TBX21 and HLX1 Polymorphisms Influence Cytokine Secretion at Birth

Vera Isabel Casaca1, Sabina Ilii1, Kathrin Suttner2, Isolde Schleich1, Nikolaus Ballenberger1, Elizabeth Klucker1, Elif Turan1, Erika von Mutius1, Michael Kabesch2, Bianca Schaub1*

1 Department of Pulmonary and Allergy, University Childrens Hospital Munich, Munich, Germany, 2 Department of Paediatric Pneumology, Allergy and Neonatology, Hannover Medical School, Hannover, Germany

Abstract

Background: TBX21 (T cell specific T-box transcription factor) and HLX1 (H.20-like homeobox 1) are crucial transcription factors of Th1-cells, inducing their differentiation and suppressing Th2 commitment, particularly important for early life immune development. This study investigated the influence of TBX21 and HLX1 single nucleotide polymorphisms (SNPs), which have previously been shown to be associated with asthma, on Th1/Th2 lineage cytokines at birth.

Methods and Findings: Cord blood mononuclear cells (CBMCs) of 200 neonates were genotyped for two TBX21 and three HLX1 SNPs. CBMCs were stimulated with innate (Lipid A, LpA; Peptidoglycan, Ppg), adaptive stimuli (house dust mite Dermatophagoides pteronyssinus 1, Derp1) or mitogen (phytohemagglutinin, PHA). Cytokines, T-cells and mRNA expression of Th1/Th2-related genes were assessed. Atopic diseases during the first 3 years of life were assessed by questionnaire answered by the parents. Carriers of TBX21 promoter SNP rs17250932 and HLX1 promoter SNP rs2738751 showed reduced or trendwise reduced (p≤0.07) IL-5, IL-13 and TNF-α secretion after LpA-stimulation. Carriers of HLX1 SNP rs2738751 had lower IL-13 levels following Ppg-stimulation (p=0.08). Carriers of HLX1 exon 1 SNP rs12141189 showed increased IL-5 (LpA, p=0.007; Ppg, p=0.10), trendwise increased IL-13 (LpA), higher GM-CSF (LpA/Ppg, p≤0.05) and trendwise decreased IFN-γ secretion (Derp1+LpA-stimulation, p=0.1). Homozygous carriers of HLX1 promoter SNP rs3806325 showed increased IL-13 and IL-6 (unstimulated, p≤0.03). In carriers of TBX21 intron 3 SNP rs11079788 no differences in cytokine secretion were observed. mRNA expression of Th1/Th2-related genes partly correlated with cytokines at protein level. TBX21 SNP rs11079788 carriers developed less symptoms of atopic dermatitis at 3 years of age (p=0.03).

Conclusions: Polymorphisms in TBX21 and HLX1 influenced primarily IL-5 and IL-13 secretion after LpA-stimulation in cord blood suggesting that genetic variations in the transcription factors essential for the Th1-pathway may contribute to modified Th1-immune responses already early in life. Further follow-up of the cohort is required to study the polymorphisms’ relevance for immune-mediated diseases such as childhood asthma.

Introduction

Asthma and inflammatory diseases are induced by a complex interplay of genetic and environmental factors influencing early immune responses [1,2,3]. Modulation of the immune system in early life via innate stimuli may play an essential role in preventing allergic responses [4], as shown in farm children, which are exposed to microbial innate stimuli and have a lower prevalence of allergic responses [4], as shown in farm children, which are exposed to microbial innate stimuli and have a lower prevalence of allergic diseases [5].

