically significant. Relative expression levels (R) were transformed before statistical analysis as indicated, to obtain a normally distributed data set. The following transformations were used: 2LogR for CD14 (maternal side only), TLR2, TLR4, GR α (maternal side only), Tbet, STAT6, GATA3, SOCS3, IL-4 and TGF β ; $R^{1/2}$ for RORc and at the foetal side for CD14 and GR α ; $1/R^{1/2}$ for GR β , STAT4 (foetal side only) IL-12(p40), IFN γ , FOXP3 and IL-10; and $1/R^{2/3}$ for STAT4 at the maternal side. A linear regression model, indicating the effect and a 95% confidence interval (CI) for every main factor, was used to analyse the influence of lifestyle and parental sensitization on the relative mRNA expression levels. Anthroposophic/conventional lifestyle, living on a farm, and sensitization of mother or father were analysed as main factors. Because of multiple testing in the linear regression model, $P < 0.05$ was considered to indicate a large effect. Statistical analysis was conducted using STATA version 10.0 (Stata-Corp, College Station, TX, USA) or GRAPHPAD PRISM software version 4.03 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Characteristics of placental gene expression

The constitutive gene expression was analysed in both maternal and foetal side of the placenta. Expression of GR α , TGF β , GATA3 and GR β were relatively high, expression of SOCS3, CD14, STAT6, TLR4, IFN γ and IL-12(p40) were intermediate, and expression of IL-10, TLR2, STAT4, RORc, Tbet and FOXP3 were low while the expression of IL-4 was low to undetectable (Fig. 1). To estimate the intra-placental gene expression variation, four samples of RNA were isolated from 10 randomly selected placentas, two samples from the maternal side and two

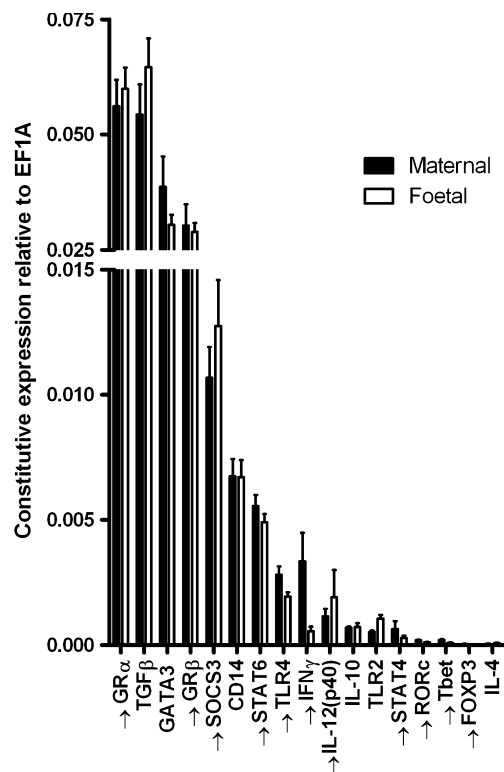


Figure 1 Constitutive expression of the 17 analysed genes at the maternal (■) and foetal (□) side of placenta. Expression was determined by qPCR and expressed relative to EF1A. Expression was determined at a threshold value of 150 for all genes and corrected for the primer pair efficiency at this threshold. Bars indicate the mean and SEM of all 36 placentas measured in duplicate. The relationship between the expression at maternal and foetal side was analysed using the relative gene expression. Primer pair specific efficiency and threshold were used, and data were normalized against both the EF1A mRNA levels and the average of the nine placentas from families with a conventional lifestyle and nonsensitized mothers. Data were 2Log transformed, and the Pearson correlation test was used to calculate the relationship. Genes with a significant correlation between relative expression at the maternal and foetal side are indicated with an arrow (→).

from the foetal side. For these forty samples, qPCR was carried out for 11 genes, with annealing temperatures at 58 and 60°C. The Spearman's rank correlation test indicated significant correlations for both the maternal ($P < 0.0001$ $r_s = 0.89$) and foetal ($P < 0.0001$ $r_s = 0.94$) side of the placenta (data not shown). In addition, when comparing the relative gene expression between the maternal and foetal side per gene, a significant correlation was detected for 11/17 genes (Fig. 1). Taken together, this indicates low intra-placental variation in gene expression levels, as suggested before (18). Therefore, we analysed one biopsy per side (maternal and foetal) of the remaining 26 placentas.

