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Research Article

ARCHIVES OF
CLINICAL AND
BIOMEDICAL RESEARCH

ISSN: 2572-5017

Cord Blood Biomarkers Predict Psoriasis Many Years Before Diagnosis: A Prospective Birth Cohort Study

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Abstract

Background: This study aimed to identify biomarkers that can serve as putative predictors for different groups based on psoriasis diagnosis, onset, and severity, with the clinical implication of decreasing the risk of developing the disease.

Material and Methods: Multiple protein biomarkers were assessed in cord blood from 115 psoriasis affected cases and 220 healthy controls in ABIS (All Babies in Southeast Sweden) by proximity extension assay (PEA).

Results: IFNGR1, COLEC12, and TNFRSF11B had higher expression in cases while 50 had a higher expression in controls, especially STX.8, COL9A1, HLA-E, PLXNA4, RAB37, EDAR, and CD84. Gene Ontology enrichment with all annotated proteins reveals that IFNGR1 is enriched in GO terms, whereas TNFRSF was enriched in GO terms. The same two proteins were found significant using ANOVA with sex, and gestational age as covariates and same GO terms were enriched. Number of significantly different biomarkers in the onset groupings were 28, 57, and 41 for "healthy - post puberty psoriasis", "healthy - pre puberty psoriasis" and "post puberty psoriasis - pre puberty psoriasis", respectively. Similarly, we found 70, 24, and 61 differentially expressed proteins for "healthy-mild", "healthy-severe", and "mild-severe" comparisons.

Conclusions: Several proteins were increased already in cord blood of individuals who later developed psoriasis, but even more proteins usually related to immune system and/or skin structure were significantly reduced in these individuals. This suggests that factors already during pregnancy may play a role for development of psoriasis. However, we found no significant association to environmental factors during pregnancy.

Keywords: Psoriasis; ABIS; IFNGR1; Normalized Protein Expression (NPX); olink; Autoimmunity; Biomarkers

What is already known about this topic?

- Psoriasis is an autoimmune disease that is known to decrease substantially the quality of life of the affected individual
- Although, list of biomarkers, particularly those measurable in blood, is present for psoriasis, it is neither complete nor adequate, and not measured long time before development of the disease

What does this study add?

- For the first time a prospective longitudinal birth cohort study makes

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Citation: Debojyoti Das, Johnny Ludvigsson. Cord Blood Biomarkers Predict Psoriasis Many Years Before Diagnosis: A Prospective Birth Cohort Study. Archives of Clinical and Biomedical Research. 8 (2025): 203-211.

Received: September 16, 2024
Accepted: October 10, 2024
Published: May 06, 2025

Das D and Ludvigsson J., Arch Clin Biomed Res 2025
DOI:10.26502/acbr.50170452

it possible to investigate biomarkers many years before onset of psoriasis

- Several proteins, especially IFNGR1, COLEC12, and TNFRSF11B, were increased already in cord blood of individuals who later developed psoriasis, but even more proteins usually related to immune system and/or skin structure were significantly reduced in these individuals
- Evidently factors during pregnancy are important for development of psoriasis. However, we found no significant association to environmental factors during pregnancy

Introduction

Psoriasis is a debilitating chronic autoimmune inflammatory skin disorder and is known to affect 0.7-3.2% of the global population [1,2]. The disease is both genetically and clinically heterogeneous, with diagnosis from early childhood as well as in adults [3]. The etiology of the disease is only partly known. Psoriasis involves an abnormal immune response with an increase of CD8-positive T-cells [4], and a specific population of myeloid "inflammatory" DCs that appears to play an important pathogenic role [5]. The disease is both genetically and clinically heterogeneous, with diagnosis from early childhood as well to the rapid turnover of skin cells, resulting in the formation of thick, scaly patches on the skin.