Th1/Th2 imbalance can lead to different inflammatory conditions and has been described in several immune-mediated diseases including asthma [6]. Th1 and Th2 cell lineages are controlled by cell-specific transcription factors (TFs) [7]. TBX21, is a specific TF of Th1-cells, which promotes their differentiation and proliferation and suppresses Th2-cell development. It activates the expression of the Th1 hallmark cytokine INF-γ and represses IL-4 production in developing Th12-cells [8]. TBX21 polymorphisms were associated with airway hyperresponsiveness in asthma [9] and increased risk for childhood asthma [10]. TBX21 interacts closely with HLX1, another Th1 transcription factor. The interaction between TBX21 and HLX1 is required to induce maximal IFN-γ secretion [11]. Both genes have the capacity to revert Th2 cell commitment of T cells already expressing Th2 cytokines [11]. Further downstream activation of Th11 and Th12 cytokines is regulated tightly by these transcription factors [8,12,13]. Like TBX21, polymorphisms in HLX1 have also been significantly associated with the development of childhood asthma [14]. Functional studies on some of these genetic variants

Citation: Casaca VI, Ilii S, Suttner K, Schleich I, Ballenberger N, et al. (2012) TBX21 and HLX1 Polymorphisms Influence Cytokine Secretion at Birth. PLoS ONE 7(1): e31069. doi:10.1371/journal.pone.0031069

Editor: Dominik Hartl, University of Tübingen, Germany

Received August 4, 2011; Accepted January 1, 2012; Published January 30, 2012

Copyright: © 2012 Casaca et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was funded by Bayerische Forschungsstiftung (DOK-111-09/VC), Comprehensive Pneumology Centre (CPC)(Dr. Schaub), and the German Ministry of Education and Research (BMBF) as part of the National Genome Research Network (NGFN), with grant NGFN 01GS0810 (Dr. Kabesch, Dr. Sutter). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal’s policy and have the following conflicts: Dr. Kabesch is a consultant for Sanofi, the Speakers Bureau of Roxall, Glaxo Wellcome, Novartis, and Allergopharma. Dr. von Mutius is a consultant for Glaxo Smith Kline, Novartis, and ProtectImmun. This does not alter the authors’ adherence to all the PLoS ONE policies on sharing data and materials.

* E-mail: Bianca.Schaub@med.uni-muenchen.de
already demonstrated their putative functional relevance [10,14]. In general, lineage-specific TFs control other TFs and genes that encode for cytokines [7].

Cytokine regulation has shown to be crucial for later development of immune-mediated diseases such as asthma. Thus, it is of particular interest in this context to study early life immune modulation. Cytokines as important mediators in the development of allergies are already modulated during pregnancy, and a T_{H2}-dominated cytokine pattern can be detected in cord blood [15,16,17,18].

We hypothesized that polymorphisms in these T_{H1}-specific TFs influence T cell immune responses already at birth. We aimed to study two TBX21 (rs17250932, rs11079788) and three HLX1 (rs2738751, rs3806325, rs12141189) polymorphisms, which have previously been associated with risk or protection from childhood allergic diseases [15]. Additionally, we investigated the development of atopic diseases of the children during the first 3 years of life.

Methods

Ethics Statement

Written informed consent was obtained from the mothers for participation. Approval was obtained from the local human research committee of the Bavarian Ethical Board, LMU Munich, Germany.

Population characteristics

Fetal cord blood was obtained from neonates (n = 200) born in the Munich metropolitan area, Germany. Informed consent was given by the mothers for participation in the study, including cord blood collection. The enrolment period was from July 2005 until September 2007. Inclusion criteria comprised healthy neonates and mothers with uncomplicated pregnancies. Exclusion criteria included preterm deliveries and perinatal infections. Maternal atopy was defined as doctor’s diagnosis of asthma and/or eczema and/or hay fever. In addition, total and specific IgE (Radio-Allergo-Sorbent Test) was measured. A positive specific IgE was defined as 1 or more positive reactions (≥0.35 IU/ml) to a panel of 20 common allergens.

Follow-up at age 3 years

A follow-up at the age of 3 years was performed. The data were assessed by detailed questionnaires answered by the parents. Wheeze was defined by wheezy symptoms in the first 3 years. Atopic dermatitis (AD) was defined by symptoms of AD in the first 3 years, and skin manifestations of food allergy were noted. A positive allergy test was defined by parental report of a positive skin Prick test or blood test with at least one positive test to one of the 20 common inhalant or food allergens.