Relative gene expression patterns

To obtain an overview of the gene expression patterns, a thumbnail overview was created (Fig. 2). Data are shown for every individual placenta (rows) and ordered by gene function (columns). A linear regression model was used to analyse whether variation in expression as seen in Fig. 2 could be explained by one of the four main factors: anthroposophic/conventional lifestyle, living on a farm and sensitization of mother or father. At the maternal side, large effects were evident of maternal sensitization for the expression of STAT4 and GATA3 and of living on a farm for the expression of IL-12(p40). For STAT4, a lower expression in placentas from families with nonsensitized compared to sensitized mothers was detected (Fig. 3A). For GATA3, a lower expression in placentas from families with sensitized compared to nonsensitized mothers was detected (Fig. 3D). For IL-12(p40), a lower expression in placentas from families living on a farm compared to not living on a farm was detected (Fig. 3G). At the foetal side, large effects were evident of living on a farm for the expression of CD14 and of paternal sensitization for the expression of IL-12(p40) (Fig. 4). A higher expression of CD14 was detected in placentas from families living on a farm compared to not living on a farm (Fig. 4A). Interestingly, a trend, with a P value < 0.10 , was also seen for the expression of TLR2 with higher expression levels in placentas of families living on a farm compared to not living on a farm (Fig. 4B). For IL-12(p40), a lower expression level was detected for placentas of families with sensitized compared to nonsensitized fathers (Fig. 4C). Additional effects with P -values < 0.10 were detected (data not shown), at the maternal side for the expression of GATA3, FOXP3 and RORc, with a trend for lower expression levels in the placentas of families living on a farm compared to not living on a farm. At the foetal side, we identified a trend for lower expression levels of IL-12(p40) and IL-4 in placentas of families living on a farm or with a sensitized mother, respectively. Furthermore, GR α and GR β showed a trend to be lower expressed in placentas from families with a sensitized compared to a nonsensitized father.

Expression of STAT4 and GATA3 could be confirmed at the protein level

Placentas with low or high mRNA expression of STAT4 or GATA3 were selected for immunohistochemical analysis. Staining of paraffin-embedded placenta sections ($n = 6$ per antibody) demonstrated the presence of STAT4 (Fig. 3B–C) and GATA3 (Fig. 3E–F) protein in the villous trophoblasts (data not shown) and within the deciduas, the maternal side of the placenta (Fig. 3). Difference between the lowest and highest mRNA expression levels was 76 times for STAT4 and 8 times for GATA3. Staining intensity of the positive cells correlated well with the mRNA expression data; placentas with the highest mRNA expression had the strongest protein staining (Fig. 3B and E), while placentas with the lowest mRNA expression had the weakest protein staining (Fig. 3C and F).

