

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## 'Barrier dysfunction in Atopic newBorns studY' (BABY): a birth cohort study protocol

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-033801
Article Type:	Protocol
Date Submitted by the Author:	22-Aug-2019
Complete List of Authors:	<p>Gerner, Trine; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Thyssen, JP; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Skov, Lone; Herlev and Gentofte Hospital, University of Copenhagen , Department og Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Halling-Sønderby, Anne-Sofie; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Rasmussen Rinnov, Maria; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Haarup Ravn, Nina; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Hjorslev Knudgaard, Mette; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Menné Bonefeld, Charlotte; University of Copenhagen, Department of Immunology and Microbiology, Skin Immunology Research Center</p> <p>Ewertsen, Caroline; Rigshospitalet, University of Copenhagen, Department of Radiology</p> <p>Trautner, Simon; Rigshospitalet, University of Copenhagen, Department of Neonatology</p> <p>Kezic, Sanja; Amsterdam Public Health Research Institute, University of Amsterdam, Coronel Institute of Occupational Health, Amsterdam UMC</p> <p>Jakaša, Ivone; University of Zagreb, Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology</p>
Keywords:	atopic dermatitis, birth cohort, Raman, skin barrier, preterm, TEWL

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1  
2  
3  
4 1 **‘Barrier dysfunction in Atopic newBorns studY’ (BABY): a birth**  
5  
6  
7 2 **cohort study protocol**  
8  
9

10 3  
11  
12 4 Trine Gerner<sup>1,2</sup>, Anne-Sofie Halling-Sønderby<sup>1,2</sup>, Maria Rasmussen Rinnov<sup>1,2</sup>, Nina Haarup Ravn<sup>1,2</sup>,  
13 5 Mette Hjorslev Knudgaard<sup>1,2</sup>, Charlotte Menné Bonefeld<sup>3</sup>, Caroline Ewertsen<sup>4</sup>, Simon Trautner<sup>5</sup>,  
14 6 Ivone Jakaša<sup>6</sup>, Sanja Kezic<sup>7</sup>, Lone Skov<sup>1,2</sup>, Jacob P. Thyssen<sup>1,2</sup>.  
15  
16  
17  
18  
19  
20 7

21  
22 8 <sup>1</sup>Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen,  
23 9 Hellerup, Denmark

24  
25  
26 10 <sup>2</sup>Copenhagen Research Group for Inflammatory Skin (CORGIS), Hellerup, Denmark

27  
28  
29 11 <sup>3</sup>University of Copenhagen, Department of Immunology and Microbiology, LEO Foundation Skin  
30 12 Immunology Research Center, Maersk Tower, Copenhagen N, Denmark

31  
32  
33 13 <sup>4</sup>Department of Radiology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

34  
35  
36 14 <sup>5</sup>Department of Neonatology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

37  
38 15 <sup>6</sup>Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of  
39 16 Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

40  
41  
42 17 <sup>7</sup>Coronel Institute of Occupational Health, Amsterdam UMC, Amsterdam Public Health Research  
43 18 Institute, University of Amsterdam, Amsterdam, Netherlands  
44  
45  
46

47 19  
48  
49 20 **Correspondence:**

50  
51 21 Jacob P. Thyssen, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Gentofte  
52 22 Hospitalsvej 15, DK-2900 Hellerup, Denmark  
53

54  
55 23 Telephone: (0045) 3867 3150

56  
57 24 E-mail: jacob.p.thyssen@regionh.dk  
58  
59  
60

1  
2  
3  
4 25 **Word count: 2198**

5  
6 26 **Number of figures: 2**

7  
8  
9 27 **Number of references: 25**

10  
11 28

12  
13 29 **Key words**

14  
15 30 Atopic dermatitis, birth cohort, filaggrin, infant, preterm, Raman, skin barrier, skin microbiome,

16  
17 31 TEWL, thymus.

18  
19  
20 32

21  
22 33 **Conflicts of interest**

23  
24 34 None declared

25  
26  
27 35

28  
29 36 **Author contributions**

30  
31 37 TG, LS and JPT designed the study, created the study protocol, and obtained approval of the study

32  
33 38 design. ST, CBM and CE contributed to revision and refinement of the study design. TG, AH,

34  
35 39 MRR, NHR, MHK and JPT were responsible for data collection. TG, AH, MRR, LS and JPT

36  
37 40 drafted the manuscript. All authors critically revised the manuscript. All authors supervised the

38  
39 41 study.

40  
41  
42 42

43  
44 43 **Acknowledgements**

45  
46 44 We thank all families for their participation in the BABY cohort. We thank all staff members at

47  
48 45 Rigshospitalet and Nordsjællands Hospital who have contributed.

49  
50  
51 46

52  
53 47

54  
55 48

56  
57 49

58  
59 50

60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

49 **Funding**

50 The study received financial support from The Leo Foundation, The Lundbeck Foundation, The  
51 Novo Nordisk Foundation, Pfizer, Aage Bangs Fond, Savværksejer Jeppe Juhl og hustru Ovita  
52 Juhls Mindelegat and The Herlev and Gentofte Hospital Research Foundation.

53

54

For peer review only

1  
2  
3  
4 55 **ABSTRACT**

5  
6  
7 56 Introduction:

8  
9 57 The skin barrier development in premature and mature newborns has been scarcely studied but may  
10  
11 58 be important for the risk of atopic dermatitis (AD).

12  
13 59 Methods and analysis:

14  
15  
16 60 The BABY Cohort is a prospective birth cohort study of 150 preterm and 300 term children. Skin  
17  
18 61 barrier function is assessed by transepidermal water loss. Biomolecules important for skin barrier  
19  
20 62 function and immune response are investigated by Raman-spectroscopy and stratum corneum (SC)  
21  
22 63 and microbiome sampling. Clinical examinations are done and DNA from buccal swabs is collected  
23  
24 64 for genetic analyses. Thymus size is assessed by ultrasound examination. Information on  
25  
26 65 pregnancy, delivery and parental exposures and diseases are collected and structured telephone  
27  
28 66 interviews are conducted at 18 and 24 months to assess exogenous exposures in the child and onset  
29  
30 67 of AD. Hanifin and Rajka criteria as well as The U.K. Working Party's Diagnostic Criteria for  
31  
32 68 Atopic Dermatitis are used to diagnose AD. Severity of AD is assessed using the Eczema Area and  
33  
34 69 Severity Index (EASI) and Patient Oriented Eczema Measure (POEM).

35  
36  
37 70 Ethics and dissemination:

38  
39 71 The study is approved by the local medical ethics committee (H-16042289 and H-16042294).

40  
41 72 Outcomes will be presented at national and international conferences and in peer-reviewed  
42  
43 73 publications.  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



## 79 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 80 • ‘BABY Cohort’ is a Danish prospective birth cohort study that examines skin barrier  
81 functions and risk factors for atopic dermatitis.
- 82 • Comprehensive and repeated measurements of skin barrier function and factors affecting  
83 immune and antimicrobial barrier in preterm and term newborns from the general  
84 population.
- 85 • Being strictly non-invasive, no blood measurements are done.

## 87 INTRODUCTION

88 Atopic dermatitis (AD) is a chronic and relapsing, inflammatory skin disease, characterized by dry  
89 and itchy skin that affects up to 20% of children in Northern Europe.[1] About 60-80% develop the  
90 disease in their first two years of life, and children with early onset are at increased risk of having  
91 severe and persistent disease.[2, 3] The risk of AD is increased in children of parents with atopic  
92 disorders such as AD, asthma and allergic rhinitis.[4-6]

93  
94 Genetic and environmental risk factors contribute to the development of AD through skin barrier  
95 dysfunction and immune dysregulation.[2] While loss-of-function mutations in the filaggrin gene  
96 (*FLG*) have been identified as the strongest genetic risk factor for AD,[7] genome wide association  
97 studies have only identified a relatively small proportion of genetic risk variants.[8] The inflammation  
98 in AD is characterized by overexpression of Th2 cytokines, including IL-4 and IL-13.[9] that together  
99 with IL-1 may lead to increased secretion of thymic stromal lymphopoietin (TSLP), decreased  
100 epidermal antimicrobial peptides and filaggrin levels, in turn worsening skin inflammation and  
101 epidermal barrier functions.[10] Changes in the skin microbiome is also associated with worsening

1  
2  
3  
4 102 of AD, showing reduced bacterial diversity[11] and increased colonization with *Staphylococcus*  
5  
6 103 *aureus* (*S. aureus*).[12]  
7  
8  
9 104  
10

11 105 While several environmental risk factors have been identified, e.g. winter birth and exposure to hard  
12  
13 106 domestic water, this has not yet led to prophylactic solutions.[13] Interestingly, the risk of AD is  
14  
15 107 decreased in premature newborns and infants undergoing heart surgery, which often includes partial  
16  
17  
18 108 or total thymectomy, perhaps due to a lower number of circulating T cells and an inappropriate  
19  
20 109 immune response to antigens encountered in the skin.[14, 15]  
21  
22

23 110  
24  
25 111 There is a need for birth cohort studies that closely examines the skin of newborns at several time  
26  
27 112 points to identify infants at risk of developing AD early in life. The BABY Cohort is a prospective  
28  
29  
30 113 birth cohort study that investigates early skin barrier development in preterm and term newborns to  
31  
32 114 identify early prognostic skin barrier changes for development of AD.  
33  
34 115

## 36 116 **OBJECTIVES**

37 117 Primary objective:

38 118 To identify predictors of AD in early childhood.  
39  
40  
41  
42  
43 119  
44

45 120 Secondary objectives:

46  
47 121 To closely describe the normal skin barrier development including immune activity and skin  
48  
49  
50 122 microbiome in preterm and term newborns during the first years of life.  
51  
52 123  
53  
54 124  
55

## 56 125 **METHODS AND ANALYSIS**

57 126 **Study population and setting**  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

127 The BABY Cohort is an ongoing prospective birth cohort study recruiting 150 preterm and 300 term  
128 newborn infants. Recruitment began in August 2017. Parents of eligible children are recruited at the  
129 maternity and neonatal wards at Rigshospitalet, Copenhagen, and Nordsjællands Hospital, Hillerød,  
130 in Denmark. Children eligible for enrolment are preterm newborns (GA below 37+0) excluding  
131 preterm newborns with severe congenital abnormality and healthy term singleton newborns (GA  
132 37+0 to 41+6) excluding mature newborns receiving antenatal steroids for fetal lung maturation.  
133 Children with parents unable to communicate in Danish are excluded.

### **Cohort design**

136 All study procedures are summarised in Figure 1 and 2, and each component of the visit is detailed  
137 below. Preterm children are scheduled for two study visits: during the first 31 days of life and  
138 approximately two months after their scheduled due date (Figure 1). Term children are scheduled for  
139 four study visits: during the first 3 days of life and approximately at 2, 6 and 12 months of age (Figure  
140 2). If a child develops AD during the first 2 years of life, an additional follow-up visit is performed.  
141 All parents participate in a structured telephone interview when the child is 18 and 24 months old.

### **Baseline interview**

144 During the first study visit, parents are interviewed to obtain information about the pregnancy and  
145 birth, including the type of delivery and maternal intrapartum antibiotic treatment. Furthermore,  
146 information about gestational age at birth, weight, height and head circumference, 1- and 5- minutes  
147 APGAR scores and medical treatment at the neonatal ward is obtained.

### **Study interview**

1  
2  
3  
4 150 At every study visit, we obtain detailed information about the child's health, vaccination status,  
5  
6 151 method of feeding, admittance to hospital, medical treatments, bathing habits and skin care.  
7  
8

9 152  
10  
11 153 **Parental questionnaires**

12  
13 154 Parents complete an online questionnaire on family structure, residential situation, pet exposure,  
14  
15  
16 155 occupation, maternal exposures during pregnancy, smoking and drinking habits, history about current  
17  
18 156 and previous skin diseases and atopic diseases in the family.  
19

20 157  
21  
22  
23 158 **Telephone interviews**

24  
25 159 At 18 and 24 months, parents participates in a structured telephone interview about the child's health,  
26  
27 160 vaccination status, method of feeding, admittance to hospital, medical treatments, bathing habits, skin  
28  
29  
30 161 care, ultraviolet exposures and AD assessment according to the U.K. Working Party's Diagnostic  
31  
32 162 Criteria for Atopic Dermatitis.[16, 17] If AD is diagnosed during the telephone interview an extra  
33  
34 163 study visit in the clinic is scheduled.  
35

36 164  
37  
38  
39 165 **Anthropometric measures**

40  
41 166 At the first visit, birth information on height, weight and head circumference is retrieved from the  
42  
43 167 birth record. At all follow-up visits, anthropometric measurements are made. A digital weight scale  
44  
45  
46 168 is used to record weight in kg without clothing and diaper. Height and head circumference are  
47  
48 169 measured in cm using a flexible non-elastic measuring tape.  
49

50 170  
51  
52 171 **Skin barrier measurements**

53  
54  
55 172 *Transepidermal water loss*  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

173 During all study visits, transepidermal water loss (TEWL) is assessed using a portable closed  
174 condenser-chamber device (Aquaflux model AF200, Biox Systems Ltd, UK).[18] TEWL is measured  
175 three times on the same skin area located on the central part of the volar forearm. No preference is  
176 given to the left or right arm but depends on how the baby is positioned.

#### 178 *Natural moisturizing factors*

179 Using a custom build device, the level of natural moisturizing factors (NMF) is measured on the  
180 thenar region using confocal Raman spectroscopy (RiverD International B.V., Rotterdam, The  
181 Netherlands).[19] Three values are recorded at all study visits, except the first study visit for the  
182 premature children. The thenar region of the child's hand is placed on the device for approximately  
183 60 seconds. Scattered light is sent towards the skin surface, exciting the molecules in the skin. Each  
184 molecule represents a specific spectrum of light, and the specific composition of molecules is thereby  
185 represented in the returned spectrum of light.[20] Again, the most accessible hand is measured, in  
186 turn depending on the child's posture at the time of examination.