Biomarkers have been an active area of research for assessment of prognosis, disease diagnosis, and treatment designs [3] in autoimmune diseases. However, the identification of biomarkers together with the requirement to confirm their validity and robustness has posed a major hurdle and, in most cases, preclude clinical usefulness. Although, list of biomarkers particularly those measurable in blood [4,5], is present for psoriasis, it is neither complete nor adequate at present. Proteomic studies have helped identify specific proteins that are dysregulated in psoriasis. For instance, researchers have found alterations in proteins involved in inflammation, immune response, and skin cell proliferation and differentiation. Understanding these changes at the protein level provides valuable insights into the pathogenesis of psoriasis and may lead to the development of targeted , at best to decrease the risk of psoriasis. There is one study [6] where the Olink platform was used to study the difference between various forms of psoriasis compared with 10 controls. They found that interleukin-17 (IL-17) and interleukin-23 (IL-23), which play key roles in the pathogenesis of psoriasis, to have elevated expression levels in cord blood samples of individuals who later developed the disease. Beside this study there is to our knowledge a lack of proteomic studies of cord-blood in individuals who later develop psoriasis to investigate factors during pregnancy which may be part of the etiology of psoriasis. Environmental factors during pregnancy, such as maternal stress, diet, smoking, and exposure to pollutants, can affect fetal immune development. These factors can induce systemic inflammation and alter cytokine profiles in maternal and fetal circulation, potentially predisposing the offspring to immune dysregulation and inflammatory disorders like psoriasis.

Understanding the interplay between maternal factors, fetal immune development, and long-term health outcomes is essential for elucidating the early-life origins of psoriasis and developing preventive strategies. With this background, we decided to study the proteomic patterns in cord blood and correlate our findings with lifestyle of the mother and environmental factors.

Materials and Methods

Participants

Cord blood was obtained from children who were registered in the All Babies in Southeast Sweden (ABIS) birth cohort. which consists of 17,055 children (78.6% of all 21,700) born between 1 Oct 1997 and 1 Oct 1999. The aim of ABIS was to study the importance of environmental factors in the development of immune-mediated diseases, especially T1D [7,8]. Parents answered comprehensive questionnaires on nutrition, infections, psychosocial situation, living conditions, life events and so on at birth and then after 1, 3, 5, 8, 11-13 years and biological samples such as blood, urine, stool, and hair were collected at these follow-ups. The ABIS register has been connected to national registers and here we have used the Swedish National Patient Register to obtain the diagnosis of psoriasis, which 121 individuals had developed by 31 Dec 2020. We had useful questionnaires on 16,415 of these individuals from which we had excluded 717 who had another autoimmune disease. Cord blood samples were collected from all ABIS individuals, and those used in this study is shown in Table 1.

Table 1: Features and distribution of samples of the Olink Cohort.

Feature	Healthy	Psoriasis	P-value	Method
Gestational age	39.91±1.48	39.71±1.53	0.268	T-test
Sex (Boy/Girl)	111/109	54/61	0.543	Pearson's Chi-squared test
Sample distribution				
Healthy		Boy	111	
		Girl	109	
Post puberty psoriasis	**Mild**	Boy	25	
		Girl	33	
	Severe	Boy	5	
		Girl	7	
Pre puberty psoriasis	**Mild**	Boy	20	
		Girl	18	
	Severe	Boy	4	
		Girl	3	

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Methods

We restricted our biomarker assay list to inflammation related biomarkers and used the Olink Inflammation panel-based approach that uses the highly sensitive and specific proximity extension assay (PEA) technology, enabling simultaneous analysis of a multitude of biomarkers [9,10]. Specifically, we used the Explore 384 Inflammation assay (Olink Proteomics, Uppsala, Sweden, www.olink.com) In total 363 proteins were tested of which 4 assays were discarded since they were detected in less than 15% of the samples, and 2 other assays were excluded since they did not meet the parametric distribution criterion. Cord blood from 115 individuals who later developed psoriasis, and from 220 randomly selected healthy controls, born in the same period, was analysed.

Using the ABIS birth questionnaire covering the pregnancy we investigated whether certain environmental factors were associated to our biomarker findings.

Statistical Analysis

Differentially expressed proteins were identified by using welch t-test on the Olink data with a P-value cutoff of 0.05. For variables based on onset, and severity which have more than two-levels we used ANOVA to arrive at differentially expressed proteins. Both sex, and gestational age was used as covariate. Post-hoc analysis was always implemented, and individual contrasts filtered before applying enrichment analysis. We made use of functions provide in the R-package OlinkAnalyze, enrichment analysis was carried out using ClusterProfiler package, and custom written R-codes based on ggplot package were used for visualizations. All reported enrichment analysis data are based on the background of all proteins since restricting the background to only tested proteins did not return any enriched term even with a very relaxed p-value cutoff. Volcano plot with the Benjamin-Hochberg adjusted p-value shows the significantly different proteins.