Polymorphisms selection and genotyping

Based on previous studies, 5 polymorphisms in TBX21 and HLX1 were selected for this analysis due to their association with asthma and their putative functional implications [10,14]. Genotyping was performed by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc., San Diego, CA, USA). Polymerase chain reaction assays and associated extension reactions were designed with the SpectroDESIGNER software (Sequenom Inc.). All amplification and extension reaction conditions have been previously described [19]. Deviations from Hardy–Weinberg Equilibrium were assessed for quality control of genotyping procedures (Table 1). Of note, wild type (WT) refers to WT alleles in absence of the particular polymorphic allele under study.

Real-time quantitative RT-PCR

Total RNA was isolated from CBMCs with TRI reagent, cDNA synthesis was performed using reverse transcriptase (Invitrogen, Karlsruhe, Germany). Vector NTI advance10 (Invitrogen, Karlsruhe, Germany) was used for specific primer pairs design (18S, GATA3, STAT6, STAT6c, TBX21, HLX1, IRF1). Gene-specific PCR-products were measured continuously by means of iCycler Real-Time PCR-Detection-System (Bio-Rad) for 40 cycles. Direct detection of the PCR product was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to dsDNA. The threshold cycle (c) of each target product was determined and set in relation to the amplification plot of 18S (Act). The level of mRNA of each gene is described as gene expression.

Cytokine secretion

CBMCs, isolated freshly within 24 hours by Ficol Hypaque density gradient, were stimulated with Lipid A (LpA, 0.1 μg/ml), Peptidoglycan (Ppg, 10 μg/ml), allergen house dust mite (Derp1, 30 μg/ml), a combination of Derp1 and LpA (D+L) or phytohemagglutinin (PHA, 5 μg/ml) for 3 days and compared with unstimulated cells as previously described [15]. Cytokine concentrations were measured in supernatants by using the Human Cytokine-Multiplex-Assay-Kit according to the manufacturer’s instructions (Bio-Rad, Munich, Germany) by LUMINEX technology. The lower limits of detection (pg/ml) were 1.8 (IL-5), 0.5 (IL-6), 3.0 (TNF-α), 0.21 (IL-13), 1.3 (IFN-γ), 1.0 (GM-CSF). Non-detectable cytokine concentrations were assigned to a value of 0.01 for inclusion into the analysis.
Flow cytometry

Cells were analyzed by using 3-color flow cytometry (FACScan; BD Biosciences, Heidelberg, Germany). For surface staining, 2 µl of anti-human CD4-fluorescein isothiocyanate (FITC) and 1 µl of CD25 RPE-Cy5 (Dako Cytomation, Glostrup, Denmark), were used. For isotype control 1 µl of IgG1-FITC (Dako Cytomation, Glostrup, Denmark), and 0.5 µl of IgG2a RPE-Cy5 (BD Biosciences) were added [4]. Data were analyzed with CellQuest software (BD Biosciences), and postacquisition analysis was performed with WinMDI 2.8 software (The Scripps Research Institute, La Jolla, CA).

Statistical analysis

Non-parametric tests (Wilcoxon/Kruskal-Wallis) were used to compare the median of cytokine concentrations, as these were generally not normally distributed and could not be transformed to normality. Data were reported as median, first and third quartile. Chi² tests were used to evaluate categorical predictor variables. Data for genetic analyses were analyzed with Kruskal-Wallis-test (three groups). Based on previous reports which described recessive effects for TH1-related SNPs, we have further analysed the SNP effects using a recessive model. Due to the fact that gene expression variables contained non-detectable measurements (censored data), summary statistics such as the median and quartiles was conducted by the Kaplan-Meier method [20]. Testing on group differences was performed by the generalized Wilcoxon test [21]. Data were not adjusted for multiple testing as this is an explorative study. In order to assess the role of maternal atopy we performed stratified analysis for the CBMCs of the neonates with or without maternal atopy. Statistical significance was defined by p<0.05; data analysis was performed with SAS 9.2 (The SAS Institute, Cary, NC, USA).