#### 188 *Superficial stratum corneum (SC) sampling*

189 During all study visits, SC is collected by tape stripping. Eight consecutive tape stripping discs (22  
190 mm) D-squame, CuDerm, Dallas, Texas) are applied on the skin followed by standardized pressure  
191 applied by a D-squame pressure application pen for 5 seconds. Tapes are stored at -80° C immediately  
192 after sampling. Preterm infants have SC collected from the skin between the shoulder blades, and at  
193 two months of age from the cheek as well. Term infants have SC collected from cheek skin and the  
194 dorsal surface of the hand. No preference is given to the left or right sides but depends on the  
195 positioning of the child. If a child develops AD, SC is collected from the dorsal surface of the hand

1  
2  
3  
4 196 and from a lesional skin site, preferably from the cheek, otherwise from a skin site with the most  
5  
6 197 severe AD. SC will be examined for NMF, proteins, cytokines, lipids and morphology.  
7  
8

### 9 198 10 11 199 **Clinical skin assessment**

12  
13 200 A complete examination of the skin is performed at each study visit. Size, number and location of  
14  
15  
16 201 both congenital and acquired naevi are registered. The palm of the hand is photographed to assess  
17  
18 202 skin hyperlinearity at 2 months of age and in case the child develops AD.  
19

### 20 203 21 22 23 204 **Atopic dermatitis assessment**

24  
25 205 The skin is evaluated for signs of AD at each study visit. A diagnosis of AD is initially given by a  
26  
27 206 physician and is subsequently diagnosed clinically using to the diagnostic criteria of Hanifin and  
28  
29  
30 207 Rajka except for IgE-levels and subcapsular cataract.[21] AD severity is assessed using the Eczema  
31  
32 208 Area and Severity Index (EASI).[22] During all following visits, AD severity is assessed using EASI  
33  
34 209 and Patient Oriented Eczema Measure (POEM) tool[17] and treatment for AD is recorded. As  
35  
36 210 mentioned, during the structured telephone interviews, AD is diagnosed using The U.K. Working  
37  
38  
39 211 Party's Diagnostic Criteria for Atopic Dermatitis.[16]  
40

### 41 212 42 43 213 **Genetics**

44  
45 214 Buccal swabs (Isohelix, Harrietsham, U.K.) are used to collect DNA to screen for the most common  
46  
47  
48 215 *FLG* mutations in Northern European populations (R501X, 2282del4 and R2447X)[23] and for single  
49  
50 216 nucleotide polymorphisms. For both analyses, cheek mucosa is rubbed for 60 seconds with a swab  
51  
52  
53 217 and stored at -80° C until analysis.  
54

### 55 218 56 57 219 **Skin swaps** 58 59 60

1  
2  
3  
4 220 During all study visits, a bacterial swab is collected from the cheek skin (ESwab Collection and  
5  
6 221 Transport System Copan Italia, Brescia, Italy) and cultured for bacterial growth by routine  
7  
8  
9 222 methodology at the Department of Microbiology, Herlev and Gentofte Hospital, Denmark. Only  
10  
11 223 samples positive for  $\beta$ -Hemolytic Streptococci isolates (groups A, B, C, G) or *S. aureus* have  
12  
13 224 antimicrobial susceptibility testing performed and are subsequently stored at -80° C for future  
14  
15  
16 225 analyses. In preterm children, skin microbiome is collected from the lumbar area of the back at first  
17  
18 226 visit and from cheek and lumbar area at two months of age. Skin microbiome samples (Isohelix,  
19  
20 227 Harrietsham, U.K.) are collected from cheek and dorsal surface of the hand in term children. If a child  
21  
22  
23 228 develops AD, skin microbiome is also collected from a lesional skin site, preferably from the cheek  
24  
25 229 otherwise from the most severe AD lesion. All samples are immediately stored at -80° C until  
26  
27 230 analysis.

28  
29  
30 231

### 31 32 232 **Ultrasound**

33  
34 233 During all study visits, ultrasound examination is performed to visualize the thymus gland and  
35  
36 234 measure its size. The thymus index is defined as the multiplication of the two measurements and  
37  
38  
39 235 represents an estimate of the thymic volume.[24] The largest transverse diameter of the thymus is  
40  
41 236 measured in a horizontal scan plane and the area of the largest lobe is measured in a sagittal scan  
42  
43 237 plane. Both measurements are performed twice. The best measurement in both planes is selected.  
44  
45  
46 238 Measurements are performed with a transportable LOGIQ V2 ultrasound system with a 2-5.5 MHz  
47  
48 239 C4-RS transducer (GE Healthcare, Milwaukee, WI).

49  
50 240

### 51 52 241 **Study settings**

53  
54  
55 242 At each visit, air humidity, outdoor and indoor temperature is registered.

56  
57 24358  
59  
60

## 244 **Sample size estimation**

245 The sample size calculation was based on a Dutch study showing a decrease in the filaggrin  
246 breakdown product 2-pyrrolidone-5-carboxylic acid (PCA) as a biomarker for the *FLG* genotype.[25]  
247 The lowest value of PCA was found in homozygote and compound *FLG* heterozygote mutation  
248 carriers (mean  $\pm$  SEM  $0.18 \pm 0.04$  mmol g<sup>-1</sup>), increasing to  $0.50 \pm 0.07$  in heterozygote mutation  
249 carriers and  $1.64 \pm 0.11$  mmol g<sup>-1</sup> protein in wild type.[25] In our cohort, we hypothesized a 5%  
250 change of NMF in children developing AD compared to children without AD. With a 5% two-sided  
251 significance level and a power of 80%, we calculated a sample size of 112 premature children and  
252 223 in term children. Because of the high risk of loss-to-follow up during the two-year follow-up  
253 period, we estimated a sample size of 150 premature children and 300 term children would be needed.

## 255 **Data management**

256 Study data are collected and entered directly into an online REDCap (Research Electronic Data  
257 Capture) database hosted at the Capital Region of Denmark.

## 259 **Patient and public involvement**

260 Patients and the public were not involved in the design of the study. All participants will be  
261 acknowledged and thanked for their contribution in future publications.

## 263 **STRENGTHS AND LIMITATIONS**

264 The major strength of this birth cohort study is the extensive and repeated skin barrier measurements  
265 beginning right after birth. We will examine the skin barrier with multiple methodologies including  
266 Raman spectroscopy, TEWL and SC biomarkers. We will collect DNA and bacteria for genetic and  
267 skin microbiome analyses at several time points increasing the chance of finding a pathogenic role.



1  
2  
3  
4 268 We will include both preterm and term newborns allowing us to study the immature skin barrier and  
5  
6 269 thymus in a large subset of children. We will use internationally accepted definitions to diagnose AD  
7  
8  
9 270 and assess severity.[21, 22] Collectively, the BABY cohort will cover a wide range of parameters  
10  
11 271 with potential importance for the development of AD. Furthermore, we already now plan for future  
12  
13  
14 272 follow-up studies on skin barrier functions, AD and allergic diseases in this birth cohort.  
15

16 273  
17  
18 274 A potential limitation of the BABY Cohort is that all term children are recruited from Copenhagen  
19  
20 275 only, possibly limiting the generalizability of the study to more rural areas. While we will register  
21  
22  
23 276 ambient room conditions including air humidity and indoor and outside temperature, seasonal and  
24  
25 277 climatic variations will affect TEWL measurements. Children receiving incubator therapy have all  
26  
27 278 measurements made directly in the incubator and the ambient conditions are recorded. As the study  
28  
29  
30 279 is strictly non-invasive, we will not make any blood measurements, and can therefore not assess the  
31  
32 280 possible role of systemic inflammation. A concern in cohort studies is that participants may be lost  
33  
34 281 to follow up. This is especially a concern for the premature children with many potential  
35  
36  
37 282 comorbidities who are recruited from Rigshospitalet; a highly specialized department responsible for  
38  
39 283 treatment of all extremely premature children in eastern Denmark. To keep track of the included  
40  
41 284 families, we gather contact information of both parents and contact them prior to follow-up visits.  
42  
43 285 However, in case a family withdraws from the study, the date and reason for withdrawal will be  
44  
45  
46 286 recorded.  
47

## 48 287 49 50 288 **ETHICS AND DISSEMINATION**

51  
52 289 The study is approved by the local ethics committee (H-16042289 and H-16042294) and the local  
53  
54  
55 290 data protection agency (ID-no.: HGH-3017-040, I-suite no.:05578). The BABY Cohort is conducted  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

291 in accordance with the Declaration of Helsinki. All relevant study results will be presented in peer-  
292 reviewed publications and presented at national and international conferences.

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60296 **REFERENCES**

1. Abuabara K, Yu AM, Okhovat JP, et al. The prevalence of atopic dermatitis beyond childhood: A systematic review and meta-analysis of longitudinal studies. *Allergy*. 2018;73(3):696-704.
2. Weidinger S, Novak N. Atopic dermatitis. *The Lancet*. 2016;387(10023):1109-22.
3. Bieber T. Atopic dermatitis. *Ann Dermatol*. 2010;22(2):125-37.
4. Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. 2011;66(2):206-13.
5. Wadonda-Kabondo N, Sterne JA, Golding J, et al. Association of parental eczema, hayfever, and asthma with atopic dermatitis in infancy: birth cohort study. *Arch Dis Child*. 2004;89(10):917-21.
6. Bohme M, Wickman M, Lennart Nordvall S, et al. Family history and risk of atopic dermatitis in children up to 4 years. *Clin Exp Allergy*. 2003;33(9):1226-31.
7. Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *British Journal of Dermatology*. 2010;163(6):1333-6.
8. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-56.
9. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130(6):1344-54.
10. Mu Z, Zhao Y, Liu X, et al. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol*. 2014;47(2):193-218.
11. Bjerre RD, Bandier J, Skov L, et al. The role of the skin microbiome in atopic dermatitis: a systematic review. *Br J Dermatol*. 2017;177(5):1272-8.
12. Totte JE, van der Feltz WT, Hennekam M, et al. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol*. 2016;175(4):687-95.
13. Engebretsen KA, Bager P, Wohlfahrt J, et al. Prevalence of atopic dermatitis in infants by domestic water hardness and season of birth: Cohort study. *J Allergy Clin Immunol*. 2017;139(5):1568-74 e1.
14. Barbarot S, Gras-Leguen C, Colas H, et al. Lower risk of atopic dermatitis among infants born extremely preterm compared with higher gestational age. *Br J Dermatol*. 2013;169(6):1257-64.
15. Thyssen JP, Andersen YMF, Zhang H, et al. Incidence of pediatric atopic dermatitis following thymectomy: A Danish register study. *Allergy*. 2018;73(8):1741-3.
16. Williams HC, Jburney PG, Hay RJ, et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. *British Journal of Dermatology*. 1994;131(3):383-96.
17. Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and initial validation of a new tool for measuring atopic eczema severity from the patients' perspective. *Arch Dermatol*. 2004;140(12):1513-9.
18. Imhof B, Xiao P, Angelova-Fischer I. TEWL, Closed-Chamber Methods: AquaFlux and VapoMeter. *Non Invasive Diagnostic Techniques in Clinical Dermatology* 2014. p. 345-52.
19. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin Immunol*. 2010;126(3):574-80 e1.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

20. Caspers PJ, Lucassen GW, Carter EA, et al. In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol.* 2001;116(3):434-42.
21. Hanifin JMR, G. Diagnostic Features of Atopic Dermatitis. *Acta Dermatol Venereologica.* 1980;60:44-7.
22. Hanifin JM, Thurston M, Omoto M, et al. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *Experimental Dermatology.* 2001;10(1):11-8.
23. Meldgaard M, Szecsi PB, Carlsen BC, et al. A novel multiplex analysis of filaggrin polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta.* 2012;413(19-20):1488-92.
24. Hasselbalch H, Nielsen MB, Jeppesen D, et al. Sonographic measurement of the thymus in infants. *European Radiology.* 1996;6(5).
25. Kezic S, Kammeyer A, Calkoen F, et al. Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br J Dermatol.* 2009;161(5):1098-104.

peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Figure 1 - Scheduled investigations for preterm children in the BABY Cohort



Figure 2 - Scheduled investigations for term children in the BABY Cohort



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

# BMJ Open

## 'Barrier dysfunction in Atopic newBorns studY' (BABY): protocol of a Danish prospective birth cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-033801.R1
Article Type:	Protocol
Date Submitted by the Author:	26-Nov-2019
Complete List of Authors:	<p>Gerner, Trine; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Halling-Sønderby, Anne-Sofie; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Rasmussen Rinnov, Maria; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Haarup Ravn, Nina; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Hjorslev Knudgaard, Mette; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Menné Bonefeld, Charlotte; University of Copenhagen, Department of Immunology and Microbiology, Skin Immunology Research Center</p> <p>Ewertsen, Caroline; Rigshospitalet, University of Copenhagen, Department of Radiology</p> <p>Trautner, Simon; Rigshospitalet, University of Copenhagen, Department of Neonatology</p> <p>Jakaša, Ivone; University of Zagreb, Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology</p> <p>Kezic, Sanja; Amsterdam Public Health Research Institute, University of Amsterdam, Coronel Institute of Occupational Health, Amsterdam UMC</p> <p>Skov, Lone; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Thyssen, JP; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p>
<b>Primary Subject Heading</b>:	Dermatology
Secondary Subject Heading:	Paediatrics, Dermatology, Immunology (including allergy)
Keywords:	atopic dermatitis, birth cohort, Raman, skin barrier, preterm, TEWL



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1  
2  
3  
4 1 **‘Barrier dysfunction in Atopic newBorns studY’ (BABY): protocol of**  
5  
6  
7  
8 2 **a Danish prospective birth cohort study**  
9

10 3  
11  
12 4 Trine Gerner<sup>1,2</sup>, Anne-Sofie Halling-Sønderby<sup>1,2</sup>, Maria Rasmussen Rinnov<sup>1,2</sup>, Nina Haarup Ravn<sup>1,2</sup>,  
13 5 Mette Hjorslev Knudgaard<sup>1,2</sup>, Charlotte Menné Bonefeld<sup>3</sup>, Caroline Ewertsen<sup>4</sup>, Simon Trautner<sup>5</sup>,  
14 6 Ivone Jakaša<sup>6</sup>, Sanja Kezic<sup>7</sup>, Lone Skov<sup>1,2</sup>, Jacob P. Thyssen<sup>1,2</sup>.  
15  
16  
17  
18  
19  
20 7

21  
22 8 <sup>1</sup>Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen,  
23 9 Hellerup, Denmark

24  
25  
26 10 <sup>2</sup>Copenhagen Research Group for Inflammatory Skin (CORGIS), Hellerup, Denmark

27  
28  
29 11 <sup>3</sup>University of Copenhagen, Department of Immunology and Microbiology, LEO Foundation Skin  
30 12 Immunology Research Center, Maersk Tower, Copenhagen N, Denmark

31  
32  
33 13 <sup>4</sup>Department of Radiology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

34  
35  
36 14 <sup>5</sup>Department of Neonatology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

37  
38 15 <sup>6</sup>Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of  
39 16 Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

40  
41  
42 17 <sup>7</sup>Coronel Institute of Occupational Health, Amsterdam UMC, Amsterdam Public Health Research  
43 18 Institute, University of Amsterdam, Amsterdam, Netherlands  
44  
45  
46

47 19  
48  
49 20 **Correspondence:**

50  
51 21 Jacob P. Thyssen, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Gentofte  
52 22 Hospitalsvej 15, DK-2900 Hellerup, Denmark  
53

54  
55 23 Telephone: (0045) 3867 3150

56  
57 24 E-mail: jacob.p.thyssen@regionh.dk  
58  
59  
60

1  
2  
3  
4 25 **Word count:** 2470

5  
6 26 **Number of figures:** 2

7  
8  
9 27 **Number of references:** 29

10  
11 28

12  
13 29 **Key words**

14  
15  
16 30 Atopic dermatitis, birth cohort, preterm, Raman, skin barrier, TEWL.