Ethics statement

The Research Ethics Committees at the Faculty of Health Science at Linköping University, Sweden, have given ethical approval to the All Babies in Southeast Sweden (ABIS) study (Dnr. 287-96, 2011/52-53) and connection to national registers (Dnr 2003-092). The parents of the participating children gave their informed consent to participate in the ABIS study after oral, written and video information. Participants were free to opt in or out of the study whenever they wished.

Results

The clinical characteristics of the participants in the Olink cohort is shown in Table 1. We found no significant differences between the controls and cases (Healthy-Psoriasis) based on gestational age or sex. The distribution of samples based on severity of psoriasis and onset of psoriasis is also delineated in the Table 1. Here we set 13 years as the cutoff age to categorise cases as pre-puberty psoriasis, and post-puberty psoriasis.

Before statistical analysis, we carried out dimensional reduction using principal component analysis (PCA). However, we did not see any clear separation between groups based on psoriasis diagnosis, Notwithstanding, controls and cases can be seen to have a very weak separation in PCA plots (Figure 1). We also plotted the inter-quartile range against the sample median but were not able to unambiguously identify outliers.

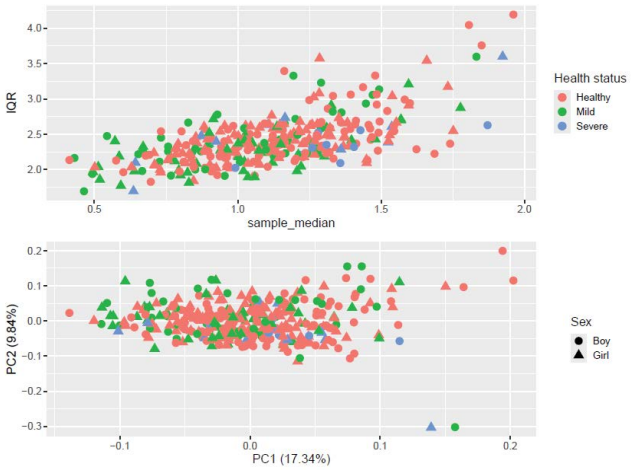

Figure 1: Plots for outlier identification. Principal component analysis (lower panel) and Inter-quartile range Vs. Sample median (upper panel). Healthy samples (red), psoriasis samples (green and blue representing mild form and severe form of psoriasis, respectively). Solid circle and solid triangle represent male and female samples, respectively.

An initial welch t-test revealed a total of 53 differentially expressed biomarkers between healthy and psoriasis samples (Figure 2). X-axis can be read as the log2 fold-change since Olink data is in log2 scale, and difference of log values is equal to log of the ratio. Three biomarkers, IFNGR1, COLEC12, and TNFRSF11B had higher expression in the cord blood of children who would eventually develop psoriasis, while remaining 50 had a higher expression in controls. Suppl Fig1 shows the normalized expression of all significant biomarkers obtained in the t-test comparison of Healthy Vs Psoriasis samples. IFGNR1 is a receptor for Interferon-gamma known to be involved in cytokine mediated pathway as is the tumour necrosis factor receptor superfamily 11B (TNFRSF11B) which belongs to the protein superfamily of cytokine receptors. COLEC12 on the other hand is known to have role in host immunity. Another remarkable finding is the fact that interleukin 5 receptor subunit alpha (IL5RA) showed

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DOI:10.26502/acbr.50170452

a more pronounced reduction in expression in samples from individuals who later developed psoriasis in comparison to healthy samples.

We also tested the role of putative covariates sex, and gestational age on the expression of proteins in the two groups using the ANOVA method with covariates. A total of 55 biomarkers were found to be significantly different between healthy and psoriasis samples. In addition to IL5RA found in t-test, the regulatory IL10 was also found to be significantly different.