Results

We assessed the effect of 5 genetic variants located in T1H1, T1H1-1T1H2, and HLX1 on cord blood immune responses of 200 neonates, namely TBX21 (rs17250932 and rs11079789) and HLX1 (rs2738751, rs3806325 and rs12141189) (Table 1) which have previously been related to the development or protection of atopic diseases [10,11]. The genotyping success rate was at least 97%. None of the polymorphisms deviated significantly from Hardy-Weinberg Equilibrium.

T1H2-related cytokine secretion upon innate stimulation was modulated in carriers of TBX21 and HLX1 polymorphisms

Assessment of cytokine responses in CBMCs showed that carriers of TBX21 SNP rs17250932 (promoter) and HLX1 SNP rs2738751 (promoter) had decreased or trendwise decreased IL-5 and IL-13 after LpA and for the latter also decreased IL-13 after Ppg-stimulation compared to wild-type (WT) and heterozygous carriers (HT). TNF-α secretion was trendwise lower or significant lower in carriers of TBX21 SNP rs17250932 and HLX1 SNP rs2738751, respectively (Fig. 1).

On the other hand, increased IL-5, IL-13 and GM-CSF secretion was observed in the carriers of HLX1 SNP rs12141189 (exon 1), after LpA and Ppg-stimulation, while the T1H1-1 cytokine IFN-γ was trendwise downregulated after D+L-stimulation (Fig. 2). Additionally we analyzed the polymorphisms using a recessive model, as previous reports described recessive effects for T1H1-related SNPs. After applying the recessive model, the majority of the findings achieved greater statistical significance (Table 2). Carriers of HLX1 SNP rs3006325 (promoter), showed increased IL-13 and IL-6 levels in unstimulated cells (Table 2), and no significant changes were observed for other cytokines or following stimulation.

Impact of TBX21 and HLX1 polymorphisms on health outcomes

The cohort was followed up to the age of 3 years and symptoms of atopic diseases and wheeze were assessed by questionnaires. The distribution of children that developed atopic diseases within the different TBX21 and HLX1 genotypes (WT, HT and SNP) is shown in the following: homozygous carriers of TBX21 SNP rs11079788 SNP showed less symptoms of atopic dermatitis (19%) compared to HT (23%) and WT (36%) (p = 0.03). No significant associations were observed for the other TBX21 and HLX1 polymorphisms with wheeze, skin manifestation of food allergy or atopic dermatitis at this time point.
Figure 1. Cytokine secretion of wildtype, heterozygous and homozygous SNP carriers of *TBX21* rs17250932 and *HLX1* rs2738751. Data were shown in boxplots (first, third quartile, median), the whiskers indicate the maximum and minimum values, dots indicate outliers, analyzed by Kruskal-Wallis-test. Values were shown in pg/ml. n (WT) = 113, n (HT) = 61, n (SNP) = 10, and *HLX1* rs2738751 n (WT) = 135, n (HT) = 45, n (SNP) = 4.

*TBX21* rs17250932: A-D. N (WT) = 113, n (HT) = 61, n (SNP) = 10.

*HLX1* rs2738751: E-H. N (WT) = 135, n (HT) = 45, n (SNP) = 4.

doi:10.1371/journal.pone.0031069.g001
Figure 2. Cytokine secretion of wildtype, heterozygous and homozygous SNP carriers of HLX1 rs12141189. Data were shown in boxplots (first, third quartile, median), the whiskers indicate the maximum and minimum values, dots indicate outliers, analyzed by Kruskal-Wallis test. Values were shown in pg/ml. n (WT) = 104, n (HT) = 68, n (SNP) = 12.