17  
18 31

19  
20 32 **Conflicts of interest**

21  
22  
23 33 None declared

24  
25 34

26  
27 35 **Author contributions**

28  
29 36 TG, LS and JPT designed the study, created the study protocol, and obtained approval of the study

30  
31 37 design. ST, CBM, CE, IJ and SK contributed to revision and refinement of the study design. TG,

32  
33 38 AH, MRR, NHR, MHK and JPT were responsible for data collection. TG, AH, MRR, LS and JPT

34  
35 39 drafted the manuscript. All authors critically revised the manuscript. All authors supervised the

36  
37 40 study.

38  
39 41

40  
41 42 **Acknowledgements**

42  
43 43 We thank all families for their participation in the BABY cohort. We thank all staff members at

44  
45 44 Rigshospitalet and Nordsjællands Hospital who have contributed.

46  
47 45

48  
49 46

50  
51 47

52  
53 46

54  
55 47

56  
57 46

58  
59 47

60

1  
2  
3  
4 **48 Funding**

5  
6  
7 49 The study received financial support from The Leo Foundation, The Lundbeck Foundation, The

8  
9 50 Novo Nordisk Foundation, Pfizer, Aage Bangs Fond, Savværksejer Jeppe Juhl og hustru Ovita

10  
11 51 Juhls Mindelegat and The Herlev and Gentofte Hospital Research Foundation. The funders had no

12  
13 52 role in study design, data collection and analysis, decision to publish, or preparation of the

14  
15 53 manuscript.

16  
17  
18 54

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3  
4 55 **ABSTRACT**

5  
6 56 Introduction:

7  
8  
9 57 Skin barrier development and dysfunction in premature and mature newborns is important for the  
10  
11 58 risk of atopic dermatitis (AD).

12  
13 59 Methods and analysis:

14  
15 60 BABY Cohort is a prospective birth cohort study of 150 preterm children (gestational age (GA)  
16  
17 below 37+0) and 300 term children (GA 37+0 to 41+6). Skin barrier is assessed through  
18 61 transepidermal water loss, tape stripping, Raman-spectroscopy and microbiome sampling. Clinical  
19  
20 62 examinations are done and DNA from buccal swabs is collected for genetic analyses. Thymus size  
21  
22 63 is assessed by ultrasound examination. Information on pregnancy, delivery and parental exposures  
23  
24 64 and diseases are collected and structured telephone interviews are conducted at 18 and 24 months to  
25  
26 65 assess exogenous exposures in the child and onset of AD. Hanifin and Rajka criteria as well as The  
27  
28 66 U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis are used to diagnose AD. Severity  
29  
30 67 of AD is assessed using the Eczema Area and Severity Index (EASI) and Patient Oriented Eczema  
31  
32 68 Measure (POEM).

33  
34  
35  
36  
37 69 Ethics and dissemination:

38  
39 70 The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
40  
41 71 H-16042294).

42  
43 72 Outcomes will be presented at national and international conferences and in peer-reviewed  
44  
45 73 publications.

46  
47  
48 74  
49  
50 75

51  
52 76

53  
54 77

55  
56

57

58

59

60

## 78 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 79 • This is a Danish prospective birth cohort study assessing skin barrier functions and risk factors  
80 for atopic dermatitis.
- 81 • The study includes both preterm and term newborns from the general population.
- 82 • Repeated and comprehensive measurements of skin barrier will be performed at several time  
83 points.
- 84 • A limitation is the lack of blood measurements as this study is strictly non-invasive.

## 86 INTRODUCTION

87 Atopic dermatitis (AD) is a chronic and relapsing, inflammatory skin disease, characterized by dry  
88 and itchy skin that affects up to 20% of children in Northern Europe.[1] About 60-80% develop the  
89 disease in their first two years of life, and children with early onset are at increased risk of having  
90 severe and persistent disease.[2, 3] The risk of AD is increased in children of parents with atopic  
91 disorders such as AD, asthma and allergic rhinitis.[4-6]

92  
93 Genetic and environmental risk factors contribute to the development of AD through skin barrier  
94 dysfunction and immune dysregulation.[2] While loss-of-function mutations in the filaggrin gene  
95 (*FLG*) have been identified as the strongest genetic risk factor for AD,[7] genome wide association  
96 studies have only identified a relatively small proportion of the genetic risk effect.[8] The  
97 inflammation in AD is characterized by overexpression of Th2 cytokines, including IL-4 and IL-  
98 13.[9] that together with IL-1 may lead to increased secretion of thymic stromal lymphopoietin  
99 (TSLP), decreased epidermal antimicrobial peptides and filaggrin levels, in turn worsening skin  
100 inflammation and epidermal barrier functions.[10] Changes in the skin microbiome is also associated

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

101 with worsening of AD, showing reduced bacterial diversity[11] and increased colonization with  
102 *Staphylococcus aureus* (*S. aureus*).[12]

103  
104 While several environmental risk factors have been identified, e.g. winter birth and exposure to hard  
105 domestic water, this has not yet led to prophylactic solutions.[13] Interestingly, the risk of AD is  
106 decreased in premature newborns and infants undergoing heart surgery, which often includes partial  
107 or total thymectomy, perhaps due their reduced number of total lymphocytes and circulation T-cells  
108 resulting in an inappropriate immune response to antigens encountered in the skin.[14-16]

109  
110 There is a need for birth cohort studies that closely examine the skin of newborns at several time  
111 points to identify infants at risk of developing AD early in life. The BABY Cohort is a prospective  
112 birth cohort study that investigates early skin barrier development in preterm and term newborns to  
113 identify early prognostic skin barrier changes for development of AD.

## 114 115 **OBJECTIVES**

116 Primary objective:

117 To identify early predictors of AD during the first two years of life.

118  
119 Secondary objectives:

120 To closely describe the normal skin barrier development including immune activity and skin  
121 microbiome in preterm and term newborns during the first two years of life.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 125 **METHODS AND ANALYSIS**

### 126 **Study population and setting**

127 The BABY Cohort is an ongoing prospective and observational birth cohort study recruiting 150  
128 preterm and 300 term newborn infants. Recruitment began in August 2017. Parents of eligible  
129 children are recruited at the maternity and neonatal wards at Rigshospitalet, Copenhagen, and  
130 Nordsjællands Hospital, Hillerød, in Denmark. Children eligible for enrolment are preterm newborns  
131 (GA below 37+0) excluding preterm newborns with severe congenital abnormality and healthy term  
132 singleton newborns (GA 37+0 to 41+6) excluding mature newborns receiving antenatal steroids for  
133 fetal lung maturation. Children with parents unable to communicate in Danish are excluded. Children  
134 are included independently of their hereditary risk for AD

135

### 136 **Cohort design**

137 All study procedures are summarised in Figure 1 and 2, and each component of the visit is detailed  
138 below. Preterm children are scheduled for two study visits: during the first 31 days of life and  
139 approximately two months after their scheduled due date (Figure 1). Term children are scheduled for  
140 four study visits: during the first 3 days of life and approximately at 2, 6 and 12 months of age (Figure  
141 2). If a child develops AD during the first 2 years of life, an additional follow-up visit is performed.  
142 Overall, all children are recruited and examined as soon as possible after their delivery. Very  
143 immature born children often receive intensive medical care, and we wait until the child is stable until  
144 we perform the examinations. For all study visits the time of the study visit is registered, to be able  
145 to adjust for any effects that occur due to age differences. All parents participate in a structured  
146 telephone interview when the child is 18 and 24 months old. All study visits are conducted by trained  
147 medical doctors.

148

### **Baseline interview**

During the first study visit, parents are interviewed to obtain information about the pregnancy and birth, including the type of delivery and maternal intrapartum antibiotic treatment. Furthermore, information about gestational age at birth, weight, height and head circumference, 1- and 5- minutes APGAR scores and medical treatment at the neonatal ward is obtained.

### **Study interview**

At every study visit, we obtain detailed information about the child's health, vaccination status, method of feeding, admittance to hospital, medical treatments, bathing habits and skin care.

### **Parental questionnaires**

Parents complete an online questionnaire on family structure, residential situation, pet exposure, occupation, maternal exposures during pregnancy, smoking and drinking habits, history about current and previous skin diseases and atopic diseases in the family.

### **Telephone interviews**

At 18 and 24 months, parents participate in a structured telephone interview about the child's health, vaccination status, method of feeding, admittance to hospital, medical treatments, bathing habits, skin care, ultraviolet exposures and AD assessment according to the U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis, with parental assessment of visible flexural dermatitis in the elbows or knees. [17] If AD is diagnosed during the telephone interview an extra study visit in the clinic is scheduled.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

#### 173 **Anthropometric measures**

174 At the first visit, birth information on height, weight and head circumference is retrieved from the  
175 birth record. At all follow-up visits, anthropometric measurements are made. A digital weight scale  
176 is used to record weight in kg without clothing and diaper. Height and head circumference are  
177 measured in cm using a flexible non-elastic measuring tape.

#### 179 **Skin barrier measurements**

##### 180 *Transepidermal water loss*

181 During all study visits, transepidermal water loss (TEWL) is assessed using a portable closed  
182 condenser-chamber device (Aquaflux model AF200, Biox Systems Ltd, UK).[18] TEWL is measured  
183 three times on the same skin area located on the central part of the volar forearm. No preference is  
184 given to the left or right arm but depends on how the baby is positioned.

##### 186 *Natural moisturizing factors*

187 Using a custom build device, the level of natural moisturizing factors (NMF) is measured on the  
188 thenar region using confocal Raman spectroscopy (RiverD International B.V., Rotterdam, The  
189 Netherlands).[19-21] Three values are recorded at all study visits, except the first study visit for the  
190 premature children. The thenar region of the child's hand is placed on the device for approximately  
191 60 seconds. Scattered light is sent towards the skin surface, exciting the molecules in the skin. Each  
192 molecule represents a specific spectrum of light, and the specific composition of molecules is thereby  
193 represented in the returned spectrum of light.[20] Again, the most accessible hand is measured, in  
194 turn depending on the child's posture at the time of examination.

#### 197 *Superficial stratum corneum (SC) sampling*

198 During all study visits, SC is collected by tape stripping as previously described.[22, 23] Eight  
199 consecutive tape stripping discs (22 mm) D-squame, CuDerm, Dallas, Texas) are applied on the skin  
200 followed by standardized pressure applied by a D-squame pressure application pen for 5 seconds and  
201 gently removed with tweezers. Tapes are stored at -80° C immediately after sampling. Preterm infants  
202 have SC collected from the skin between the shoulder blades, and at two months of age from the  
203 cheek as well. Term infants have SC collected from cheek skin and the dorsal surface of the hand. No  
204 preference is given to the left or right sides but depends on the positioning of the child. If a child  
205 develops AD, SC is collected from the dorsal surface of the hand and from a lesional skin site,  
206 preferably from the cheek, otherwise from a skin site with the most severe AD. SC samples will be  
207 analyzed for biomarkers of the immune response by multiplex immuno-assays, NMF using a liquid  
208 chromatography previously described by Kezic et al. [22] and corneocyte surface morphology by  
209 atomic force microscopy. [24]

#### 211 **Clinical skin assessment**

212 A complete examination of the skin is performed at each study visit. Size, number and location of  
213 both congenital and acquired naevi are registered. The palm of the hand is photographed to assess  
214 skin hyperlinearity at 2 months of age and in case the child develops AD.

#### 216 **Atopic dermatitis assessment**

217 The skin is evaluated for signs of AD at each study visit. A diagnosis of AD is initially given by a  
218 physician and is subsequently diagnosed clinically using to the diagnostic criteria of Hanifin and  
219 Rajka except for IgE-levels and subcapsular cataract.[25] AD severity is assessed using the Eczema  
220 Area and Severity Index (EASI).[26] During all following visits, AD severity is assessed using EASI

1  
2  
3  
4 221 and Patient Oriented Eczema Measure (POEM) tool[27] and treatment for AD is recorded. As  
5  
6 222 mentioned, during the structured telephone interviews, AD is diagnosed using The U.K. Working  
7  
8  
9 223 Party's Diagnostic Criteria for Atopic Dermatitis.[17]  
10

11 224

### 13 225 **Genetics**

15  
16 226 Buccal swabs (Isohelix, Harrietsham, U.K.) are used to collect DNA to screen for the most common  
17  
18 227 *FLG* mutations in Northern European populations (R501X, 2282del4 and R2447X)[28] and for single  
19  
20 228 nucleotide polymorphisms. For both analyses, cheek mucosa is rubbed for 60 seconds with a swab  
21  
22  
23 229 and stored at -80° C until analysis.  
24

25 230

### 27 231 **Skin swabs**

29  
30 232 During all study visits, a bacterial swab is collected from the cheek skin (ESwab Collection and  
31  
32 233 Transport System Copan Italia, Brescia, Italy) and cultured for bacterial growth by routine  
33  
34 234 methodology at the Department of Microbiology, Herlev and Gentofte Hospital, Denmark. Only  
35  
36 235 samples positive for  $\beta$ -Hemolytic Streptococci isolates (groups A, B, C, G) or *S. aureus* have  
37  
38  
39 236 antimicrobial susceptibility testing performed and are subsequently stored at -80° C for future  
40  
41 237 analyses. In preterm children, skin microbiome is collected from the lumbar area of the back at first  
42  
43 238 visit and from cheek and lumbar area at two months of age. Skin microbiome samples (Isohelix,  
44  
45  
46 239 Harrietsham, U.K.) are collected from cheek and dorsal surface of the hand in term children. If a child  
47  
48 240 develops AD, skin microbiome is also collected from a lesional skin site, preferably from the cheek  
49  
50 241 otherwise from the most severe AD lesion. Skin swabs are rubbed on the skin for 60 seconds and are  
51  
52  
53 242 immediately stored at -80° C until analysis.  
54

55 243

57 244

59  
60

## 245 **Ultrasound**

246 During all study visits, ultrasound examination is performed to visualize the thymus gland and  
247 measure its size. The thymus index is defined as the multiplication of the two measurements and  
248 represents an estimate of the thymic volume.[29] The largest transverse diameter of the thymus is  
249 measured in a horizontal scan plane and the area of the largest lobe is measured in a sagittal scan  
250 plane. Both measurements are performed twice. The best measurement in both planes is selected.  
251 Measurements are performed with a transportable LOGIQ V2 ultrasound system with a 2-5.5 MHz  
252 C4-RS transducer (GE Healthcare, Milwaukee, WI).