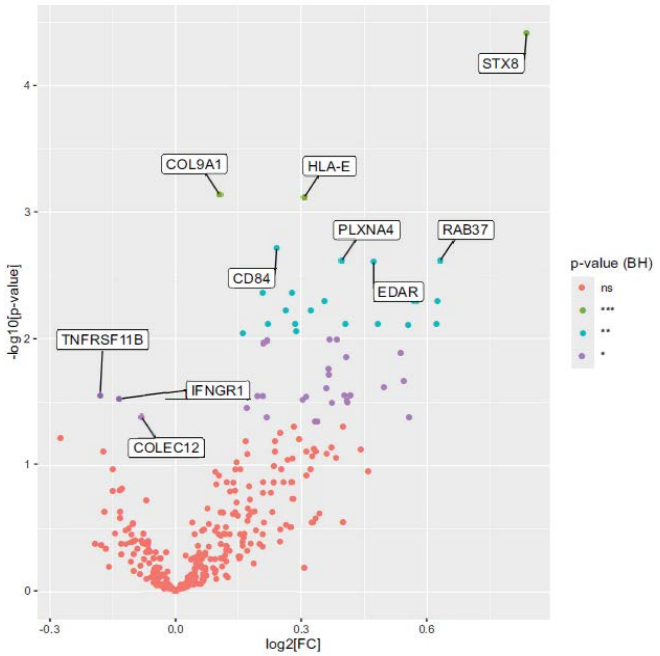

Figure 2: Volcano plot for Healthy Vs Psoriasis using results of t-test with Benjamin-Hochberg (BH) adjusted P-value. Where * = p-value < 0.05 (purple solid circle), ** = p-value < 0.01 (blue solid circle), *** = p-value < 0.001 (green solid circle), and ns represents not-significant.

To rigorously examine the potential functionalities of the proteins exhibiting differential expression, we conducted gene ontology (GO) enrichment analysis based on various backgrounds. Using t-test results and a background of all annotated proteins, proteins were found to be enriched in several GO terms, such as cytokine–mediated signalling pathway (IFNGR1 higher in psoriasis whereas CCL26 higher in healthy samples), positive regulation of cytokine production (IFGNR1), and negative regulation of immune system process (TNFRSF11B higher in psoriasis samples, and CCL28 higher in healthy samples). Heat plot of enriched biological pathways is shown in Figure 3. Almost all the pathways, specifically related to cytokine production, negative regulation of immune system process, and tumour necrosis factor productions were obtained in the ANOVA with covariates gene sets like t-test results. In addition, negative regulation of interleukin-6 production was also found to be enriched (Figure 4).

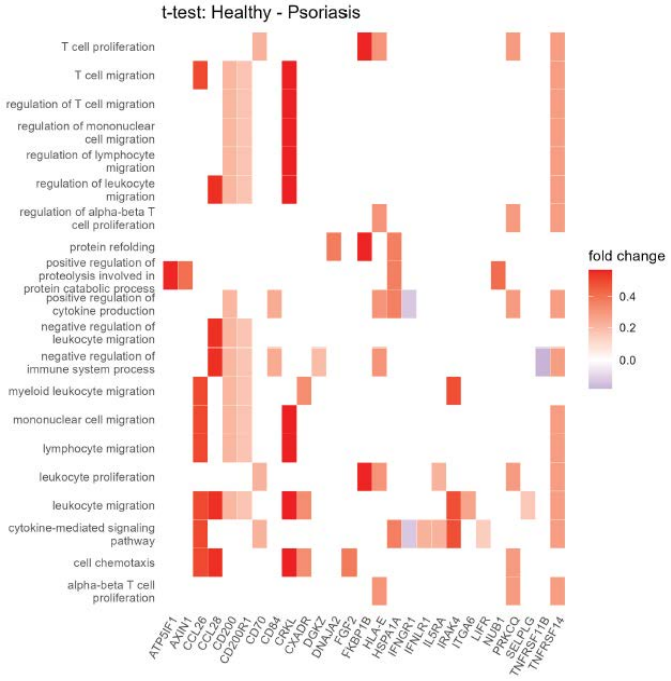

Figure 3: Gene Ontology (GO) enrichment analysis of the differentially expressed inflammation-related biomarkers obtained from t-test (Healthy Vs Psoriasis). Background all annotated gene Entrez IDs.