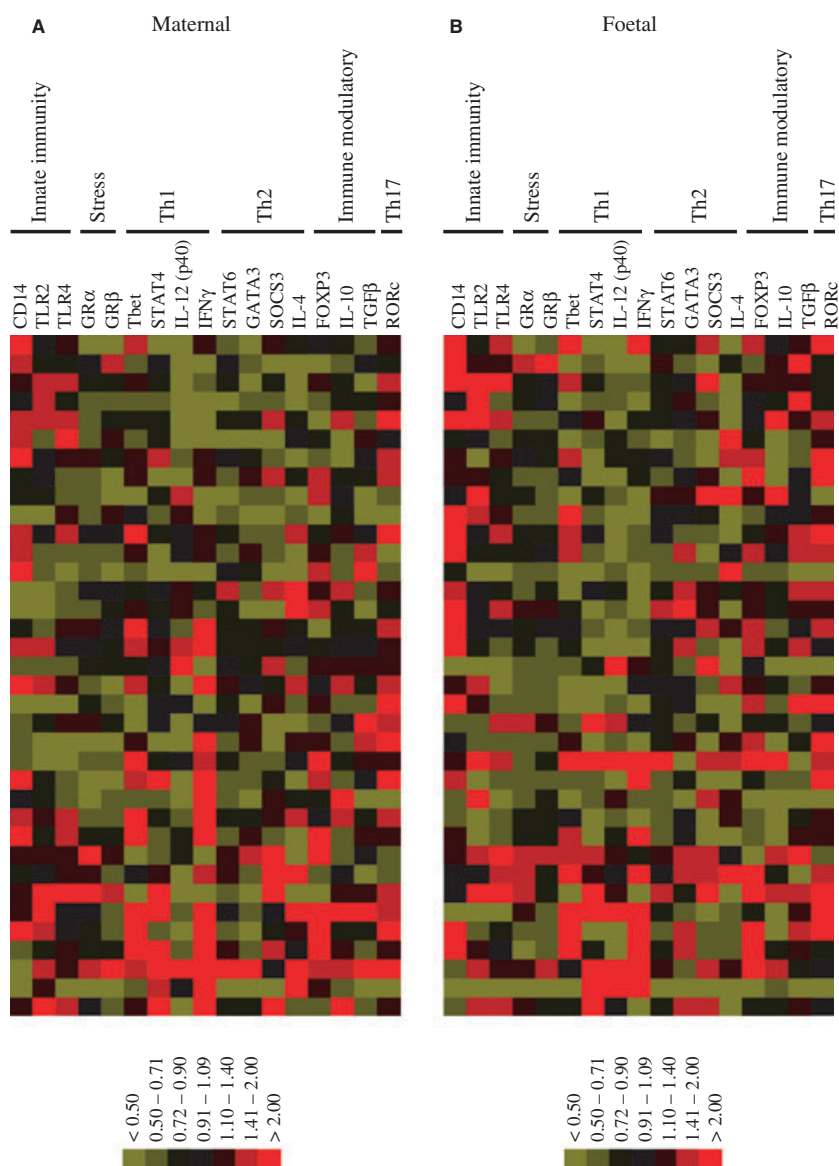


Figure 2 Thumbnail overview of the gene expression of 17 genes at the maternal (A) and foetal (B) side of the 36 placentas. mRNA levels were determined by qPCR, primer pair specific efficiency and threshold were used, and data were normalized against both the EF1A mRNA levels and the average of the nine placentas from

families with a conventional lifestyle and nonsensitized mothers. Relative expression (R) is indicated by colours ranging from green (less than half the expression) to red (more than double the expression). Data are shown for every individual placenta (rows, $n = 36$) and ordered by gene function (columns).

Discussion

Here, we describe for the first time the expression of immune-related genes in placenta of families with different lifestyles (conventional/anthroposophic and living on a farm) and parental sensitization status. The aim was to determine whether these factors are reflected in the intrauterine environment at the level of gene expression. We detected large effects of living on a farm for the expression of IL-12(p40) and CD14, of maternal sensitization for the expression of STAT4 and GATA3 and of paternal sensitiza-

tion for the expression of IL-12(p40) and confirmed the differences on the protein level for STAT4 and GATA3. Furthermore, a number of trends, P -values < 0.10 , were identified for the effects of living on a farm, maternal sensitization, and paternal sensitization with regard to the expression of GATA3, IL-12(p40), FOXP3, RORc, IL-4, GR α and GR β . Conventional/anthroposophic lifestyle was not reflected at the intrauterine gene expression of the here-analysed genes.