## 254 **Study settings**

255 At each visit, air humidity, outdoor and indoor temperature is registered.

## 257 **Data management**

258 Study data are collected and entered directly into an online REDCap (Research Electronic Data  
259 Capture) database hosted at the Capital Region of Denmark.

## 261 **Patient and public involvement**

262 Patients and the public were not involved in the design of the study. All participants will be  
263 acknowledged and thanked for their contribution in future publications.

## 265 **STRENGTHS AND LIMITATIONS**

266 The major strength of this birth cohort study is the extensive and repeated skin barrier measurements  
267 beginning shortly after birth. We will examine the skin barrier with multiple methodologies including  
268 Raman spectroscopy, TEWL and SC biomarkers. We will collect DNA and bacteria for genetic and

1  
2  
3  
4 269 skin microbiome analyses at several time points increasing the chance of finding a pathogenic role.  
5  
6  
7 270 We will include both preterm and term newborns allowing us to study the immature skin barrier and  
8  
9 271 thymus in a large subset of children. We will use internationally accepted definitions to diagnose AD  
10  
11 272 and assess severity.[25, 26] Collectively, the BABY cohort will cover a wide range of parameters  
12  
13  
14 273 with potential importance for the development of AD. Furthermore, we already now plan for future  
15  
16 274 follow-up studies on skin barrier functions, AD and allergic diseases in this birth cohort.  
17

18 275  
19  
20 276 A potential limitation of the BABY Cohort is that all term children are recruited from Copenhagen  
21  
22  
23 277 only, possibly limiting the generalizability of the study to more rural areas. While we will register  
24  
25 278 ambient room conditions including air humidity and indoor and outside temperature, seasonal and  
26  
27 279 climatic variations will affect TEWL measurements. Children receiving incubator therapy have all  
28  
29  
30 280 measurements made directly in the incubator and the ambient conditions are recorded. As the study  
31  
32 281 is strictly non-invasive, we will not make any blood measurements, and can therefore not assess the  
33  
34 282 possible role of systemic inflammation. A concern in cohort studies is that participants may be lost  
35  
36  
37 283 to follow up. This is especially a concern for the premature children with many potential  
38  
39 284 comorbidities who are recruited from Rigshospitalet; a highly specialized department responsible for  
40  
41 285 treatment of all extremely premature children in eastern Denmark. To keep track of the included  
42  
43 286 families, we gather contact information of both parents and contact them prior to follow-up visits.  
44  
45  
46 287 However, in case a family withdraws from the study, the date and reason for withdrawal will be  
47  
48 288 recorded.  
49

50 289  
51  
52  
53 290  
54  
55 291  
56  
57 292  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

#### 293 **ETHICS AND DISSEMINATION**

294 The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
295 H-16042294) and the local data protection agency (ID-no.: HGH-3017-040, I-suite no.:05578). Both  
296 parents or guardians will give written informed consent prior to entry to the study.

297 The BABY Cohort is conducted in accordance with the Declaration of Helsinki. All relevant study  
298 results will be presented in peer-reviewed publications and presented at national and international  
299 conferences.

For peer review only



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60**REFERENCES**

1. Abuabara K, Yu AM, Okhovat JP, et al. The prevalence of atopic dermatitis beyond childhood: A systematic review and meta-analysis of longitudinal studies. *Allergy*. 2018;73(3):696-704.
2. Weidinger S, Novak N. Atopic dermatitis. *The Lancet*. 2016;387(10023):1109-22.
3. Bieber T. Atopic dermatitis. *Ann Dermatol*. 2010;22(2):125-37.
4. Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. 2011;66(2):206-13.
5. Wadonda-Kabondo N, Sterne JA, Golding J, et al. Association of parental eczema, hayfever, and asthma with atopic dermatitis in infancy: birth cohort study. *Arch Dis Child*. 2004;89(10):917-21.
6. Bohme M, Wickman M, Lennart Nordvall S, et al. Family history and risk of atopic dermatitis in children up to 4 years. *Clin Exp Allergy*. 2003;33(9):1226-31.
7. Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *British Journal of Dermatology*. 2010;163(6):1333-6.
8. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-56.
9. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130(6):1344-54.
10. Mu Z, Zhao Y, Liu X, et al. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol*. 2014;47(2):193-218.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 326 11. Bjerre RD, Bandier J, Skov L, et al. The role of the skin microbiome in atopic dermatitis: a  
327 systematic review. *Br J Dermatol*. 2017;177(5):1272-8.
- 328 12. Totte JE, van der Feltz WT, Hennekam M, et al. Prevalence and odds of *Staphylococcus aureus*  
329 carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol*.  
330 2016;175(4):687-95.
- 331 13. Engebretsen KA, Bager P, Wohlfahrt J, et al. Prevalence of atopic dermatitis in infants by  
332 domestic water hardness and season of birth: Cohort study. *J Allergy Clin Immunol*.  
333 2017;139(5):1568-74 e1.
- 334 14. Barbarot S, Gras-Leguen C, Colas H, et al. Lower risk of atopic dermatitis among infants born  
335 extremely preterm compared with higher gestational age. *Br J Dermatol*. 2013;169(6):1257-64.
- 336 15. Thyssen JP, Andersen YMF, Zhang H, et al. Incidence of pediatric atopic dermatitis following  
337 thymectomy: A Danish register study. *Allergy*. 2018;73(8):1741-3.
- 338 16. Eysteinsdottir JH, Freysdottir J, Haraldsson A, et al. The influence of partial or total  
339 thymectomy during open heart surgery in infants on the immune function later in life. *Clin Exp*  
340 *Immunol*. 2004;136(2):349-55.
- 341 17. Williams HC, Jburney PG, Hay RJ, et al. The U.K. Working Party's Diagnostic Criteria for  
342 Atopic Dermatitis. *British Journal of Dermatology*. 1994;131(3):383-96.
- 343 18. Imhof B, Xiao P, Angelova-Fischer I. TEWL, Closed-Chamber Methods: AquaFlux and  
344 VapoMeter. *Non Invasive Diagnostic Techniques in Clinical Dermatology*2014. p. 345-52.
- 345 19. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum  
346 define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin*  
347 *Immunol*. 2010;126(3):574-80 e1.

- 1  
2  
3  
4 348 20. Caspers PJ, Lucassen GW, Carter EA, et al. In vivo confocal Raman microspectroscopy of the  
5 skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol.*  
6 349 2001;116(3):434-42.  
7  
8  
9 350  
10  
11 351 21. Caspers PJ, Lucassen GW, Puppels GJ. Combined In Vivo Confocal Raman Spectroscopy and  
12 Confocal Microscopy of Human Skin. *Biophysical Journal.* 2003;85(1):572-80.  
13 352  
14  
15  
16 353 22. Kezic S, Kammeyer A, Calkoen F, et al. Natural moisturizing factor components in the stratum  
17 corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br J*  
18 354 *Dermatol.* 2009;161(5):1098-104.  
19 355  
20  
21 356 23. McAleer MA, Jakasa I, Raj N, et al. Early-life regional and temporal variation in filaggrin-  
22 derived natural moisturizing factor, filaggrin-processing enzyme activity, corneocyte  
23 357 phenotypes and plasmin activity: implications for atopic dermatitis. *Br J Dermatol.*  
24 358 2018;179(2):431-41.  
25 359  
26  
27 360 24. Riethmuller C, McAleer MA, Koppes SA, et al. Filaggrin breakdown products determine  
28 361 corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol.*  
29 362 2015;136(6):1573-80 e2.  
30  
31  
32 363 25. Hanifin JMR, G. Diagnostic Features of Atopic Dermatitis. *Acta Dermatol Venereologica.*  
33 364 1980;60:44-7.  
34  
35  
36 365 26. Hanifin JM, Thurston M, Omoto M, et al. The eczema area and severity index (EASI):  
37 366 assessment of reliability in atopic dermatitis. *Experimental Dermatology.* 2001;10(1):11-8.  
38  
39  
40  
41 367 27. Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and  
42 368 initial validation of a new tool for measuring atopic eczema severity from the patients'  
43 369 perspective. *Arch Dermatol.* 2004;140(12):1513-9.  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 370 28. Meldgaard M, Szecsi PB, Carlsen BC, et al. A novel multiplex analysis of filaggrin  
371 polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta*. 2012;413(19-  
372 20):1488-92.
- 373 29. Hasselbalch H, Nielsen MB, Jeppesen D, et al. Sonographic measurement of the thymus in  
374 infants. *European Radiology*. 1996;6(5).

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

377 Figure legend 1:

378 Scheduled investigations for preterm children in the BABY Cohort

379

380 Figure legend 2:

381 Scheduled investigations for term children in the BABY Cohort

382

For peer review only

Figure 1 - Scheduled investigations for preterm children in the BABY Cohort



Figure 2 - Scheduled investigations for term children in the BABY Cohort



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only



# BMJ Open

## 'Barrier dysfunction in Atopic newBorns studY' (BABY): protocol of a Danish prospective birth cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-033801.R2
Article Type:	Protocol
Date Submitted by the Author:	04-Feb-2020
Complete List of Authors:	<p>Gerner, Trine; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Halling-Sønderby, Anne-Sofie; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Rasmussen Rinnov, Maria; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Haarup Ravn, Nina; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Hjorslev Knudgaard, Mette; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Menné Bonefeld, Charlotte; University of Copenhagen, Department of Immunology and Microbiology, Skin Immunology Research Center</p> <p>Ewertsen, Caroline; Rigshospitalet, University of Copenhagen, Department of Radiology</p> <p>Trautner, Simon; Rigshospitalet, University of Copenhagen, Department of Neonatology</p> <p>Jakaša, Ivone; University of Zagreb, Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology</p> <p>Kezic, Sanja; Amsterdam Public Health Research Institute, University of Amsterdam, Coronel Institute of Occupational Health, Amsterdam UMC</p> <p>Skov, Lone; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Thyssen, JP; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p>
<b>Primary Subject Heading</b>:	Dermatology
Secondary Subject Heading:	Paediatrics, Dermatology, Immunology (including allergy)
Keywords:	atopic dermatitis, birth cohort, Raman, skin barrier, preterm, TEWL

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1  
2  
3  
4 1 **‘Barrier dysfunction in Atopic newBorns studY’ (BABY): protocol of**  
5  
6  
7  
8 2 **a Danish prospective birth cohort study**  
9

10 3  
11  
12 4 Trine Gerner<sup>1,2</sup>, Anne-Sofie Halling-Sønderby<sup>1,2</sup>, Maria Rasmussen Rinnov<sup>1,2</sup>, Nina Haarup Ravn<sup>1,2</sup>,  
13 5 Mette Hjorslev Knudgaard<sup>1,2</sup>, Charlotte Menné Bonefeld<sup>3</sup>, Caroline Ewertsen<sup>4</sup>, Simon Trautner<sup>5</sup>,  
14 6 Ivone Jakaša<sup>6</sup>, Sanja Kezic<sup>7</sup>, Lone Skov<sup>1,2</sup>, Jacob P. Thyssen<sup>1,2</sup>.  
15  
16  
17  
18  
19  
20 7

21  
22 8 <sup>1</sup>Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen,  
23 9 Hellerup, Denmark

24  
25  
26 10 <sup>2</sup>Copenhagen Research Group for Inflammatory Skin (CORGIS), Hellerup, Denmark

27  
28  
29 11 <sup>3</sup>University of Copenhagen, Department of Immunology and Microbiology, LEO Foundation Skin  
30 12 Immunology Research Center, Maersk Tower, Copenhagen N, Denmark

31  
32  
33 13 <sup>4</sup>Department of Radiology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

34  
35  
36 14 <sup>5</sup>Department of Neonatology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

37  
38 15 <sup>6</sup>Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of  
39 16 Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

40  
41  
42 17 <sup>7</sup>Coronel Institute of Occupational Health, Amsterdam UMC, Amsterdam Public Health Research  
43 18 Institute, University of Amsterdam, Amsterdam, Netherlands  
44  
45  
46

47 19  
48  
49 20 **Correspondence:**

50  
51 21 Jacob P. Thyssen, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Gentofte  
52 22 Hospitalsvej 15, DK-2900 Hellerup, Denmark  
53

54  
55 23 Telephone: (0045) 3867 3150

56  
57 24 E-mail: jacob.p.thyssen@regionh.dk  
58  
59  
60

1  
2  
3  
4 25 **Word count:** 2538

5  
6 26 **Number of figures:** 2

7  
8  
9 27 **Number of references:** 29

10  
11 28

12  
13 29 **Key words**

14  
15  
16 30 Atopic dermatitis, birth cohort, preterm, Raman, skin barrier, TEWL.

17  
18 31

19  
20 32 **Conflicts of interest**

21  
22  
23 33 JPT reports grants from The Leo Foundation, The Novo Nordisk Foundation, Pfizer, The Lundbeck

24  
25 34 Foundation and grants from Savværksejer Jeppe Juhl og hustru Ovita Juhls Mindelegat, during the

26  
27 35 conduct of the study. JPT has been an advisor, investigator and speaker for Abbvie, Regeneron,

28  
29 36 Pfizer, Sanofi-Genzyme, LEO Pharma, and Eli Lilly & Co.