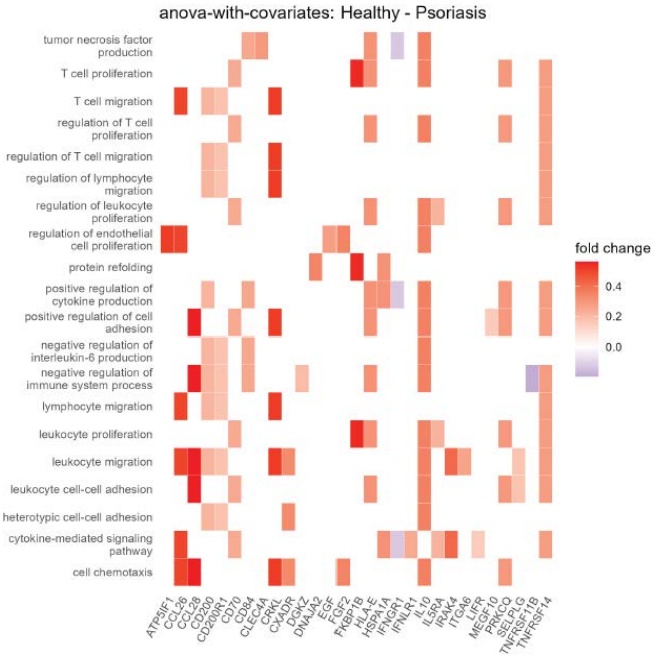

Figure 4: Gene Ontology (GO) enrichment analysis of the differentially expressed inflammation-related biomarkers obtained from ANOVA (Healthy Vs Psoriasis) with covariates(sex, and gestational age). Background all annotated gene Entrez IDs.

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Table 2: Number of significant biomarkers (P-value < 0.05) for different contrasts obtained from post-hoc ANOVA with covariates.

Contrast	Number of biomarkers
Healthy - Psoriasis	55
Healthy - Post puberty psoriasis	28
Healthy - Pre puberty psoriasis	57
Post puberty psoriasis - Pre puberty psoriasis	41
Healthy - Mild	70
Healthy - Severe	24
Mild - Severe	61

