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

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ABSTRACT

Introduction Skin barrier development and dysfunction in premature and mature newborns is important for the risk of atopic dermatitis (AD).

Methods and analysis The Barrier dysfunction in Atopic newBorns studY (BABY) Cohort is a prospective birth cohort study of 150 preterm children (gestational age (GA) below 37+0) and 300 term children (GA 37+0 to 41+6). Skin barrier is assessed through transepidermal water loss, tape stripping, Raman-spectroscopy and microbiome sampling. Clinical examinations are done and DNA from buccal swabs is collected for genetic analyses. Thymus size is assessed by ultrasound examination. Information on pregnancy, delivery, parental exposures and diseases are collected, and structured telephone interviews are conducted at 18 and 24 months to assess exogenous exposures in the child and onset of AD. Hanifin and Rajka criteria as well as The UK Working Party's Diagnostic Criteria for Atopic Dermatitis are used to diagnose AD. Severity of AD is assessed using the Eczema Area and Severity Index (EASI) and Patient Oriented Eczema Measure (POEM).

Ethics and dissemination The study is approved by the scientific Ethical Committee of the Capital Region (H-16042289 and H-16042294).

Outcomes will be presented at national and international conferences and in peer-reviewed publications.

Strengths and limitations of this study

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

INTRODUCTION

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

Genetic and environmental risk factors contribute to the development of AD through skin barrier dysfunction and immune dysregulation.[2] While loss-of-function mutations in the filaggrin gene (*FLG*) have been identified as the strongest genetic risk factor for AD,[7] genome-wide association studies have only identified a relatively small proportion of the genetic risk effect.[8] The inflammation in AD is characterised by overexpression of Th2 cytokines, including interleukin (IL)-4 and IL-13.[9] That, together with IL-1, may lead to increased secretion of thymic stromal lymphopoietin, decreased epidermal antimicrobial peptides and filaggrin levels, which, in turn, can worsen skin inflammation and epidermal barrier functions.[10] Changes in the skin microbiome is also associated with worsening of AD, showing reduced bacterial diversity[11] and increased colonisation with *Staphylococcus aureus*.[12]

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

There is a need for birth cohort studies that closely examine the skin of newborns at several time points to identify infants at risk of developing AD early in life. The Barrier dysfunction in Atopic newBorns studY (BABY) Cohort is a prospective birth cohort study that investigates early skin barrier development in preterm and term newborns to identify early prognostic skin barrier changes for the development of AD.

OBJECTIVES

Primary objective

To identify early predictors of AD during the first 2 years of life, including skin barrier dysfunction and exogenous exposures during pregnancy and in infancy. The study will assess patient and parental characteristics, family history of atopic comorbidities, exposures during pregnancy and in infancy, and skin barrier function and development.

Secondary objectives

To closely describe the normal skin barrier development, including immune activity and skin microbiome, in preterm and term newborns during the first 2 years of life.

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, Denmark. Children eligible for enrolment are preterm newborns (gestational age (GA) below 37+0), excluding preterm newborns with severe congenital abnormality or conditions affecting their life expectancy, and full-term healthy 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 independent of their hereditary risk for AD

Cohort design

All study procedures are summarised in figures 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 2 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 prematurely born children continue to have many hospital visits after their discharge, and many of the families live far away from the hospital, that is, 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.

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

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

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 GA at birth, weight, height and head circumference, 1 and 5 min 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 using a modification to the UK 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.

Anthropometric measures

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

Figure 1 Scheduled investigations for preterm children in the BABY Cohort. AD, atopic dermatitis; BABY, Barrier dysfunction in Atopic newBorns studY; mo, months; TEWL, transepidermal water loss.