At the maternal side of the placenta, large effects were demonstrated for the expression of STAT4, GATA3 and IL-12(p40) (Fig. 3). STAT4 and GATA3 are mainly known

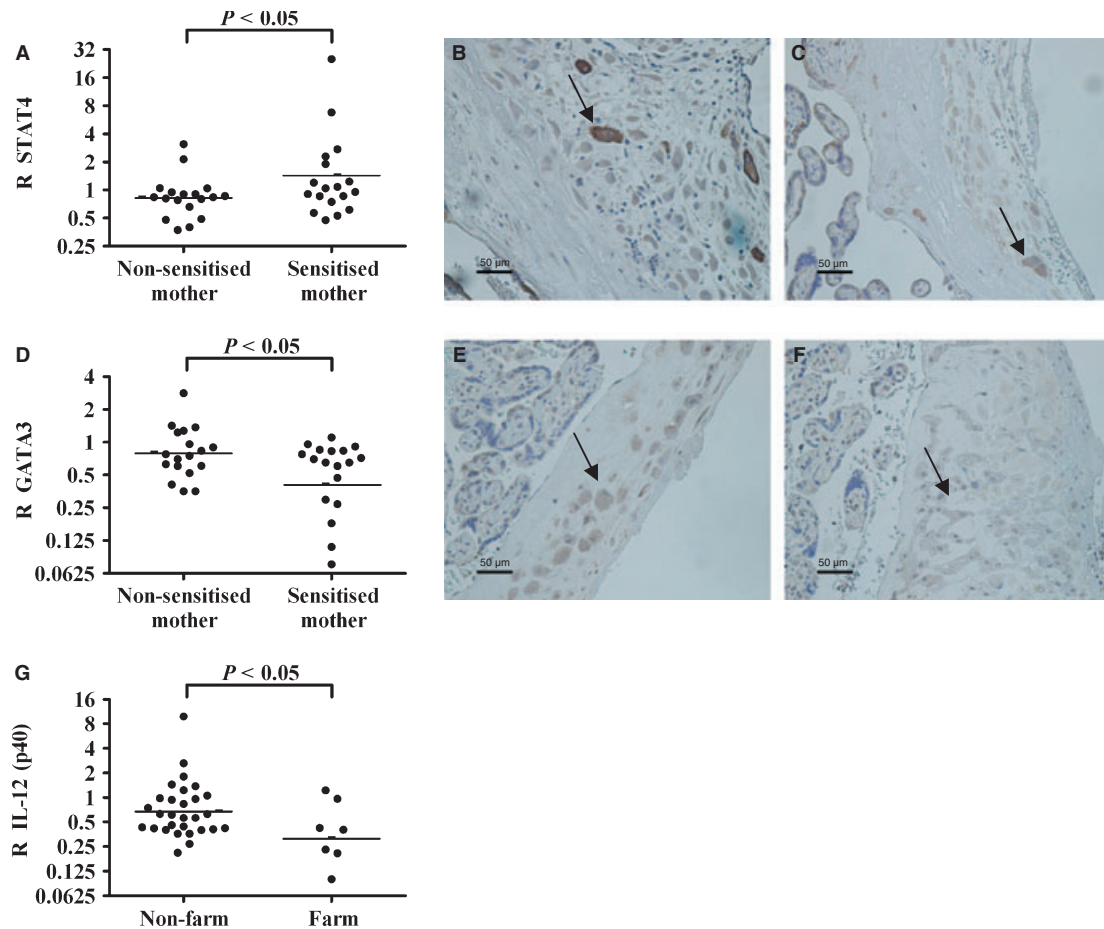


Figure 3 mRNA and protein expression of STAT4 (A–C), GATA3 (D–F), and IL-12(p40) (G) at the maternal side of the placenta. mRNA levels were determined by qPCR, primer pair specific efficiency and threshold were used, and data were normalized against both the EF1A mRNA levels and the average of the nine placentas from families with a conventional lifestyle and nonsensitized mothers. Relative expression levels (R) were transformed (see Materials and methods) before analysis with a linear regression model taking the four main factors: anthroposophic/conventional lifestyle, living on a farm, and sensitization of mother or father into account. For the mRNA expression of STAT4 at the maternal side of the placenta, two interactions (between living on a farm and maternal sensitization; and between living on a farm and anthroposophic/conventional lifestyle) were included next to the four main factors. This revealed a large effect for maternal sensitization (A). Maternal sensitization also had a large effect on GATA3 expression (D). The