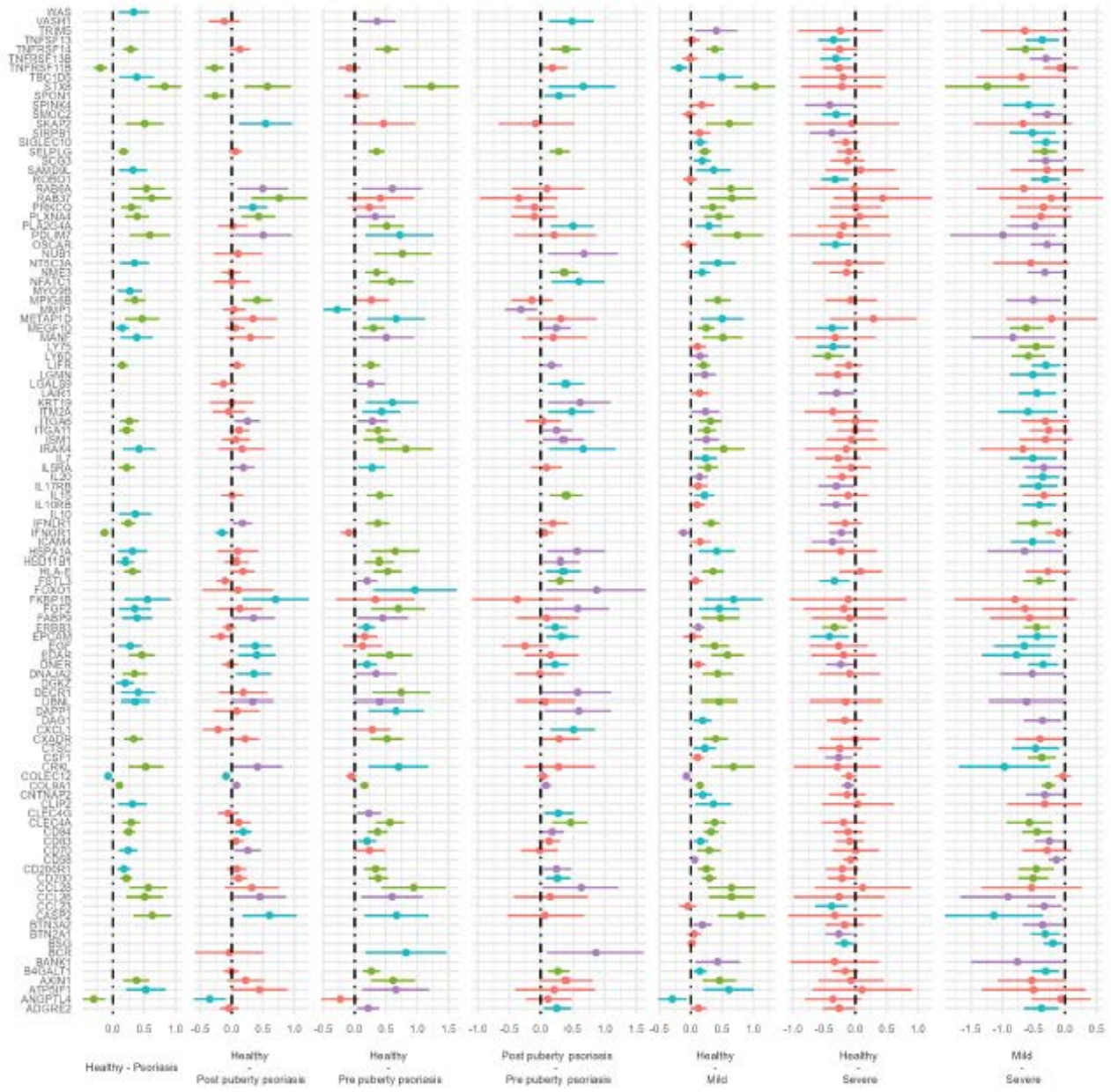

Figure 5: Significant biomarkers across all combinations obtained from post-hoc ANOVA analysis. In the preceding ANOVA analysis step sex, and gestational age was used as covariates. Difference in mean NPX between variables (from contrast) are plotted against biomarkers. Where red = not-significant, purple = p-value < 0.05, blue = p-value < 0.01, and green = p-value < 0.001, respectively.

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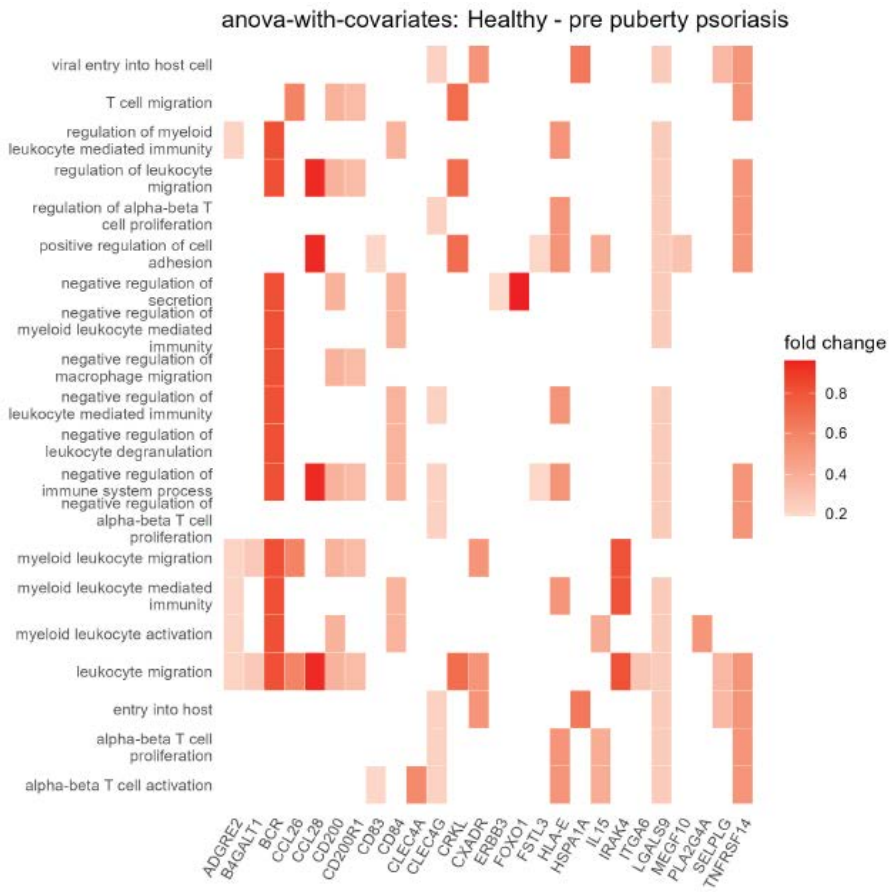

Figure 6: Gene Ontology (GO) enrichment analysis of the differentially expressed inflammation-related biomarkers obtained from ANOVA (Healthy Vs Pre puberty psoriasis). Background all annotated gene Entrez IDs.