30  
31 37 LS reports personal fees from Abbvie, Eli Lilly, Novatis, Sanofi, Celegen Leo pharma, and

32  
33 38 Almirall, outside the submitted work. LS reports non-financial support from Abbvie, Sanofi,

34  
35 39 Janssen and grants from Novatis, Janssen and Sanofi.

36  
37 40 TG, AH, MRR, NHR and MHK reports grants from Herlev and Gentofte Hospital Research

38  
39 41 Foundation, during the conduct of the study.

40  
41 42

42  
43 43 **Author contributions**

44  
45 44 TG, LS and JPT designed the study, created the study protocol, and obtained approval of the study

46  
47 45 design. ST, CBM, CE, IJ and SK contributed to revision and refinement of the study design. TG,

48  
49 46 AH, MRR, NHR, MHK and JPT were responsible for data collection. TG, AH, MRR, LS and JPT

50  
51 47 drafted the manuscript. All authors critically revised the manuscript. All authors supervised the

52  
53 48 study.

54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 49  
5  
6  
7 50  
8  
9 51  
10  
11 52  
12  
13  
14 53  
15  
16 54  
17  
18 55  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Acknowledgements**

We thank all families for their participation in the BABY cohort. We thank all staff members at Rigshospitalet and Nordsjællands Hospital who have contributed.

For peer review only

1  
2  
3  
4 **56 Funding**

5  
6 57 The study received financial support from The Leo Foundation, The Lundbeck Foundation, The  
7  
8  
9 58 Novo Nordisk Foundation, Pfizer, Aage Bangs Fond, Savværksejer Jeppe Juhl og hustru Ovita  
10  
11 59 Juhls Mindelegat and The Herlev and Gentofte Hospital Research Foundation. The funders had no  
12  
13 60 role in study design, data collection and analysis, decision to publish, or preparation of the  
14  
15 61 manuscript, as well as no role in future publications.  
16  
17  
18 62

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3  
4 **63 ABSTRACT**

5  
6  
7 **64 Introduction:**

8  
9 **65** Skin barrier development and dysfunction in premature and mature newborns is important for the  
10  
11 **66** risk of atopic dermatitis (AD).

12  
13  
14 **67 Methods and analysis:**

15  
16 **68** BABY Cohort is a prospective birth cohort study of 150 preterm children (gestational age (GA)  
17  
18 **69** below 37+0) and 300 term children (GA 37+0 to 41+6). Skin barrier is assessed through  
19  
20 **70** transepidermal water loss, tape stripping, Raman-spectroscopy and microbiome sampling. Clinical  
21  
22 **71** examinations are done and DNA from buccal swabs is collected for genetic analyses. Thymus size  
23  
24 **72** is assessed by ultrasound examination. Information on pregnancy, delivery and parental exposures  
25  
26 **73** and diseases are collected and structured telephone interviews are conducted at 18 and 24 months to  
27  
28 **74** assess exogenous exposures in the child and onset of AD. Hanifin and Rajka criteria as well as The  
29  
30 **75** U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis are used to diagnose AD. Severity  
31  
32 **76** of AD is assessed using the Eczema Area and Severity Index (EASI) and Patient Oriented Eczema  
33  
34 **77** Measure (POEM).

35  
36  
37  
38  
39 **78 Ethics and dissemination:**

40  
41 **79** The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
42  
43 **80** H-16042294).

44  
45  
46 **81** Outcomes will be presented at national and international conferences and in peer-reviewed  
47  
48 **82** publications.

49  
50 **83**

51  
52 **84**

53  
54  
55 **85**



## 86 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 87 • This is a Danish prospective birth cohort study assessing skin barrier functions and risk factors  
88 for atopic dermatitis.
- 89 • The study includes both preterm and term newborns from the general population.
- 90 • Repeated and comprehensive measurements of skin barrier will be performed at several time  
91 points.
- 92 • A limitation is the lack of blood measurements as this study is strictly non-invasive.

## 94 INTRODUCTION

95 Atopic dermatitis (AD) is a chronic and relapsing, inflammatory skin disease, characterized by dry  
96 and itchy skin that affects up to 20% of children in Northern Europe.[1] About 60-80% develop the  
97 disease in their first two years of life, and children with early onset are at increased risk of having  
98 severe and persistent disease.[2, 3] The risk of AD is increased in children of parents with atopic  
99 disorders such as AD, asthma and allergic rhinitis.[4-6]

100  
101 Genetic and environmental risk factors contribute to the development of AD through skin barrier  
102 dysfunction and immune dysregulation.[2] While loss-of-function mutations in the filaggrin gene  
103 (*FLG*) have been identified as the strongest genetic risk factor for AD,[7] genome wide association  
104 studies have only identified a relatively small proportion of the genetic risk effect.[8] The  
105 inflammation in AD is characterized by overexpression of Th2 cytokines, including IL-4 and IL-  
106 13.[9] that together with IL-1 may lead to increased secretion of thymic stromal lymphopietin  
107 (TSLP), decreased epidermal antimicrobial peptides and filaggrin levels, in turn worsening skin  
108 inflammation and epidermal barrier functions.[10] Changes in the skin microbiome is also associated

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

109 with worsening of AD, showing reduced bacterial diversity[11] and increased colonization with  
110 *Staphylococcus aureus* (*S. aureus*).[12]

111  
112 While several environmental risk factors have been identified, e.g. winter birth and exposure to hard  
113 domestic water, this has not yet led to prophylactic solutions.[13] Interestingly, the risk of AD is  
114 decreased in premature newborns and infants undergoing heart surgery, which often includes partial  
115 or total thymectomy, perhaps due their reduced number of total lymphocytes and circulation T-cells  
116 resulting in an inappropriate immune response to antigens encountered in the skin.[14-16]

117  
118 There is a need for birth cohort studies that closely examine the skin of newborns at several time  
119 points to identify infants at risk of developing AD early in life. The BABY Cohort is a prospective  
120 birth cohort study that investigates early skin barrier development in preterm and term newborns to  
121 identify early prognostic skin barrier changes for development of AD.

## 122 123 **OBJECTIVES**

124 Primary objective:

125 To identify early predictors of AD during the first two years of life.

126

127 Secondary objectives:

128 To closely describe the normal skin barrier development including immune activity and skin  
129 microbiome in preterm and term newborns during the first two years of life.

130

131

132

## **METHODS AND ANALYSIS**

### **Study population and setting**

The BABY Cohort is an ongoing prospective and observational birth cohort study recruiting 150 preterm and 300 term newborn infants. Recruitment began in August 2017. Parents of eligible children are recruited at the maternity and neonatal wards at Rigshospitalet, Copenhagen, and Nordsjællands Hospital, Hillerød, in Denmark. Children eligible for enrolment are preterm newborns (GA below 37+0) excluding preterm newborns with severe congenital abnormality or conditions affecting their life expectancy and fullterm healthy term singleton newborns (GA 37+0 to 41+6) excluding mature newborns receiving antenatal steroids for fetal lung maturation. Children with parents unable to communicate in Danish are excluded, since it is not possible to use (for practical and financial reasons) interpreters right after birth given that we have to be very flexible and recruit at odd hours. Children are included independently of their hereditary risk for AD

### **Cohort design**

All study procedures are summarised in Figure 1 and 2, and each component of the visit is detailed below. Preterm children are scheduled for two study visits: during the first 31 days of life and approximately two months after their scheduled due date (Figure 1). Term children are scheduled for four study visits: during the first 3 days of life and approximately at 2, 6 and 12 months of age (Figure 2). Many premature born children continue to have many hospital visits after their discharge, and many of the families lives far away from the hospital, i.e. other parts of Denmark. Therefore, preterm children are only scheduled to participate in one follow-up visit. We can therefore only make certain comparisons across the two groups.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

156 If a child develops AD during the first 2 years of life, an additional follow-up visit is performed.

157 Overall, all children are recruited and examined as soon as possible after their delivery. Very  
158 immature born children often receive intensive medical care, and we wait until the child is stable until  
159 we perform the examinations.

160  
161 For all study visits the time of the study visit is registered, to be able to adjust for any effects that  
162 occur due to age differences. All parents participate in a structured telephone interview when the child  
163 is 18 and 24 months old. All study visits are conducted by trained medical doctors.

#### 165 **Baseline interview**

166 During the first study visit, parents are interviewed to obtain information about the pregnancy and  
167 birth, including the type of delivery and maternal intrapartum antibiotic treatment. Furthermore,  
168 information about gestational age at birth, weight, height and head circumference, 1- and 5- minutes  
169 APGAR scores and medical treatment at the neonatal ward is obtained.

#### 171 **Study interview**

172 At every study visit, we obtain detailed information about the child's health, vaccination status,  
173 method of feeding, admittance to hospital, medical treatments, bathing habits and skin care.

#### 175 **Parental questionnaires**

176 Parents complete an online questionnaire on family structure, residential situation, pet exposure,  
177 occupation, maternal exposures during pregnancy, smoking and drinking habits, history about current  
178 and previous skin diseases and atopic diseases in the family.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 180 **Telephone interviews**

181 At 18 and 24 months, parents participate in a structured telephone interview about the child's health,  
182 vaccination status, method of feeding, admittance to hospital, medical treatments, bathing habits, skin  
183 care, ultraviolet exposures and AD assessment using a modification to the U.K. Working Party's  
184 Diagnostic Criteria for Atopic Dermatitis, with parental assessment of visible flexural dermatitis in  
185 the elbows or knees.[17] If AD is diagnosed during the telephone interview an extra study visit in the  
186 clinic is scheduled.

## 188 **Anthropometric measures**

189 At the first visit, birth information on height, weight and head circumference is retrieved from the  
190 birth record. At all follow-up visits, anthropometric measurements are made. A digital weight scale  
191 is used to record weight in kg without clothing and diaper. Height and head circumference are  
192 measured in cm using a flexible non-elastic measuring tape.

## 194 **Skin barrier measurements**

### 195 *Transepidermal water loss*

196 During all study visits, transepidermal water loss (TEWL) is assessed using a portable closed  
197 condenser-chamber device (Aquaflux model AF200, Biox Systems Ltd, UK).[18] TEWL is measured  
198 three times on the same skin area located on the central part of the volar forearm. No preference is  
199 given to the left or right arm but depends on how the baby is positioned.

### 201 *Natural moisturizing factors*

202 Using a custom build device, the level of natural moisturizing factors (NMF) is measured on the  
203 thenar region using confocal Raman spectroscopy (RiverD International B.V., Rotterdam, The

1  
2  
3  
4 204 Netherlands).[19-21] Three values are recorded at all study visits, except the first study visit for the  
5  
6 205 premature children. The thenar region of the child's hand is placed on the device for approximately  
7  
8  
9 206 60 seconds. Scattered light is sent towards the skin surface, exciting the molecules in the skin. Each  
10  
11 207 molecule represents a specific spectrum of light, and the specific composition of molecules is thereby  
12  
13 208 represented in the returned spectrum of light.[20] Again, the most accessible hand is measured, in  
14  
15  
16 209 turn depending on the child's posture at the time of examination.  
17

18 210

19  
20 211 *Superficial stratum corneum (SC) sampling*

21  
22  
23 212 During all study visits, SC is collected by tape stripping as previously described.[22, 23] Eight  
24  
25 213 consecutive tape stripping discs (22 mm) D-squame, CuDerm, Dallas, Texas) are applied on the skin  
26  
27 214 followed by standardized pressure applied by a D-squame pressure application pen for 5 seconds and  
28  
29  
30 215 gently removed with tweezers. Tapes are stored at -80° C immediately after sampling. Preterm infants  
31  
32 216 have SC collected from the skin between the shoulder blades, and at two months of age from the  
33  
34 217 cheek as well. Term infants have SC collected from cheek skin and the dorsal surface of the hand. No  
35  
36  
37 218 preference is given to the left or right sides but depends on the positioning of the child. If a child  
38  
39 219 develops AD, SC is collected from the dorsal surface of the hand and from a lesional skin site,  
40  
41 220 preferably from the cheek, otherwise from a skin site with the most severe AD. SC samples will be  
42  
43 221 analyzed for biomarkers of the immune response by multiplex immuno-assays, NMF using a liquid  
44  
45  
46 222 chromatography previously described by Kezic et al. [22] and corneocyte surface morphology by  
47  
48 223 atomic force microscopy. [24]  
49

50 224

51  
52  
53 225 **Clinical skin assessment**

54  
55 226 A complete examination of the skin is performed at each study visit to describe the normal skin barrier  
56  
57 227 development.  
58  
59  
60

1  
2  
3  
4 228 Size, number and location of both congenital and acquired naevi are registered. Studies and meta-  
5  
6 229 analysis have shown that the number of nevi is inverse with AD. However, we are not aware of  
7  
8  
9 230 prospective data collection. The palm of the hand is photographed to assess skin hyperlinearity at 2  
10  
11 231 months of age and in case the child develops AD.

### 12 13 14 232 15 16 233 **Atopic dermatitis assessment**

17  
18 234 The skin is evaluated for signs of AD at each study visit. A diagnosis of AD is initially given by a  
19  
20 235 physician and is subsequently diagnosed clinically using to the diagnostic criteria of Hanifin and  
21  
22 236 Rajka except for IgE-levels and subcapsular cataract.[25] AD severity is assessed using the Eczema  
23  
24 237 Area and Severity Index (EASI).[26] During all following visits, AD severity is assessed using EASI  
25  
26 238 and Patient Oriented Eczema Measure (POEM) tool[27] and treatment for AD is recorded. As  
27  
28 239 mentioned, during the structured telephone interviews, AD is diagnosed using The U.K. Working  
29  
30 240 Party's Diagnostic Criteria for Atopic Dermatitis.[17]

### 31 32 241 33 34 242 **Genetics**

35  
36 243 Buccal swabs (Isohelix, Harrietsham, U.K.) are used to collect DNA to screen for the most common  
37  
38 244 *FLG* mutations in Northern European populations (R501X, 2282del4 and R2447X)[28] by TaqMan  
39  
40 245 genotyping assay, a routine analysis in our Biochemical department, and for single nucleotide  
41  
42 246 polymorphisms. For both analyses, cheek mucosa is rubbed for 60 seconds with a swab and stored at  
43  
44 247 -80° C until analysis.