Skin barrier measurements

Transepidermal water loss

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

Natural moisturising factors

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

Superficial stratum corneum sampling

During all study visits, stratum corneum (SC) is collected by tape stripping as previously described.[22 23] Eight consecutive tape stripping discs (22 mm) D-squame (CuDerm, Dallas, Texas, USA) are applied on the skin followed by standardised pressure applied by a D-squame

Figure 2 Scheduled investigations for term children in the BABY Cohort. AD, atopic dermatitis; BABY, Barrier dysfunction in Atopic newBorns studY; mo, months; TEWL, transepidermal water loss.

pressure application pen for 5 s and gently removed with tweezers. Tapes are stored at −80°C immediately after sampling. Preterm infants have SC collected from the skin between the shoulder blades, and at 2 months of age from the cheek as well. Term infants have SC collected from cheek skin and the dorsal surface of the hand. No preference is given to the left or right side but depends on the positioning of the child. If a child develops AD, SC is collected from the dorsal surface of the hand and from a lesional skin site, preferably from the cheek, otherwise from a skin site with the most severe AD. SC samples are analysed for biomarkers of the immune response by multiplex immuno-assays, NMF using a liquid chromatography previously described by Kezic *et al*[22] and corneocyte surface morphology by atomic force microscopy.[24]

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 naevi 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 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 UK Working Party's Diagnostic Criteria for Atopic Dermatitis.[17]

Genetics

Buccal swabs (Isohelix, Harrietsham, UK) 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, the cheek mucosa is rubbed for 60 s with a swab and stored at −80°C until analysis.

Skin swabs

During all study visits, a bacterial swab is collected from the cheek skin (ESwab Collection and Transport System, Copan Italia, Brescia, Italy) and cultured for bacterial growth by routine methodology at the Department of Microbiology, Herlev and Gentofte Hospital, Denmark. Only samples positive for β-haemolytic streptococci isolates (groups A, B, C and G) or *S. aureus* have antimicrobial susceptibility testing performed and are subsequently stored at −80°C for future analyses. In preterm children, skin microbiome is collected from the lumbar area of the back at first visit, and from cheek and lumbar area at 2 months of age. Skin microbiome samples (Isohelix, Harrietsham, UK) are collected from cheek and dorsal surface of the hand in term children. If a child develops AD, skin microbiome is also collected from a lesional skin site, preferably from the cheek, otherwise from the most severe AD lesion. Skin swabs are rubbed on the skin for 60 s and are immediately stored at −80°C until analysis.

Ultrasound

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

Study settings

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

Sample size estimation

The sample size calculation was based on including preterm and mature children in a 1:2 ratio. The power calculation was based on an expected prevalence of AD in 20% of the cohort population, assessing changes in NMF, which is one of multiple important endpoints in our study. Based on a previous study, where adult controls had an NMF of 0.095±0.029,[30] we hypothesised a 12% change in NMF in newborns developing AD compared with children without developing AD. Using a two-sided parametric test with an alpha of 5% and a power of 80%, we calculated at sample size of 366 children. In order to account for possible drop-outs, and the intention to study many other predictors for AD and skin barrier function in general, we decided on a study population of 450 participants in total, that is, 150 preterm and 300 mature children.

Data management

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

Patient and public involvement

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

STRENGTHS AND LIMITATIONS

The major strength of this birth cohort study is the extensive and repeated skin barrier measurements. We will examine the skin barrier with multiple methodologies, including Raman spectroscopy, TEWL and SC biomarkers. We will collect DNA and bacteria for genetic and skin microbiome analyses at several time points, increasing the chance of finding a pathogenic role. We will include both preterm and term newborns, allowing us to study the immature skin barrier and thymus in a large subset of children. We will use internationally accepted definitions to diagnose AD and assess severity.[25 26] Collectively, the BABY cohort will cover a wide range of parameters with potential importance for the development of AD. Since approximately 80% of AD patients develop their disease within the first 2 years of life, we expect to identify children with both transient and more established AD, as well as being able to differentiate between early features and predictors. Furthermore, we already now plan for future follow-up studies on skin barrier functions, AD and allergic diseases in this birth cohort.