Table 3: Pregnancy related variables. P-values were obtained using t-test and Fisher exact test for continuous variables and categorical variables, respectively. P-value < 0.05 = *.

Term	Healthy	Psoriasis	P.value
Mother age at birth			
Mean(± Sd)	30(±4.09)	28.96(±4.58)	0.042*
Mother infection pregnancy			
Yes	60	29	0.658
No	147	82	
Do not know	2	2	
Missing	11	2	
Antibiotics pregnancy			
Yes	38	15	0.303
No	156	89	
Do not know	3	0	
Missing	23	11	
Smoking pregnancy			
No	193	100	0.449
Yes	20	14	
Missing	7	1	

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Vitamin/ Mineral pregnancy			
Yes	129	60	0.19
No	80	52	
Do not know	0	0	
Missing	11	3	
Fish Lake or Baltic Sea			
Every Day	1	0	0.228
3-5 Times/Week	0	0	
1-2 Times/Week	14	3	
More Seldom Than 1-2 Times /Week	194	109	
Missing	11	3	
Mother worked with petroleum products			
Yes	3	0	0.267
No	166	88	
Do not know	1	2	
Missing	50	25	
Mushroom intake frequency			
Every Day	0	0	0.887
3-5 Times/Week	1	1	
1-2 Times/Week	8	3	
More Seldom Than 1-2 Times /Week	199	108	
Missing	12	3	

Next, we implemented ANOVA with covariates (Table 2) on contrasts with three levels, based on onset of psoriasis, and severity of disease. For onset of psoriasis, we observed 28 significant proteins for "Healthy – Post puberty psoriasis" comparison, and 57 significant proteins for "Healthy – Pre puberty psoriasis". There are more than twice the number of significant proteins for the latter compared to the former contrast, because the deviation between the controls ("Healthy") and cases ("Pre puberty psoriasis") is much more pronounced at a nascent stage. Notably, 36 significant proteins are shared between "Healthy – Psoriasis", and "Healthy – Post puberty psoriasis". "Post puberty psoriasis – Pre puberty psoriasis" had 41 significant proteins from the analysis.

For the severity groupings, we observed 70 significant proteins between "Healthy - Mild", and only 24 significant proteins between "Healthy - Severe". However, 61 proteins differed significantly between "Mild - Severe", an indication of the different pathogenesis in mild-form, and severe-form psoriasis. Proteins that were significant in at least one of the comparisons are plotted in Figure 5 and colour coded based on P-value significance level.

Like t-test significant proteins, we investigated GO enrichment patterns for significant biomarkers in the ANOVA with covariates results. Here too several GO terms were enriched, notably the negative regulation immune system process. Figure 6 shows heat plot for "Healthy – Pre puberty psoriasis" comparison.

Our investigations of environmental factors and/or life style during pregnancy such as infections, intake of vitamins, exposure to certain toxic agents via working place, or smoking (Table 3) showed no significant associations to the biomarker patterns.

Discussion

In this unique prospective study of proteomic biomarkers in cord blood, with cases comprising of samples from individuals who later develop psoriasis, we have identified three biomarkers, IFNGR1, COLEC12, and TNFRSF11B to be significantly upregulated in the disease samples. IFNGR1 (Interferon Gamma Receptor 1 is a part of the receptor complex for interferon-gamma (IFN-γ), a critical cytokine in the immune response. This receptor is involved in activating macrophages, enhancing antigen presentation, and promoting the expression of various genes involved in the immune response. It plays a vital role in the defense against pathogens, particularly viruses and intracellular bacteria, and has previously been described to have a role in relation to psoriasis [11,12], COLEC12 (Collectin Sub-Family Member 12) is a scavenger receptor that is involved in binding and clearing various ligands, including pathogen-associated

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molecular patterns (PAMPs) on microbes and apoptotic cells. It plays a role in innate immunity by recognizing and binding to pathogens and facilitating their clearance from the body [13]. Additionally, COLEC12 is involved in maintaining homeostasis by clearing damaged cells and cellular debris. but it´s role in development of psoriasis is to our knowledge not studied TNFRSF11B (Tumor Necrosis Factor Receptor Superfamily Member 11B) has roles in modulating immune responses and apoptosis., and has earlier been described to be involved in psoriatic arthritis [14,15].