HLX1 rs12141189: A-G. N (WT) = 104, n (HT) = 68, n (SNP) = 12.
Discussion

In this study, we assessed the effect of single nucleotide polymorphisms (SNPs) of TH1 transcription factors, namely TBX21 and HLX1 on T cell lineages and in particular TH1/TH2 cytokine secretion in cord blood. Carriers of TBX21 and HLX1 polymorphisms showed modulated IL-5 and IL-13 secretion upon innate stimulation with Lipid A or peptidoglycan in cord blood. TH1/TH2-related mRNA expression partly correlated with cytokine secretion.

The influence of a child's genotype on the development of immune-mediated diseases such as allergic diseases has been shown in several studies, e.g. for IRF1 or TLR polymorphisms [22,23,24]. For TBX21 polymorphisms, several studies have shown an association with the development of asthma [25,26,27] indicating the relevance of TH1 TFs in TH2-associated allergic diseases. Therefore we investigated the effect of SNPs in the TH1 pathway (TBX21 and HLX1) on primarily TH1 and TH2 cytokine regulation early in life, a critical time window, which is relevant for early immune maturation and subsequent determination of TH2-mediated allergic diseases [20,29,30]. We detected a decrease of IL-5 and a trend for decreased IL-13 secretion after Lipid A or peptidoglycan stimulation in homozygous carriers of TBX21 SNP rs17250932. This decrease may potentially be explained by an overexpression of TBX21. Indeed, it has previously been shown experimentally that this polymorphism increases promoter activity [10]. Szabo et al. have shown that expression of TBX21 in TH2-cells by retroviral gene transduction leads to lower IL-4 and IL-5 [8]. Furthermore, IL-13 mRNA expression and its promoter activity were suppressed upon TBX21 expression suggesting a regulation of IL-13 at gene transcription level [12]. In parallel, T-bet null mice showed elevated production of IL-4, IL-5 and IL-13, and diminished production of interferon-γ [31]. In unstimulated conditions the TBX21 SNP rs17250932 carriers showed reduced STAT6 mRNA expression, which represents a STAT6 isoform (splice variant including both intron 17 and intron 18) [32]. The second TBX21 SNP (rs11079788) investigated in our study was not associated with any change in cytokine responses. Yet, homozygous carriers

Table 2. Effects of TBX21 and HLX1 polymorphisms on cytokine secretion, T cells and mRNA regulation.

| Gene/rs number | Cytokine secretion/T cell regulation | P Overall | P recessive model | mRNA regulation | P Overall |
|----------------|------------------------------------|-----------|-----------------|-----------------|-----------|
| TBX21 rs17250932 | IL-5 (LpA) ↓ | 0.03 | 0.03 | No changes with LpA | - |
| | IL-13 (LpA) ↓ | 0.06 | 0.28 | STAT6e (U) ↓ | 0.01 |
| | TNF-α (LpA) ↓ | 0.07 | 0.16 | | |
| TBX21 rs11079788 | CD4+CD25+ (U) ↑ | 0.04 | 0.23 | GATA3 (U) ↑ | 0.08 |
| | HLX1 (U) ↑ | | | | 0.02 |
| | IRF1 (U) ↑ | | | | 0.01 |
| HLX1 rs2738751 | IL-5 (LpA) ↓ | 0.05 | 0.02 | No changes with LpA or Ppg | - |
| | IL-13 (LpA) ↓ | 0.05 | 0.02 | | |
| | IL-13 (Ppg) ↓ | 0.08 | 0.03 | | |
| | TNF-α (LpA) ↓ | 0.04 | 0.01 | | |
| HLX1 rs3806325 | IL-13 (U) ↑ | 0.005 | 0.003 | GATA3 (LpA) ↑ | 0.003 |
| | IL-6 (U) ↑ | 0.03 | 0.05 | STAT6e (LpA) ↑ | 0.007 |
| HLX1 rs12141189 | IL-5 LpA ↑ | 0.007 | 0.002 | TBX21 (D+L) ↓ | 0.02 |
| | IL-13 LpA ↑ | | | | |
| | GM-CSF LpA ↑ | 0.03 | 0.009 | | |
| | IL-5 (Ppg) ↑ | 0.1 | 0.09 | | |
| | GM-CSF (Ppg) ↑ | 0.05 | 0.02 | | |
| | IFN-γ (D+U) ↓ | 0.1 | 0.03 | | |