A potential limitation of the BABY Cohort is that all term children are recruited from Copenhagen only, possibly limiting the generalisability of the study to more rural areas. While we will register ambient room conditions, including air humidity and indoor and outside temperature, seasonal and climatic variations, which affect TEWL measurements. Since bathing habits prior to study visits are not standardised, but only registered, this might impact our skin barrier assessments. Children receiving incubator therapy have all measurements made directly in the incubator and the ambient conditions are recorded. As the study is strictly non-invasive, we will not make any blood measurements, and can therefore not assess the possible role of systemic inflammation. Due to our study design, we cannot discriminate clearly between early features and predictors. A concern in cohort studies is that participants may be lost to follow-up. This is especially a concern for the premature children with many potential comorbidities who are recruited from Rigshospitalet, a highly specialised department responsible for treatment of all extremely premature children in eastern Denmark. To keep track of the included families, we

gather contact information of both parents and contact them prior to follow-up visits. However, in case a family withdraws from the study, the date and reason for withdrawal will be recorded.

ETHICS AND DISSEMINATION

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

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

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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REFERENCES

1 Abuabara K, Yu AM, Okhovat J-P, *et al*. The prevalence of atopic dermatitis beyond childhood: a systematic review and meta-analysis of longitudinal studies. *Allergy* 2018;73:696–704.
2 Weidinger S, Novak N. Atopic dermatitis. *The Lancet* 2016;387:1109–22.
3 Bieber T. Atopic dermatitis. *Ann Dermatol* 2010;22:125–37.
4 Apfelbacher CJ, Diepgen TL, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy* 2011;66:206–13.
5 Wadonda-Kabondo N, Sterne JAC, Golding J, *et al*. Association of parental eczema, hayfever, and asthma with atopic dermatitis in infancy: birth cohort study. *Arch Dis Child* 2004;89:917–21.
6 Böhme 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;33:1226–31.
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. *Br J Dermatol* 2010;163:1333–6.
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;47:1449–56.
9 Gittler JK, Shemer A, Suárez-Fariñas 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;130:1344–54.
10 Mu Z, Zhao Y, Liu X, *et al*. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol* 2014;47:193–218.
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;177:1272–8.
12 Totté JEE, 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;175:687–95.
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;139:1568–74.
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;169:1257–64.
15 Thyssen JP, Andersen YMF, Zhang H, *et al*. Incidence of pediatric atopic dermatitis following thymectomy: a Danish register study. *Allergy* 2018;73:1741–3.
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;136:349–55.
17 Williams HC, Jburney PG, Hay RJ, *et al*. The U.K. Working Party's diagnostic criteria for atopic dermatitis.. *Br J Dermatol* 1994;131:383–96.
18 Imhof B, Xiao P, Angelova-Fischer I. *TEWL, Closed-Chamber methods: AquaFlux and VapoMeter.Non invasive diagnostic techniques in clinical dermatology*, 2014: 345–52.
19 O'Regan GM, Kemperman PMJH, Sandilands A, *et al*. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin Immunol* 2010;126:574–80.
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;116:434–42.
21 Caspers PJ, Lucassen GW, Puppels GJ. Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin. *Biophys J* 2003;85:572–80.
22 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;161:1098–104.
23 McAleer MA, Jakasa I, Raj N, *et al*. Early-Life regional and temporal variation in filaggrin-derived natural moisturizing factor,

filaggrin-processing enzyme activity, corneocyte phenotypes and plasmin activity: implications for atopic dermatitis. *Br J Dermatol* 2018;179:431–41.
24 Riethmuller C, McAleer MA, Koppes SA, *et al*. Filaggrin breakdown products determine corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol* 2015;136:1573–80.
25 Hanifin JMR G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereologica* 1980;60:44–7.
26 Hanifin JM, Thurston M, Omoto M, *et al*. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI evaluator group. *Exp Dermatol* 2001;10:11–18.
27 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;140:1513–9.
28 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;413:1488–92.
29 Hasselbalch H, Nielsen MB, Jeppesen D, *et al*. Sonographic measurement of the thymus in infants. *Eur Radiol* 1996;6:700–3.
30 Simonsen S, Thyssen JP, Heegaard S, *et al*. Expression of filaggrin and its degradation products in human skin following Erythemal doses of ultraviolet B irradiation. *Acta Derm Venereol* 2017;97:797–801.