effect of living on a farm was large for IL-12(p40) expression (G). Dots (●) indicate the relative expression (R) of individual placentas before transformation, and horizontal lines indicate the estimated effects of maternal sensitization (A and D) or living on a farm (G) as predicted by the linear regression model. Note the 2Log scale. For the immunohistochemical staining, placentas with high ($n = 3$ per antibody) and low ($n = 3$ per antibody) mRNA expression levels of STAT4 and GATA3 were selected. Staining of the paraffin-embedded placenta sections was performed using the ABC-ELITE method and DAB (brown/red) and counterstained with haematoxylin (blue). Antibodies used were against STAT4 (B and C) and GATA3 (E and F). For each antibody, a placenta with high (B and E) and low (C and F) mRNA expression is shown. Intensity of the observed protein staining correlated well with the mRNA expression. Pictures show maternal side of the placenta with villi on the left side. Arrows indicate positive staining. Scale bars: 50 µm.

as T-cell-specific transcription factors. In normal healthy pregnancy, there are very few T cells present in the placenta. However, trophoblasts are important immune cells within the placenta, which can produce Th1 and Th2 cytokines as well (19). Our study demonstrates protein expression of STAT4 and GATA3 mainly in villous trophoblasts and in cells that most likely are extravillous trophoblasts (Fig. 3). Furthermore, protein staining intensities correlated well with the mRNA expression levels. Together this indicates that tropho-

blasts might be able to skew the intra-uterine cytokine environment. Analysis of the constitutive gene expression (Fig. 1) revealed higher basal expression levels of Th2-related transcription factors compared to Th1-related transcription factors. This is in agreement with the notion that established pregnancy is a Th2-skewed phenomenon (20, 21). We, however, revealed a relatively higher expression of the Th1-related transcription factor STAT4 and a relatively lower expression of the Th2-related transcription factor GATA3

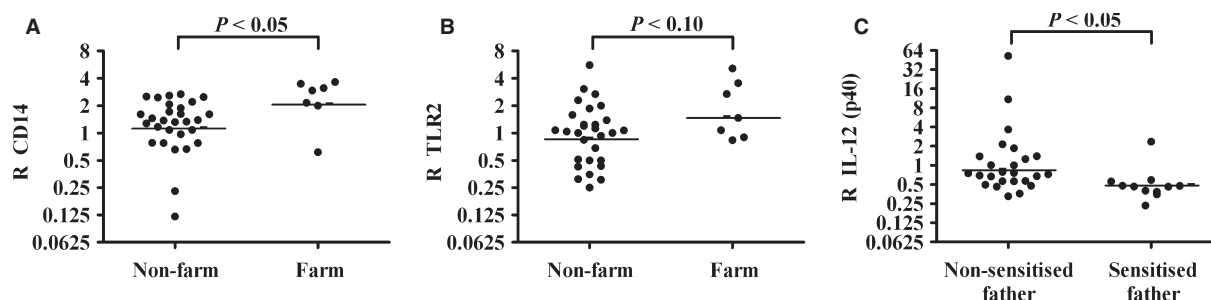


Figure 4 mRNA expression of CD14 (A), TLR2 (B), and IL-12(p40) (C) at the foetal side of the placenta. mRNA levels were determined by qPCR, primer pair specific efficiency and threshold were used, and data were normalized against both the EF1A mRNA levels and the average of the nine placentas from families with a conventional lifestyle and nonsensitized mothers. Relative expression levels (R) were transformed (see Materials and methods)

when the mothers were sensitized, suggesting an imbalance in the intrauterine Th2 skewing depending on the sensitization status of the mother. The Th1 type cytokine IL-12(p40) was lower expressed in placentas of families living on a farm compared to not living on a farm (Fig. 3G). If in our study living on a farm is a surrogate marker for exposure to microbial products such as endotoxins, the results on IL-12 are in agreement with a previous study where lower IL-12 production by peripheral blood leucocytes was detected in school children with high compared to low endotoxin exposure (22).