LpA = Lipid A; Ppg = Peptidoglycan and U = unstimulated, D+L = Dermatophagoides pteronyssinus 1 and Lipid A;
1: expression upregulated;
2: expression downregulated. P values analyzed by Kruskal-Wallis-test (overall, 3 categories) or Wilcoxon-test (recessive model, 2 categories). Genotype comparison includes the comparison of the respective genotype groups used for statistical analysis of mRNA expression; statistics performed by generalized Wilcoxon test.
Significance (p<0.05) is marked in bold.

doi:10.1371/journal.pone.0031069.t002

Table 3. Minor Allele Frequency (MAF) in children of atopic and non-atopic mothers.

| Gene/rs number | Maternal atopy | MAF |
|----------------|----------------|-----|
| TBX21 rs17250932 | No | 0.25 |
| | Yes | 0.17 |
| TBX21 rs11079788 | No | 0.32 |
| | Yes | 0.25 |
| HLX1 rs2738751 | No | 0.15 |
| | Yes | 0.15 |
| HLX1 rs3806325 | No | 0.16 |
| | Yes | 0.21 |
| HLX1 rs12141189 | No | 0.20 |
| | Yes | 0.23 |

Minor Allele Frequency of TBX21 and HLX1 polymorphisms in children of atopic and non-atopic mothers.
doi:10.1371/journal.pone.0031069.t003
had a higher number of activated T cells, combined with an increased expression of GATA3, HXL1, IRF1 mRNA expression, representing Th1 as well as Th2-genes. Similar to TBX21 rs17250932, homozygous carriers of HXL1 SNP rs2738751 showed lower Th2 and inflammatory cytokine secretion (IL-5, IL-13 and TNF-α) after Lpa-stimulation.

Carriers of HXL1 polymorphism rs12141109 showed increased Th1 cytokines such as IL-5, IL-13 and also GM-CSF following in vitro stimulation (Lpa or Ppg). In parallel to higher Th2 cytokines, we detected also lower Th1 IFN-γ secretion and lower TBX21 mRNA expression. This could lead to HXL1 downregulation, as HXL1 can be induced by TBX21 [11]. This increase of Th2 cytokines at birth in our study may potentially be in contrast with an asthma-protective effect observed in school-age children [14]. Although without stimulation no differences at mRNA-level of Th1/Th2-related genes were detected, the Lpa-induced increase of GATA3 and STAT6 mRNA expression in homozygous carriers of the HXL1 SNP rs3806325 may be supportive of a Th2-dominated immune response. However different influences on immune maturation during childhood may modify the T cell lineage fate over time. Thus, a direct comparison between findings in cord blood and school-age children needs to be interpreted with caution.

Homozygous carriers of HXL1 SNP rs3806325, which was previously associated with an enhanced risk for asthma, especially non-atopic asthma in childhood [14], showed increased levels of IL-13 (Th2) and additionally enhanced IL-6 secretion in this cord blood study. Previous functional studies have demonstrated that this polymorphism significantly influences HXL1 gene expression levels due to an altered transcription factor binding to the HXL1 promoter, indicating its potential functional relevance [14]. Results comparing HXL1 SNP carriers vs heterozygous and WT carriers became more significant when applying the recessive model, which compares the homozygous SNP carriers vs heterozygous carriers and wild type. Overall, immune development is influenced by a variety of factors over time, and cord blood responses may not be translated to childhood atopic phenotypes without taking additional regulatory factors into account. It is likely that certain genotypes may be more relevant than others and that the interplay with specific environmental exposures will impact Th1 cell lineages and disease development differently.

In our study most of the effects of TBX21 and HXL1 polymorphisms of Th1 pathway were observed upon Lpa-stimulation, partly upon Ppg-stimulation and almost no differences were observed following Derp1-stimulation. Thus, the effects seem to be influenced by innate immune regulation, mainly via TLR4-stimulation. For further functional explanation, more detailed studies are required.