We also found a number of proteins with lower expression levels in individuals who later develop psoriasis than in healthy controls, such as STX.8, COL9A1, HLA-E, PLXNA4, RAB37, EDAR, and CD84. This is interesting as they have important immunological functions. For instance, STX8 (Syntaxin 8) is involved in vesicle trafficking within cells, particularly in the endosomal-lysosomal pathway. Dysregulation in vesicle trafficking can affect the delivery and presentation of antigens and cytokine release, potentially contributing to inflammatory processes seen in psoriasis. COL9A1 (Collagen Type IX Alpha 1 Chain) is a component of type IX collagen, which is involved in maintaining the integrity of the extracellular matrix. Lower levels of COL9A1 might impact the structural integrity of the skin and joints, potentially contributing to the susceptibility to psoriatic arthritis and the skin manifestations of psoriasis. HLA-E (Human Leukocyte Antigen E) is a non-classical MHC class I molecule that interacts with NK cells and certain T cell receptors to modulate immune responses. Reduced expression of HLA-E could affect immune regulation and tolerance, leading to an increased risk of autoimmune conditions like psoriasis. PLXNA4 (Plexin A4) is known to be involved in immune cell signaling and lower levels of PLXNA4 might disrupt normal immune cell trafficking and signaling, contributing to the aberrant immune responses. RAB37 (Ras-Related Protein Rab-37) is involved in the regulation of exocytosis. Reduced RAB37 levels could affect the secretion of inflammatory mediators, altering the inflammatory milieu EDAR (Ectodysplasin A Receptor) which in turn is involved in the development of ectodermal tissues. Lower expression of EDAR could make the skin more susceptible to the inflammatory and proliferative changes seen in psoriasis. Finally, CD84 (Cluster of Differentiation 84) plays a role in the regulation of immune cell interactions and signaling. Reduced CD84 expression might impair immune cell communication and regulation, contributing to the dysregulated immune responses observed in psoriasis.

We notice some differences related to when psoriasis is diagnosed, and some differences in biomarker patterns related to the severity of the psoriasis later in life.

Thus there are several biomarkers already in cord blood which may predict development of psoriasis and could be a consequence of environmental or life style factors during pregnancy. However, we have analysed the relation to such factors based on the birth questionnaire which the parents filled in after birth of the child, and cannot show any significant associations.

Strengths and limitations

A strength of our study is the prospective design of birth cohort including a general population without restrictions to certain genetic risks or family history. Furthermore, the long follow-up and connection to the Swedish National Patient Register give us unique possibilities to follow who have developed psoriasis, and we have good opportunities to compare the biomarker pattern in those who develop psoriasis with healthy controls. There is of course always some lacking information and risk of incorrect registration of data, but this risk should be quite low in mothers who shortly after birth are very motivated and tend to remember very well what has happened during pregnancy.

Regarding the Olink technique there are limitations(16), and without clear hypothesis there is a risk of statistical significance based on multiple comparisons. However, we have corrected for this, and our results show reasonable results regarding the proteomic markers.

Conclusions

Several proteins were increased already in cord blood of individuals who later develop psoriasis, but even more proteins were significantly reduced in these individuals. This shows that factors already before birth play a significant role for the development of psoriasis. However, we have not been able to show any significant association to the mother´s life style or any environmental factors during pregnancy. Further studies are needed to be able to recommend preventive measures.

Disclosure of conflicts

None of the authors has anything to disclose.

Data availability

Data may be obtained from the corresponding author upon reasonable request after ethical approval.

Authorship Contributions

JL had the idea, created ABIS and contributed with all material that is biological samples analyzed by olink, and questionnaire data, funding, while DD made statistical analyses, produced all tables and figures. Both authors wrote together the first draft, and then critically reviewed and finally approved the manuscript.

Acknowledgments

We are grateful to all ABIS individuals and parents participating in the study.

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DOI:10.26502/acbr.50170452

Funding sources: ABIS was supported by Barndiabetesfonden (Swedish Child Diabetes Foundation); Swedish Research Council, Grant/Award Numbers: K2005-72X-11242-11A and K2008-69X-20826-01-4, K2008-69X-20826-01-4; Medical Research Council of Southeast Sweden (FORSS); JDRF Wallenberg Foundation, Grant/Award Number: K 98-99D-12813-01A; ALF grants from Region Östergötland and Linköping University, Sweden, and the Joanna Cocozza Foundation.

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