### 45 46 248 47 48 249 **Skin swabs**

49  
50 250 During all study visits, a bacterial swab is collected from the cheek skin (ESwab Collection and  
51  
52 251 Transport System Copan Italia, Brescia, Italy) and cultured for bacterial growth by routine

1  
2  
3  
4 252 methodology at the Department of Microbiology, Herlev and Gentofte Hospital, Denmark. Only  
5  
6 253 samples positive for  $\beta$ -Hemolytic Streptococci isolates (groups A, B, C, G) or *S. aureus* have  
7  
8  
9 254 antimicrobial susceptibility testing performed and are subsequently stored at  $-80^{\circ}$  C for future  
10  
11 255 analyses. In preterm children, skin microbiome is collected from the lumbar area of the back at first  
12  
13  
14 256 visit and from cheek and lumbar area at two months of age. Skin microbiome samples (Isohelix,  
15  
16 257 Harrietsham, U.K.) are collected from cheek and dorsal surface of the hand in term children. If a child  
17  
18 258 develops AD, skin microbiome is also collected from a lesional skin site, preferably from the cheek  
19  
20 259 otherwise from the most severe AD lesion. Skin swabs are rubbed on the skin for 60 seconds and are  
21  
22  
23 260 immediately stored at  $-80^{\circ}$  C until analysis.  
24

25 261

26

27 262

28

29

30 263

31

32 264

33

34 265

35

36 266

37

38

39 267

40

41 268

42

43 269

44

45

46 270

47

48 271

49

50 272

51

52

53 273

54

55 274

56

57

58

59

60

## 262 **Ultrasound**

263 During all study visits, ultrasound examination is performed to visualize the thymus gland and  
264 measure its size. The thymus index is defined as the multiplication of the two measurements and  
265 represents an estimate of the thymic volume.[29] The largest transverse diameter of the thymus is  
266 measured in a horizontal scan plane and the area of the largest lobe is measured in a sagittal scan  
267 plane. Both measurements are performed twice. The best images with a full visualization of the gland  
268 are selected by a trained radiologist. Measurements are performed with a transportable LOGIQ V2  
269 ultrasound system with a 2-5.5 MHz C4-RS transducer (GE Healthcare, Milwaukee, WI).

## 271 **Study settings**

272 At each visit, air humidity, outdoor and indoor temperature is registered.

## 274 **Sample size estimation**



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

275 The sample size calculation is based on including preterm and mature children in a 1:2 ratio. The  
276 power calculation was based on an expected prevalence of AD in 20 % of the cohort population.  
277 Based on previous knowledge, where adult controls have and NMF of 0.095 +/-0.029,[30] we  
278 hypothesized a 12% change in NMF in children developing AD compared with children without AD.  
279 With a 5% two-sided significance level and a power of 80%. AD, as we calculated at sample size of  
280 366 children. In order to account for possible drop-outs we decided on a study population of 450  
281 participants in total, whereas 150 were preterm and 300 mature children.

### 283 **Data management**

284 Study data are collected and entered directly into an online REDCap (Research Electronic Data  
285 Capture) database hosted at the Capital Region of Denmark.

### 287 **Patient and public involvement**

288 Patients and the public were not involved in the design of the study. All participants will be  
289 acknowledged and thanked for their contribution in future publications.

### 291 **STRENGTHS AND LIMITATIONS**

292 The major strength of this birth cohort study is the extensive and repeated skin barrier measurements.  
293 We will examine the skin barrier with multiple methodologies including Raman spectroscopy, TEWL  
294 and SC biomarkers. We will collect DNA and bacteria for genetic and skin microbiome analyses at  
295 several time points increasing the chance of finding a pathogenic role. We will include both preterm  
296 and term newborns allowing us to study the immature skin barrier and thymus in a large subset of  
297 children. We will use internationally accepted definitions to diagnose AD and assess severity.[25, 26]  
298 Collectively, the BABY cohort will cover a wide range of parameters with potential importance for

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

299 the development of AD. Since approximately 80% of AD patients develop their disease within the  
300 first two years of life, we expect to identify children with both transient and more established AD, as  
301 well as being able to differentiate between early features and predictors. Furthermore, we already  
302 now plan for future follow-up studies on skin barrier functions, AD and allergic diseases in this birth  
303 cohort.

304  
305 A potential limitation of the BABY Cohort is that all term children are recruited from Copenhagen  
306 only, possibly limiting the generalizability of the study to more rural areas. While we will register  
307 ambient room conditions including air humidity and indoor and outside temperature, seasonal and  
308 climatic variations will affect TEWL measurements. Since bathing habits prior to study visits are not  
309 standardized, but only registered, this might impact our skin barrier assessments. Children receiving  
310 incubator therapy have all measurements made directly in the incubator and the ambient conditions  
311 are recorded. As the study is strictly non-invasive, we will not make any blood measurements, and  
312 can therefore not assess the possible role of systemic inflammation. Due to our study design, we  
313 cannot discriminate clearly between early features and predictors. A concern in cohort studies is that  
314 participants may be lost to follow up. This is especially a concern for the premature children with  
315 many potential comorbidities who are recruited from Rigshospitalet; a highly specialized department  
316 responsible for treatment of all extremely premature children in eastern Denmark. To keep track of  
317 the included families, we gather contact information of both parents and contact them prior to follow-  
318 up visits. However, in case a family withdraws from the study, the date and reason for withdrawal  
319 will be recorded.

## ETHICS AND DISSEMINATION

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

322 The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
323 H-16042294) and the local data protection agency (ID-no.: HGH-3017-040, I-suite no.:05578). Both  
324 parents or guardians will give written informed consent prior to entry to the study.

325 The BABY Cohort is conducted in accordance with the Declaration of Helsinki. All relevant study  
326 results will be presented in peer-reviewed publications and presented at national and international  
327 conferences.

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60**REFERENCES**

1. Abuabara K, Yu AM, Okhovat JP, et al. The prevalence of atopic dermatitis beyond childhood: A systematic review and meta-analysis of longitudinal studies. *Allergy*. 2018;73(3):696-704.
2. Weidinger S, Novak N. Atopic dermatitis. *The Lancet*. 2016;387(10023):1109-22.
3. Bieber T. Atopic dermatitis. *Ann Dermatol*. 2010;22(2):125-37.
4. Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. 2011;66(2):206-13.
5. Wadonda-Kabondo N, Sterne JA, Golding J, et al. Association of parental eczema, hayfever, and asthma with atopic dermatitis in infancy: birth cohort study. *Arch Dis Child*. 2004;89(10):917-21.
6. Bohme M, Wickman M, Lennart Nordvall S, et al. Family history and risk of atopic dermatitis in children up to 4 years. *Clin Exp Allergy*. 2003;33(9):1226-31.
7. Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *British Journal of Dermatology*. 2010;163(6):1333-6.
8. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-56.
9. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130(6):1344-54.
10. Mu Z, Zhao Y, Liu X, et al. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol*. 2014;47(2):193-218.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 355 11. Bjerre RD, Bandier J, Skov L, et al. The role of the skin microbiome in atopic dermatitis:  
356 a systematic review. *Br J Dermatol.* 2017;177(5):1272-8.
- 357 12. Totte JE, van der Feltz WT, Hennekam M, et al. Prevalence and odds of *Staphylococcus*  
358 *aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol.*  
359 2016;175(4):687-95.
- 360 13. Engebretsen KA, Bager P, Wohlfahrt J, et al. Prevalence of atopic dermatitis in infants  
361 by domestic water hardness and season of birth: Cohort study. *J Allergy Clin Immunol.*  
362 2017;139(5):1568-74 e1.
- 363 14. Barbarot S, Gras-Leguen C, Colas H, et al. Lower risk of atopic dermatitis among  
364 infants born extremely preterm compared with higher gestational age. *Br J Dermatol.*  
365 2013;169(6):1257-64.
- 366 15. Thyssen JP, Andersen YMF, Zhang H, et al. Incidence of pediatric atopic dermatitis  
367 following thymectomy: A Danish register study. *Allergy.* 2018;73(8):1741-3.
- 368 16. Eysteinsdottir JH, Freysdottir J, Haraldsson A, et al. The influence of partial or total  
369 thymectomy during open heart surgery in infants on the immune function later in life. *Clin Exp*  
370 *Immunol.* 2004;136(2):349-55.
- 371 17. Williams HC, Jburney PG, Hay RJ, et al. The U.K. Working Party's Diagnostic Criteria  
372 for Atopic Dermatitis. *British Journal of Dermatology.* 1994;131(3):383-96.
- 373 18. Imhof B, Xiao P, Angelova-Fischer I. TEWL, Closed-Chamber Methods: AquaFlux  
374 and VapoMeter. *Non Invasive Diagnostic Techniques in Clinical Dermatology*2014. p. 345-52.
- 375 19. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum  
376 corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin*  
377 *Immunol.* 2010;126(3):574-80 e1.

- 1  
2  
3  
4 378 20. Caspers PJ, Lucassen GW, Carter EA, et al. In vivo confocal Raman microspectroscopy  
5  
6 379 of the skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol.*  
7  
8 380 2001;116(3):434-42.  
9  
10  
11 381 21. Caspers PJ, Lucassen GW, Puppels GJ. Combined In Vivo Confocal Raman  
12  
13 382 Spectroscopy and Confocal Microscopy of Human Skin. *Biophysical Journal.* 2003;85(1):572-80.  
14  
15  
16 383 22. Kezic S, Kammeyer A, Calkoen F, et al. Natural moisturizing factor components in the  
17  
18 384 stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br*  
19  
20 385 *J Dermatol.* 2009;161(5):1098-104.  
21  
22  
23 386 23. McAleer MA, Jakasa I, Raj N, et al. Early-life regional and temporal variation in  
24  
25 387 filaggrin-derived natural moisturizing factor, filaggrin-processing enzyme activity, corneocyte  
26  
27 388 phenotypes and plasmin activity: implications for atopic dermatitis. *Br J Dermatol.* 2018;179(2):431-  
28  
29 389 41.  
30  
31  
32 390 24. Riethmuller C, McAleer MA, Koppes SA, et al. Filaggrin breakdown products  
33  
34 391 determine corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol.*  
35  
36 392 2015;136(6):1573-80 e2.  
37  
38  
39 393 25. Hanifin JMR, G. Diagnostic Features of Atopic Dermatitis. *Acta Dermatol*  
40  
41 394 *Venereologica.* 1980;60:44-7.  
42  
43 395 26. Hanifin JM, Thurston M, Omoto M, et al. The eczema area and severity index (EASI):  
44  
45 396 assessment of reliability in atopic dermatitis. *Experimental Dermatology.* 2001;10(1):11-8.  
46  
47  
48 397 27. Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure:  
49  
50 398 development and initial validation of a new tool for measuring atopic eczema severity from the  
51  
52 399 patients' perspective. *Arch Dermatol.* 2004;140(12):1513-9.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 400 28. Meldgaard M, Szecsi PB, Carlsen BC, et al. A novel multiplex analysis of filaggrin  
401 polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta*. 2012;413(19-  
402 20):1488-92.
- 403 29. Hasselbalch H, Nielsen MB, Jeppesen D, et al. Sonographic measurement of the thymus  
404 in infants. *European Radiology*. 1996;6(5).
- 405 30. Simonsen S, Thyssen JP, Heegaard S, et al. Expression of Filaggrin and its Degradation  
406 Products in Human Skin Following Erythematous Doses of Ultraviolet B Irradiation. *Acta Derm  
407 Venereol*. 2017;97(7):797-801.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

409 Figure legend 1:

410 Scheduled investigations for preterm children in the BABY Cohort

411

412 Figure legend 2:

413 Scheduled investigations for term children in the BABY Cohort

414

For peer review only



Figure 1 - Scheduled investigations for preterm children in the BABY Cohort



Figure 2 - Scheduled investigations for term children in the BABY Cohort



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

# BMJ Open

## 'Barrier dysfunction in Atopic newBorns study' (BABY): protocol of a Danish prospective birth cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-033801.R3
Article Type:	Protocol
Date Submitted by the Author:	24-Apr-2020
Complete List of Authors:	<p>Gerner, Trine; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Halling-Sønderby, Anne-Sofie; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Rasmussen Rinnov, Maria; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Haarup Ravn, Nina; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Hjorslev Knudgaard, Mette; Herlev and Gentofte Hospital, University of Copenhagen, Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Menné Bonefeld, Charlotte; University of Copenhagen, Department of Immunology and Microbiology, Skin Immunology Research Center</p> <p>Ewertsen, Caroline; Rigshospitalet, University of Copenhagen, Department of Radiology</p> <p>Trautner, Simon; Rigshospitalet, University of Copenhagen, Department of Neonatology</p> <p>Jakaša, Ivone; University of Zagreb, Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology</p> <p>Kezic, Sanja; Amsterdam Public Health Research Institute, University of Amsterdam, Coronel Institute of Occupational Health, Amsterdam UMC</p> <p>Skov, Lone; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p> <p>Thyssen, JP; Herlev and Gentofte Hospital, University of Copenhagen , Department of Dermatology and Allergy; 2. Copenhagen Research Group for Inflammatory Skin (CORGIS)</p>
<b>Primary Subject Heading</b>:	Dermatology
Secondary Subject Heading:	Paediatrics, Dermatology, Immunology (including allergy)
Keywords:	atopic dermatitis, birth cohort, Raman, skin barrier, preterm, TEWL

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1  
2  
3  
4 1 **‘Barrier dysfunction in Atopic newBorns studY’ (BABY): protocol of**  
5  
6  
7  
8 2 **a Danish prospective birth cohort study**  
9

10 3  
11  
12 4 Trine Gerner<sup>1,2</sup>, Anne-Sofie Halling-Sønderby<sup>1,2</sup>, Maria Rasmussen Rinnov<sup>1,2</sup>, Nina Haarup Ravn<sup>1,2</sup>,  
13 5 Mette Hjorslev Knudgaard<sup>1,2</sup>, Charlotte Menné Bonefeld<sup>3</sup>, Caroline Ewertsen<sup>4</sup>, Simon Trautner<sup>5</sup>,  
14 6 Ivone Jakaša<sup>6</sup>, Sanja Kezic<sup>7</sup>, Lone Skov<sup>1,2</sup>, Jacob P. Thyssen<sup>1,2</sup>.  
15  
16  
17  
18  
19  
20 7

21  
22 8 <sup>1</sup>Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen,  
23 9 Hellerup, Denmark

24  
25  
26 10 <sup>2</sup>Copenhagen Research Group for Inflammatory Skin (CORGIS), Hellerup, Denmark

27  
28  
29 11 <sup>3</sup>University of Copenhagen, Department of Immunology and Microbiology, LEO Foundation Skin  
30 12 Immunology Research Center, Maersk Tower, Copenhagen N, Denmark

31  
32  
33 13 <sup>4</sup>Department of Radiology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

34  
35  
36 14 <sup>5</sup>Department of Neonatology, Rigshospitalet, University of Copenhagen, Copenhagen Ø, Denmark

37  
38 15 <sup>6</sup>Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of  
39 16 Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

40  
41  
42 17 <sup>7</sup>Coronel Institute of Occupational Health, Amsterdam UMC, Amsterdam Public Health Research  
43 18 Institute, University of Amsterdam, Amsterdam, Netherlands  
44  
45  
46

47 19  
48  
49 20 **Correspondence:**

50  
51 21 Jacob P. Thyssen, Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Gentofte  
52 22 Hospitalsvej 15, DK-2900 Hellerup, Denmark  
53

54  
55 23 Telephone: (0045) 3867 3150

56  
57 24 E-mail: jacob.p.thyssen@regionh.dk  
58  
59  
60

1  
2  
3  
4 25 **Word count:** 2607

5  
6 26 **Number of figures:** 2

7  
8  
9 27 **Number of references:** 30

10  
11 28

12  
13 29 **Key words**

14  
15  
16 30 Atopic dermatitis, birth cohort, preterm, Raman, skin barrier, TEWL.