Furthermore, we described in our previous studies that maternal atopy modulated cord blood immune responses [15,22]. Yet, in stratified analysis in this study, all observed effects were of similar magnitude for children with and without maternal atopy.

In regards to allergic outcomes assessed at age 3 years, one TBX21′ polymorphism was associated with less symptoms of atopic dermatitis. Therefore, further investigations on both TBX21 and HXL1 polymorphisms are required in larger cohorts to confirm the effects of the polymorphisms on atopic diseases in childhood. It may be of particular interest to investigate children at risk for atopic diseases, in order to disentangle the effects of the SNPs on early immune development and subsequent disease.

Additional points need to be considered for interpretation of our data. The results were not adjusted for multiple testing as this is an explorative study and further replication by other studies rather than correction for multiple testing is required. Another important point is the assessment of cytokines in bulk culture due to logistic possibilities and low cell numbers per child. Thus, we were not able to clearly differentiate the origin of the secreting cell. Additionally, we do not have details about leukocyte subsets available. The low frequency of SNPs resulted in low numbers of homozygous carriers of the SNPs in our cohort, therefore some findings could possibly be masked or power was limited due to the number of affected children.

The strength of this study is a detailed investigation of the early immune system regarding Th1-cell lineages at mRNA and cytokine protein level in relation to Th1 genotypes in a well documented birth cohort, including the follow-up for atopic diseases until the age of 3 years. It reveals that polymorphisms in crucial transcription factors of Th1 cells lead to alterations in the Th1 pathway already in early life. The further follow-up of our cohort will help to elucidate which of these children will also develop atopic diseases and/or asthma and whether the TBX21 and HXL1 genotypes have an impact on their later immune development.

Supporting Information

Table S1 Distribution of TLR2 polymorphisms within HXL1 genotypes. Results are presented as % and below absolute numbers shown in brackets. (DOC)

Acknowledgments

We acknowledge the families for their participation in the study and midwives for taking the cord blood samples. We thank Anna Lluis and Diana Radler for review and thoughtful critique of this manuscript.

Author Contributions

Conceived and designed the experiments: BS. Performed the experiments: VIC KS EVM MK BS. Analyzed the data: VIC SI NB BS. Contributed reagents/materials/analysis tools: BS. Wrote the paper: VIC KS EVM MK BS.

References

1. von Mutius E (2009) Gene-environment interactions in asthma. J Allergy Clin Immunol 123: 3–11; quiz 12–13.
2. Pinto LA, Stein RT, Kalesch M (2008) Impact of genetics in childhood asthma. J Pediatr (Rio J) 84: 660–73.
3. Finckh-Kruse F, Vercelli D (2007) Advances in asthma, allergy mechanisms, and genetics in 2006. J Allergy Clin Immunol 120: 544–550.
4. Schaaf B, Liu J, Hoppeler S, Schleich I, Hueln J, et al. (2009) Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol 123: 774–782 e775.
5. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, et al. (2002) Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 346: 869–877.
6. Woodfine K, Modlicek B, Glay DF, Jia G, Abbas AR, et al. (2009) T-helper type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med 180: 388–395.
7. Wilson CB, Rosell E, Sekimata M (2009) Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol 9: 91–103.
8. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, et al. (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100: 655–669.
9. Raby BA, Hwang ES, Van Steen K, Tantisira K, Peng S, et al. (2006) T-bet polymorphisms are associated with asthma and airway hyperresponsiveness. Am J Respir Crit Care Med 175: 64–70.
10. Suttner K, Rosenstiel P, Depner M, Schedel M, Pinto LA, et al. (2009) TBX21 gene variants increase childhood asthma risk in combination with HXL1 variants. J Allergy Clin Immunol 123: 1062–1068, 1068 e1061–1068.
11. Muller AC, Hutchins AS, High FA, Lee HW, Sykes KJ, et al. (2002) Hbs is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. Nat Immunol 3: 632–638.
12. Suzuki K, Kaminuma O, Hiroi T, Kitamura F, Miyatake S, et al. (2008) Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. Science 293: 330–342.
14. Suttner K, Ruoss I, Rosenstiel P, Depner M, Pinto LA, et al. (2009) HLX1 gene variants influence the development of childhood asthma. J Allergy Clin Immunol 123: 32–38 e86.