Interestingly, effects of living on a farm were also detected for the innate immunity-related genes CD14 and TLR2. Higher expression of CD14 and a trend for higher expression of TLR2 were detected at the foetal side of the placenta from families living on a farm compared to not living on a farm (Fig. 4). Similar results have been reported previously with increased mRNA expression levels of CD14 and TLR2 in peripheral blood mononuclear cells isolated from 10-year-old children of farmers compared to nonfarmers' children (23). Furthermore, decreased levels of CD14 and TLR2 mRNA in maternal peripheral blood mononuclear cells and cord blood mononuclear cells of their children have been associated with parental allergy (24). CD14 and TLR2 can bind bacterial components and trigger the innate immune response. Increased levels of CD14 and TLR2 indirectly suggest a higher microbial exposure, which has been reported to have a protective effect against allergy development (7). It has been suggested that this protective effect is a result of innate immune stimulation leading to the development of tolerance (22). The influence of CD14 and TLR2 on allergy development could also be more direct because single nucleotide polymorphisms in these genes can modify the associations between farming and allergy development or asthma (25–27). Additional trends, with P -values < 0.10, for the effect of living on a farm were detected for GATA3, FOXP3 and RORc at the maternal side of the placenta and for the expression of IL-12(p40) at the foetal

side of the placenta further emphasizing the importance of the farming environment. All effects of paternal sensitization were detected at the foetal side of placenta, suggesting that this effect might be genetic, because the cells at this side are mainly of foetal origin and thus genetically half paternal. We detected lower mRNA levels of the Th1 type cytokine IL-12(p40) and a trend for lower expression levels of the stress-related $GR\alpha$ and $GR\beta$ at the foetal side of the placenta of families with sensitized compared to nonsensitized fathers. Glucocorticoids are known to negatively regulate GR expression (16); lower GR levels could therefore indicate higher levels of placental glucocorticoids. Higher intrauterine glucocorticoid levels are suggested to influence the development of the foetal immune system and increase the risk of allergy development (6, 28). The expression levels of $GR\alpha$ and $GR\beta$ are relatively high (Fig. 1) and could be influenced by other more stress-related factors not analysed here, such as mode of delivery.

Relative gene expression patterns were calculated and revealed variation between the placentas (Fig. 2). A linear regression model was used to analyse whether this variation could be explained by one of the four main factors: anthroposophic/conventional lifestyle, living on a farm and sensitization of mother or father. Because of the relatively small sample size, we could not analyse additional factors. Confounding factors could, however, influence our results. A recent study reported that mRNA expression levels of five cytokines in placenta were influenced only when the foetus was a girl (29). We did not include foetal sex as a main factor; however, the number of boys/girls was not significantly different for the four main factors analysed.

Functional influences of the differential expressed genes remain speculative, because differences in expression levels are relatively small. Intrauterine expression differences can however possibly predict a risk of allergy development later in life. The children within this study will be followed for at least up to 5 years of age. Future analysis will therefore indicate whether mRNA expression levels within placenta can predict a risk of allergy development.

In conclusion, even though it was not possible to identify which factors affect the expression of the analysed genes most, we identified differences in gene and protein expression in placenta depending on lifestyle and sensitization of the parents.

Acknowledgments

The authors would like to acknowledge the families participating in ALADDIN for their trust and contribution to this study, the 'ALADDIN-group' for their involvement in this work, Sven-Göran Eriksson (Institute of Environmental Medicine, Karolinska Institutet) for help with the statistical analysis, Niko Jokilaakso and Bart van Riel for immunohistochemical stainings, Nikos Papadogiannakis

(Department of Laboratory Medicine, Karolinska Institutet) for help with the evaluation of the immunohistochemical stainings and Phadia AB for supply of reagents for IgE analyses.

This work was supported by grants from the Swedish Research Council, the Stockholm County Council, the 'Mjölkdroppen' Society, the Centre for Allergy Research Karolinska Institutet, the Swedish Research Council for Working Life and Social Research, the Swedish Society of Medicine and the following Foundations: Hesselman, Gyllenberg, 'Frimurare Barnhuset in Stockholm', Ekhaga, the Vårdal Foundation for Health Care Sciences and Allergy Research and the Swedish Asthma and Allergy Association's Research. M.J. was supported by a Marie Curie Intra-European Fellowship (PIEF-GA-2008-219680).

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