17  
18 31

19  
20 32 **Conflicts of interest**

21  
22  
23 33 JPT reports grants from The Leo Foundation, The Novo Nordisk Foundation, Pfizer, The Lundbeck

24  
25 34 Foundation and grants from Savværksejer Jeppe Juhl og hustru Ovita Juhls Mindelegat, during the

26  
27 35 conduct of the study. JPT has been an advisor, investigator and speaker for Abbvie, Regeneron,

28  
29 36 Pfizer, Sanofi-Genzyme, LEO Pharma, and Eli Lilly & Co.

30  
31 37 LS reports personal fees from Abbvie, Eli Lilly, Novatis, Sanofi, Celegen Leo pharma, and

32  
33 38 Almirall, outside the submitted work. LS reports non-financial support from Abbvie, Sanofi,

34  
35 39 Janssen and grants from Novatis, Janssen and Sanofi.

36  
37 40 TG, AH, MRR, NHR and MHK reports grants from Herlev and Gentofte Hospital Research

38  
39 41 Foundation, during the conduct of the study.

40  
41  
42  
43 42

44  
45 43 **Author contributions**

46  
47 44 TG, LS and JPT designed the study, created the study protocol, and obtained approval of the study

48  
49 45 design. ST, CMB, CE, IJ and SK contributed to revision and refinement of the study design. TG,

50  
51 46 AH, MRR, NHR, MHK and JPT were responsible for data collection. TG, AH, MRR, LS and JPT

52  
53 47 drafted the manuscript. All authors critically revised the manuscript. All authors supervised the

54  
55 48 study.

56  
57  
58  
59  
60



1  
2  
3  
4 49  
5  
6  
7 50  
8  
9 51  
10  
11 52  
12  
13  
14 53  
15  
16 54  
17  
18 55  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Acknowledgements**

We thank all families for their participation in the BABY cohort. We thank all staff members at Rigshospitalet and Nordsjællands Hospital who have contributed.

For peer review only

1  
2  
3  
4 **56 Funding**

5  
6 57 The study received financial support from The Leo Foundation, The Lundbeck Foundation, The  
7  
8  
9 58 Novo Nordisk Foundation, Pfizer, Aage Bangs Fond, Savværksejer Jeppe Juhl og hustru Ovita  
10  
11 59 Juhls Mindelegat and The Herlev and Gentofte Hospital Research Foundation. The funders had no  
12  
13 60 role in study design, data collection and analysis, decision to publish, or preparation of the  
14  
15 61 manuscript, as well as no role in future publications.  
16  
17  
18 62

19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3  
4 63 **ABSTRACT**

5  
6 64 Introduction:

7  
8  
9 65 Skin barrier development and dysfunction in premature and mature newborns is important for the  
10  
11 66 risk of atopic dermatitis (AD).

12  
13 67 Methods and analysis:

14  
15 68 BABY Cohort is a prospective birth cohort study of 150 preterm children (gestational age (GA)  
16  
17 below 37+0) and 300 term children (GA 37+0 to 41+6). Skin barrier is assessed through  
18 69 transepidermal water loss, tape stripping, Raman-spectroscopy and microbiome sampling. Clinical  
19  
20 70 examinations are done and DNA from buccal swabs is collected for genetic analyses. Thymus size  
21  
22 71 is assessed by ultrasound examination. Information on pregnancy, delivery and parental exposures  
23  
24 72 and diseases are collected and structured telephone interviews are conducted at 18 and 24 months to  
25  
26 73 assess exogenous exposures in the child and onset of AD. Hanifin and Rajka criteria as well as The  
27  
28 74 U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis are used to diagnose AD. Severity  
29  
30 75 of AD is assessed using the Eczema Area and Severity Index (EASI) and Patient Oriented Eczema  
31  
32 76 Measure (POEM).  
33  
34  
35  
36  
37  
38

39 78 Ethics and dissemination:

40  
41 79 The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
42  
43 80 H-16042294).

44  
45 81 Outcomes will be presented at national and international conferences and in peer-reviewed  
46  
47 82 publications.  
48  
49

50 83

51 84

52 85

53

54

55

56

57

58

59

60

## 86 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 87 • This is a Danish prospective birth cohort study assessing skin barrier functions and risk factors  
88 for atopic dermatitis.
- 89 • The study includes both preterm and term newborns from the general population.
- 90 • Repeated and comprehensive measurements of skin barrier will be performed at several time  
91 points.
- 92 • A limitation is the lack of blood measurements as this study is strictly non-invasive.

## 94 INTRODUCTION

95 Atopic dermatitis (AD) is a chronic and relapsing, inflammatory skin disease, characterized by dry  
96 and itchy skin that affects up to 20% of children in Northern Europe.[1] About 60-80% develop the  
97 disease in their first two years of life, and children with early onset are at increased risk of having  
98 severe and persistent disease.[2, 3] The risk of AD is increased in children of parents with atopic  
99 disorders such as AD, asthma and allergic rhinitis.[4-6]

100  
101 Genetic and environmental risk factors contribute to the development of AD through skin barrier  
102 dysfunction and immune dysregulation.[2] While loss-of-function mutations in the filaggrin gene  
103 (*FLG*) have been identified as the strongest genetic risk factor for AD,[7] genome wide association  
104 studies have only identified a relatively small proportion of the genetic risk effect.[8] The  
105 inflammation in AD is characterized by overexpression of Th2 cytokines, including IL-4 and IL-  
106 13.[9] that together with IL-1 may lead to increased secretion of thymic stromal lymphopietin  
107 (TSLP), decreased epidermal antimicrobial peptides and filaggrin levels, in turn worsening skin  
108 inflammation and epidermal barrier functions.[10] Changes in the skin microbiome is also associated

1  
2  
3  
4 109 with worsening of AD, showing reduced bacterial diversity[11] and increased colonization with  
5  
6 110 *Staphylococcus aureus* (*S. aureus*).[12]  
7  
8

9 111  
10  
11 112 While several environmental risk factors have been identified, e.g. winter birth and exposure to hard  
12  
13 113 domestic water, this has not yet led to prophylactic solutions.[13] Interestingly, the risk of AD is  
14  
15  
16 114 decreased in premature newborns and infants undergoing heart surgery, which often includes partial  
17  
18 115 or total thymectomy, perhaps due their reduced number of total lymphocytes and circulation T-cells  
19  
20 116 resulting in an inappropriate immune response to antigens encountered in the skin.[14-16]  
21  
22

23 117  
24  
25 118 There is a need for birth cohort studies that closely examine the skin of newborns at several time  
26  
27 119 points to identify infants at risk of developing AD early in life. The BABY Cohort is a prospective  
28  
29  
30 120 birth cohort study that investigates early skin barrier development in preterm and term newborns to  
31  
32 121 identify early prognostic skin barrier changes for development of AD.  
33

34 122

## 36 123 **OBJECTIVES**

38  
39 124 Primary objective:

40  
41 125 To identify early predictors of AD during the first two years of life, including skin barrier dysfunction  
42  
43 126 and exogenous exposures during pregnancy and in infancy. The study will assess patient and parental  
44  
45  
46 127 characteristics, family history of atopic comorbidities, exposures during pregnancy and in infancy  
47  
48 128 and skin barrier function and development.  
49

50 129

52  
53 130 Secondary objectives:

54  
55 131 To closely describe the normal skin barrier development including immune activity and skin  
56  
57 132 microbiome in preterm and term newborns during the first two years of life.  
58  
59  
60

## METHODS AND ANALYSIS

### Study population and setting

The BABY Cohort is an ongoing prospective and observational birth cohort study recruiting 150 preterm and 300 term newborn infants. Recruitment began in August 2017. Parents of eligible children are recruited at the maternity and neonatal wards at Rigshospitalet, Copenhagen, and Nordsjællands Hospital, Hillerød, in Denmark. Children eligible for enrolment are preterm newborns (GA below 37+0) excluding preterm newborns with severe congenital abnormality or conditions affecting their life expectancy and fullterm healthy term singleton newborns (GA 37+0 to 41+6) excluding mature newborns receiving antenatal steroids for fetal lung maturation. Children with parents unable to communicate in Danish are excluded, since it is not possible to use (for practical and financial reasons) interpreters right after birth given that we have to be very flexible and recruit at odd hours. Children are included independently of their hereditary risk for AD

### Cohort design

All study procedures are summarised in Figure 1 and 2, and each component of the visit is detailed below. Preterm children are scheduled for two study visits: during the first 31 days of life and approximately two months after their scheduled due date (Figure 1). Term children are scheduled for four study visits: during the first 3 days of life and approximately at 2, 6 and 12 months of age (Figure 2). Many premature born children continue to have many hospital visits after their discharge, and many of the families lives far away from the hospital, i.e. other parts of Denmark. Therefore, preterm

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

156 children are only scheduled to participate in one follow-up visit. We can therefore only make certain  
157 comparisons across the two groups.

158  
159 If a child develops AD during the first 2 years of life, an additional follow-up visit is performed.  
160 Overall, all children are recruited and examined as soon as possible after their delivery. Very  
161 immature born children often receive intensive medical care, and we wait until the child is stable until  
162 we perform the examinations.

163  
164 For all study visits the time of the study visit is registered, to be able to adjust for any effects that  
165 occur due to age differences. All parents participate in a structured telephone interview when the child  
166 is 18 and 24 months old. All study visits are conducted by trained medical doctors.

### 167 168 **Baseline interview**

169 During the first study visit, parents are interviewed to obtain information about the pregnancy and  
170 birth, including the type of delivery and maternal intrapartum antibiotic treatment. Furthermore,  
171 information about gestational age at birth, weight, height and head circumference, 1- and 5- minutes  
172 APGAR scores and medical treatment at the neonatal ward is obtained.

### 173 174 **Study interview**

175 At every study visit, we obtain detailed information about the child's health, vaccination status,  
176 method of feeding, admittance to hospital, medical treatments, bathing habits and skin care.

### 177 178 **Parental questionnaires**

1  
2  
3  
4 179 Parents complete an online questionnaire on family structure, residential situation, pet exposure,  
5  
6 180 occupation, maternal exposures during pregnancy, smoking and drinking habits, history about current  
7  
8  
9 181 and previous skin diseases and atopic diseases in the family.  
10

### 11 182 12 13 183 **Telephone interviews**

14  
15  
16 184 At 18 and 24 months, parents participate in a structured telephone interview about the child's health,  
17  
18 185 vaccination status, method of feeding, admittance to hospital, medical treatments, bathing habits, skin  
19  
20 186 care, ultraviolet exposures and AD assessment using a modification to the U.K. Working Party's  
21  
22  
23 187 Diagnostic Criteria for Atopic Dermatitis, with parental assessment of visible flexural dermatitis in  
24  
25 188 the elbows or knees.[17] If AD is diagnosed during the telephone interview an extra study visit in the  
26  
27 189 clinic is scheduled.  
28

### 29 190 30 31 191 **Anthropometric measures**

32  
33  
34 192 At the first visit, birth information on height, weight and head circumference is retrieved from the  
35  
36 193 birth record. At all follow-up visits, anthropometric measurements are made. A digital weight scale  
37  
38  
39 194 is used to record weight in kg without clothing and diaper. Height and head circumference are  
40  
41 195 measured in cm using a flexible non-elastic measuring tape.  
42

### 43 196 44 45 197 **Skin barrier measurements**

#### 46 198 *Transepidermal water loss*

47  
48  
49  
50 199 During all study visits, transepidermal water loss (TEWL) is assessed using a portable closed  
51  
52 200 condenser-chamber device (Aquaflux model AF200, Biox Systems Ltd, UK).[18] TEWL is measured  
53  
54  
55 201 three times on the same skin area located on the central part of the volar forearm. No preference is  
56  
57 202 given to the left or right arm but depends on how the baby is positioned.  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

203

204 *Natural moisturizing factors*

205 Using a custom build device, the level of natural moisturizing factors (NMF) is measured on the  
206 thenar region using confocal Raman spectroscopy (RiverD International B.V., Rotterdam, The  
207 Netherlands).[19-21] Three values are recorded at all study visits, except the first study visit for the  
208 premature children. The thenar region of the child's hand is placed on the device for approximately  
209 60 seconds. Scattered light is sent towards the skin surface, exciting the molecules in the skin. Each  
210 molecule represents a specific spectrum of light, and the specific composition of molecules is thereby  
211 represented in the returned spectrum of light.[20] Again, the most accessible hand is measured, in  
212 turn depending on the child's posture at the time of examination.