15. Schaub B, Liu J, Hoppler S, Haug S, Sattler G, et al. (2008) Impairment of T-regulatory cells in cord blood of atopic mothers. J Allergy Clin Immunol 121: 1491–1499, 1499 e1491–1413.

16. Peifferle P, Buchele G, Blumer N, Roponen M, Ege MJ, et al. (2010) Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study. J Allergy Clin Immunol 125: 108–115 e101–103.

17. Sandberg M, Frykman A, Emernadh J, Berg G, Matthiesen L, et al. (2009) Cord blood cytokines and chemokines and development of allergic disease. Pediatr Allergy Immunol 20: 519–527.

18. Fusaro AE, de Brito CA, Taniguchi EF, Muniz BP, Victor JR, et al. (2009) Balance between early life tolerance and sensitization in allergy: dependence on the timing and intensity of prenatal and postnatal allergen exposure of the mother. Immunology 125: e541–550.

19. Schaedel M, Carr D, Klopp N, Weinsch B, Illig T, et al. (2004) A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels. J Allergy Clin Immunol 114: 1100–1105.

20. Kaplan EL, Meier P (1958) Nonparametric-Estimation from Incomplete Observations. Journal of the American Statistical Association 53: 457–481.

21. Prentice RL (1978) Linear Rank-Tests with Right Censored Data. Biometrika 65: 167–179.

22. Liu J, Radler D, Illi S, Knicker E, Tarun E, et al. (2011) TLR2 polymorphisms influence neonatal regulatory T cells depending on maternal atopy. Allergy.

23. Schaedel M, Pinto LA, Schaub B, Rosenstiel P, Cherkassov D, et al. (2008) IRF-1 gene variants influence IgE regulation and atopy. Am J Respil Crit Care Med 177: 615–621.

24. Kormann MS, Fesel R, Depner M, Klopp N, Spiller S, et al. (2009) Rare TLR2 mutations reduce TLR2 receptor function and can increase atopy risk. Allergy 64: 636–642.

25. Chung HT, Kim LH, Park BL, Lee JH, Park HS, et al. (2003) Association analysis of novel T3X21 variants with asthma phenotypes. Hum Mutat 22: 257.

26. Munthe-Kaes MC, Carlsen KH, Haland G, Devulapali CS, Gervin K, et al. (2008) T cell-specific T-box transcription factor haplotype is associated with allergic asthma in children. J Allergy Clin Immunol 121: 51–56.

27. Akahoshi M, Obaza K, Hirota T, Matsuda A, Hasegawa K, et al. (2005) Functional promoter polymorphism in the T3X21 gene associated with aspirin-induced asthma. Hum Genet 117: 16–26.

28. Macaubas C, de Klerk NH, Holt RJ, Woe C, Kendall G, et al. (2003) Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. Lancet 362: 1192–1197.

29. Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA (2000) Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. J Allergy Clin Immunol 105: 951–959.

30. Tsukiki H, Arakawa H, Sugiyama M, Otsu H, Minura T, et al. (2009) Association of cord blood cytokine levels with wheezy infants in the first year of life. Pediatr Allergy Immunol 20: 227–233.

31. Lakos G, Melchian D, Wu M, Varga J (2006) Increased bleomycin-induced skin fibrosis in mice lacking the Th1-specific transcription factor T-bet. Padoiology 73: 224–237.

32. Schaedel M, Friei R, Bieli C, Cameron I, Adamski J, et al. (2009) An IgE-associated polymorphism in STAT6 alters NF-kappaB binding, STAT6 promoter activity, and mRNA expression. J Allergy Clin Immunol 124: 583–589, 589 e581–586.