213

214 *Superficial stratum corneum (SC) sampling*

215 During all study visits, SC is collected by tape stripping as previously described.[22, 23] Eight  
216 consecutive tape stripping discs (22 mm) D-squame, CuDerm, Dallas, Texas) are applied on the skin  
217 followed by standardized pressure applied by a D-squame pressure application pen for 5 seconds and  
218 gently removed with tweezers. Tapes are stored at -80° C immediately after sampling. Preterm infants  
219 have SC collected from the skin between the shoulder blades, and at two months of age from the  
220 cheek as well. Term infants have SC collected from cheek skin and the dorsal surface of the hand. No  
221 preference is given to the left or right sides but depends on the positioning of the child. If a child  
222 develops AD, SC is collected from the dorsal surface of the hand and from a lesional skin site,  
223 preferably from the cheek, otherwise from a skin site with the most severe AD. SC samples will be  
224 analyzed for biomarkers of the immune response by multiplex immuno-assays, NMF using a liquid  
225 chromatography previously described by Kezic et al. [22] and corneocyte surface morphology by  
226 atomic force microscopy. [24]

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

227

### **Clinical skin assessment**

A complete examination of the skin is performed at each study visit to describe the normal skin barrier development.

Size, number and location of both congenital and acquired naevi are registered. Studies and meta-analysis have shown that the number of nevi is inverse with AD. However, we are not aware of prospective data collection. The palm of the hand is photographed to assess skin hyperlinearity at 2 months of age and in case the child develops AD.

### **Atopic dermatitis assessment**

The skin is evaluated for signs of AD at each study visit. A diagnosis of AD is initially given by a physician and is subsequently diagnosed clinically using to the diagnostic criteria of Hanifin and Rajka except for IgE-levels and subcapsular cataract.[25] AD severity is assessed using the Eczema Area and Severity Index (EASI).[26] During all following visits, AD severity is assessed using EASI and Patient Oriented Eczema Measure (POEM) tool[27] and treatment for AD is recorded. As mentioned, during the structured telephone interviews, AD is diagnosed using The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis.[17]

### **Genetics**

Buccal swabs (Isohelix, Harrietsham, U.K.) are used to collect DNA to screen for the most common *FLG* mutations in Northern European populations (R501X, 2282del4 and R2447X)[28] by TaqMan genotyping assay, a routine analysis in our Biochemical department, and for single nucleotide polymorphisms. For both analyses, cheek mucosa is rubbed for 60 seconds with a swab and stored at -80° C until analysis.

1  
2  
3  
4 251  
5

6  
7 252 **Skin swabs**

8  
9 253 During all study visits, a bacterial swab is collected from the cheek skin (ESwab Collection and  
10  
11 254 Transport System Copan Italia, Brescia, Italy) and cultured for bacterial growth by routine  
12  
13 255 methodology at the Department of Microbiology, Herlev and Gentofte Hospital, Denmark. Only  
14  
15  
16 256 samples positive for  $\beta$ -Hemolytic Streptococci isolates (groups A, B, C, G) or *S. aureus* have  
17  
18 257 antimicrobial susceptibility testing performed and are subsequently stored at  $-80^{\circ}$  C for future  
19  
20 258 analyses. In preterm children, skin microbiome is collected from the lumbar area of the back at first  
21  
22  
23 259 visit and from cheek and lumbar area at two months of age. Skin microbiome samples (Isohelix,  
24  
25 260 Harrietsham, U.K.) are collected from cheek and dorsal surface of the hand in term children. If a child  
26  
27 261 develops AD, skin microbiome is also collected from a lesional skin site, preferably from the cheek  
28  
29  
30 262 otherwise from the most severe AD lesion. Skin swabs are rubbed on the skin for 60 seconds and are  
31  
32 263 immediately stored at  $-80^{\circ}$  C until analysis.  
33

34 264  
35

36  
37 265 **Ultrasound**

38  
39 266 During all study visits, ultrasound examination is performed to visualize the thymus gland and  
40  
41 267 measure its size. The thymus index is defined as the multiplication of the two measurements and  
42  
43 268 represents an estimate of the thymic volume.[29] The largest transverse diameter of the thymus is  
44  
45  
46 269 measured in a horizontal scan plane and the area of the largest lobe is measured in a sagittal scan  
47  
48 270 plane. Both measurements are performed twice. The best images with a full visualization of the gland  
49  
50 271 are selected by a trained radiologist. Measurements are performed with a transportable LOGIQ V2  
51  
52  
53 272 ultrasound system with a 2-5.5 MHz C4-RS transducer (GE Healthcare, Milwaukee, WI).  
54

55 273  
56

57 274 **Study settings**  
58  
59  
60

1  
2  
3  
4 275 At each visit, air humidity, outdoor and indoor temperature is registered.  
5  
6  
7 276

### 9 277 **Sample size estimation**

10  
11 278 The sample size calculation was based on including preterm and mature children in a 1:2 ratio. The  
12  
13 279 power calculation was based on an expected prevalence of AD in 20% of the cohort population,  
14  
15 280 assessing changes in NMF, which is one of multiple important endpoints in our study. Based on a  
16  
17 281 previous study, where adult controls had an NMF of 0.095 +/-0.029,[30] we hypothesized a 12%  
18  
19 282 change in NMF in newborns developing AD compared with children without developing AD. Using  
20  
21 283 a two-sided parametric test with an alpha of 5% and a power of 80%, we calculated at sample size of  
22  
23 284 366 children. In order to account for possible drop-outs, and the intention to study many other  
24  
25 285 predictors for AD and skin barrier function in general, we decided on a study population of 450  
26  
27 286 participants in total, i.e. 150 preterm and 300 mature children.  
28  
29  
30  
31  
32 287  
33

### 34 288 **Data management**

35  
36 289 Study data are collected and entered directly into an online REDCap (Research Electronic Data  
37  
38 290 Capture) database hosted at the Capital Region of Denmark.  
39  
40  
41 291  
42

### 43 292 **Patient and public involvement**

44  
45 293 Patients and the public were not involved in the design of the study. All participants will be  
46  
47 294 acknowledged and thanked for their contribution in future publications.  
48  
49  
50 295

### 52 296 **STRENGTHS AND LIMITATIONS**

53  
54  
55 297 The major strength of this birth cohort study is the extensive and repeated skin barrier measurements.  
56

57 298 We will examine the skin barrier with multiple methodologies including Raman spectroscopy, TEWL  
58  
59  
60

1  
2  
3  
4 299 and SC biomarkers. We will collect DNA and bacteria for genetic and skin microbiome analyses at  
5  
6 300 several time points increasing the chance of finding a pathogenic role. We will include both preterm  
7  
8  
9 301 and term newborns allowing us to study the immature skin barrier and thymus in a large subset of  
10  
11 302 children. We will use internationally accepted definitions to diagnose AD and assess severity.[25, 26]  
12  
13 303 Collectively, the BABY cohort will cover a wide range of parameters with potential importance for  
14  
15 304 the development of AD. Since approximately 80% of AD patients develop their disease within the  
16  
17  
18 305 first two years of life, we expect to identify children with both transient and more established AD, as  
19  
20 306 well as being able to differentiate between early features and predictors. Furthermore, we already  
21  
22  
23 307 now plan for future follow-up studies on skin barrier functions, AD and allergic diseases in this birth  
24  
25 308 cohort.

26  
27 309  
28  
29 310 A potential limitation of the BABY Cohort is that all term children are recruited from Copenhagen  
30  
31 311 only, possibly limiting the generalizability of the study to more rural areas. While we will register  
32  
33 312 ambient room conditions including air humidity and indoor and outside temperature, seasonal and  
34  
35 313 climatic variations will affect TEWL measurements. Since bathing habits prior to study visits are not  
36  
37 314 standardized, but only registered, this might impact our skin barrier assessments. Children receiving  
38  
39 315 incubator therapy have all measurements made directly in the incubator and the ambient conditions  
40  
41 316 are recorded. As the study is strictly non-invasive, we will not make any blood measurements, and  
42  
43 317 can therefore not assess the possible role of systemic inflammation. Due to our study design, we  
44  
45 318 cannot discriminate clearly between early features and predictors. A concern in cohort studies is that  
46  
47 319 participants may be lost to follow up. This is especially a concern for the premature children with  
48  
49 320 many potential comorbidities who are recruited from Rigshospitalet; a highly specialized department  
50  
51 321 responsible for treatment of all extremely premature children in eastern Denmark. To keep track of  
52  
53 322 the included families, we gather contact information of both parents and contact them prior to follow-  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 323 up visits. However, in case a family withdraws from the study, the date and reason for withdrawal  
5  
6 324 will be recorded.  
7  
8

9 325  
10  
11 326 **ETHICS AND DISSEMINATION**  
12

13 327 The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and  
14  
15 H-16042294) and the local data protection agency (ID-no.: HGH-3017-040, I-suite no.:05578). Both  
16 328  
17  
18 329 parents or guardians will give written informed consent prior to entry to the study.  
19

20 330 The BABY Cohort is conducted in accordance with the Declaration of Helsinki. All relevant study  
21  
22  
23 331 results will be presented in peer-reviewed publications and presented at national and international  
24  
25 332 conferences.  
26

27 333  
28  
29  
30 334  
31  
32 335  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60**REFERENCES**

1. Abuabara K, Yu AM, Okhovat JP, et al. The prevalence of atopic dermatitis beyond childhood: A systematic review and meta-analysis of longitudinal studies. *Allergy*. 2018;73(3):696-704.
2. Weidinger S, Novak N. Atopic dermatitis. *The Lancet*. 2016;387(10023):1109-22.
3. Bieber T. Atopic dermatitis. *Ann Dermatol*. 2010;22(2):125-37.
4. Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. 2011;66(2):206-13.
5. Wadonda-Kabondo N, Sterne JA, Golding J, et al. Association of parental eczema, hayfever, and asthma with atopic dermatitis in infancy: birth cohort study. *Arch Dis Child*. 2004;89(10):917-21.
6. Bohme M, Wickman M, Lennart Nordvall S, et al. Family history and risk of atopic dermatitis in children up to 4 years. *Clin Exp Allergy*. 2003;33(9):1226-31.
7. Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *British Journal of Dermatology*. 2010;163(6):1333-6.
8. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-56.
9. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130(6):1344-54.
10. Mu Z, Zhao Y, Liu X, et al. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol*. 2014;47(2):193-218.
11. Bjerre RD, Bandier J, Skov L, et al. The role of the skin microbiome in atopic dermatitis: a systematic review. *Br J Dermatol*. 2017;177(5):1272-8.
12. Totte JE, van der Feltz WT, Hennekam M, et al. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol*. 2016;175(4):687-95.
13. Engebretsen KA, Bager P, Wohlfahrt J, et al. Prevalence of atopic dermatitis in infants by domestic water hardness and season of birth: Cohort study. *J Allergy Clin Immunol*. 2017;139(5):1568-74 e1.
14. Barbarot S, Gras-Leguen C, Colas H, et al. Lower risk of atopic dermatitis among infants born extremely preterm compared with higher gestational age. *Br J Dermatol*. 2013;169(6):1257-64.
15. Thyssen JP, Andersen YMF, Zhang H, et al. Incidence of pediatric atopic dermatitis following thymectomy: A Danish register study. *Allergy*. 2018;73(8):1741-3.
16. Eysteinsdottir JH, Freysdottir J, Haraldsson A, et al. The influence of partial or total thymectomy during open heart surgery in infants on the immune function later in life. *Clin Exp Immunol*. 2004;136(2):349-55.
17. Williams HC, Jburney PG, Hay RJ, et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. *British Journal of Dermatology*. 1994;131(3):383-96.
18. Imhof B, Xiao P, Angelova-Fischer I. TEWL, Closed-Chamber Methods: AquaFlux and VapoMeter. *Non Invasive Diagnostic Techniques in Clinical Dermatology* 2014. p. 345-52.
19. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin Immunol*. 2010;126(3):574-80 e1.



- 1  
2  
3  
4 383 20. Caspers PJ, Lucassen GW, Carter EA, et al. In vivo confocal Raman microspectroscopy  
5 384 of the skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol.*  
6 385 2001;116(3):434-42.  
7 386 21. Caspers PJ, Lucassen GW, Puppels GJ. Combined In Vivo Confocal Raman  
8 387 Spectroscopy and Confocal Microscopy of Human Skin. *Biophysical Journal.* 2003;85(1):572-80.  
9 388 22. Kezic S, Kammeyer A, Calkoen F, et al. Natural moisturizing factor components in the  
10 389 stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br*  
11 390 *J Dermatol.* 2009;161(5):1098-104.  
12 391 23. McAleer MA, Jakasa I, Raj N, et al. Early-life regional and temporal variation in  
13 392 filaggrin-derived natural moisturizing factor, filaggrin-processing enzyme activity, corneocyte  
14 393 phenotypes and plasmin activity: implications for atopic dermatitis. *Br J Dermatol.* 2018;179(2):431-  
15 394 41.  
16 395 24. Riethmuller C, McAleer MA, Koppes SA, et al. Filaggrin breakdown products  
17 396 determine corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol.*  
18 397 2015;136(6):1573-80 e2.  
19 398 25. Hanifin JMR, G. Diagnostic Features of Atopic Dermatitis. *Acta Dermatol*  
20 399 *Venereologica.* 1980;60:44-7.  
21 400 26. Hanifin JM, Thurston M, Omoto M, et al. The eczema area and severity index (EASI):  
22 401 assessment of reliability in atopic dermatitis. *Experimental Dermatology.* 2001;10(1):11-8.  
23 402 27. Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure:  
24 403 development and initial validation of a new tool for measuring atopic eczema severity from the  
25 404 patients' perspective. *Arch Dermatol.* 2004;140(12):1513-9.  
26 405 28. Meldgaard M, Szecsi PB, Carlsen BC, et al. A novel multiplex analysis of filaggrin  
27 406 polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta.* 2012;413(19-  
28 407 20):1488-92.  
29 408 29. Hasselbalch H, Nielsen MB, Jeppesen D, et al. Sonographic measurement of the thymus  
30 409 in infants. *European Radiology.* 1996;6(5).  
31 410 30. Simonsen S, Thyssen JP, Heegaard S, et al. Expression of Filaggrin and its Degradation  
32 411 Products in Human Skin Following Erythematous Doses of Ultraviolet B Irradiation. *Acta Derm*  
33 412 *Venereol.* 2017;97(7):797-801.  
34 413  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

414 Figure legend 1:

415 Scheduled investigations for preterm children in the BABY Cohort

416

417 Figure legend 2:

418 Scheduled investigations for term children in the BABY Cohort

419

For peer review only

Figure 1 - Scheduled investigations for preterm children in the BABY Cohort



Figure 2 - Scheduled investigations for term children in the BABY Cohort



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only