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II Lillemäe K*, Ress K*, Harro J, Merenäkk L, Maaroos H-I, Uibo R, Uibo O. A 10-year serological follow-up of coeliac disease in an Estonian population. Eur J Gastroenterol Hepatol 2012;24:55–8

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IV Ress K, Annus T, Putnik U, Luts K, Uibo O, Uibo R. Celiac disease in children with atopic dermatitis. Ped Dermatol 2014, doi: 10.1111/pde.12372

V Ress K, Metsküla K, Annus T, Putnik U, Lepik K, Luts K, Uibo O, Uibo R. Antinuclear antibodies in atopic dermatitis: a cross-sectional study on 346 children. Int J Dermatol 2014, doi: 10.1111/ijd.12535

VI Ress K, Teesalu K, Annus T, Putnik U, Lepik K, Luts K, Uibo O, Uibo R. Low prevalence of IgA anti-transglutaminase 1, 2, and 3 autoantibodies in children with atopic dermatitis (submitted)

Contributions by Krista Ress:

Papers I–II:	participation in experimental work, data analysis and writing the manuscript.
Paper III:	participation in data analysis and writing the manuscript.
Papers IV, VI:	participation in study design, data collection, experimental work, data analysis and writing the manuscript.
Paper V:	participation in study design, data collection, data analysis and writing the manuscript.

ABBREVIATIONS

AD	atopic dermatitis
ANA	antinuclear antibodies
anti-DGP	antibodies against deamidated form of gliadin peptides
anti-TG2	antibodies against tissue transglutaminase (TG2)
AU	arbitrary units
CD	coeliac disease
CI	confidential interval
DH	dermatitis herpetiformis
dsDNA	double stranded DNA
ELISA	enzyme-linked immunosorbent assay
EmA	antibodies against endomysium
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
HLA	human leukocyte antigen
IFN-γ	interferon-γ
IgA	immunoglobulin A
IgA-anti-DGP	IgA type antibodies to DGP
IgA-anti-TG1	IgA type antibodies to TG1
IgA-anti-TG2	IgA type antibodies to TG2
IgA-anti-TG3	IgA type antibodies to TG3
IgE	immunoglobulin E
IgG	immunoglobulin G
IgG-anti-DGP	IgG type anti-DGP
IgG-anti-TG2	IgG type anti-TG2
IgM	immunoglobulin M
IL	interleukin
IL-3	interleukin 3
IL-4	interleukin 4
IL-5	interleukin 5
IL-10	interleukin 10
IL-12	interleukin 12
IL-13	interleukin 13
IL-18	interleukin 18
IR	incidence rate

OR	odds ratio
PBS	phosphate-buffered saline
Tc	cytotoxic T-cell
TG1	transglutaminase 1, keratinocyte transglutaminase
TG2	transglutaminase 2, tissue transglutaminase
TG3	transglutaminase 3, epidermal transglutaminase
Th	helper T-cell
Th1	type 1 helper T-cell
Th2	type 2 helper T-cell
TNF-α	tumour necrosis factor α
Treg	regulatory T-cell

1. INTRODUCTION

Coeliac disease (CD) and atopic dermatitis (AD) are two of the most common immune mediated chronic diseases in childhood.

CD is an autoimmune disorder of the small intestine in which the ingestion of gluten and related cereals leads to chronic inflammation and damage of the small intestinal mucosa in genetically susceptible individuals (Fasano et al 2012; Kneepkens et al 2012). Traditionally CD has been considered a rare disease of childhood, manifesting during the first years of life as mainly gastrointestinal symptoms. On the basis of prevalence figures (0.1% or less) based mainly on clinical diagnosis, CD was previously regarded as rare in different populations and countries, including Estonia (Uibo 1994a; Mäki et al 2003; Myleus et al 2009; Mustalahti et al 2010). In recent years it has become evident, that CD occurs more frequently worldwide than previously thought and the clinical spectrum of the disease is highly variable. Nowadays, most of patients tend to present with atypical clinical symptoms and an increasing number of patients are diagnosed based on screening the risk groups (Kang et al 2013; Mustalahti et al 2010). CD has been associated with a list of diseases and conditions, type 1 diabetes being the most common comorbidity (Holmes 2001; Barker et al 2008; Volta et al 2011; Elli et al 2012). In case of comorbidities, CD often presents with atypical or asymptomatic clinical picture, leading to late or even missed diagnosis.

AD is a multifactorial chronic inflammatory skin disease, characterised by intense pruritus and relapsing eczema (Bieber 2008). Previously considered a disease purely mediated by type 2 helper T-cell (Th2) type responses, but the role of type 1 helper T-cell (Th1) responses and autoimmune reactions has since been revealed, especially in the chronic phase of the disease (Simpson et al 2002; Bieber 2008; Valenta et al 2009; Tang et al 2012). It has been proposed, that AD is the cutaneous manifestation of a systemic allergic disease, therefore being associated with other allergic diseases (Spergel et al 2003). As AD is often linked to food allergy, it suggests a potential causative role for changes at the small intestinal mucosal level in the pathogenesis of AD.

Both CD and AD have complex etiologies with genetic predisposition, environmental factors and immunoregulatory mechanisms playing an important role in their pathogenesis (Kupfer et al 2012; Meresse et al 2012; Leung 2013). So far, only a few studies have investigated the coexistence of CD and atopic disorders, especially AD. Although it has been suggested that these two diseases may coexist, it is still not known, whether the coexistence of CD and AD could be coincidental or whether there is a real association.

2. BACKGROUND TO THE STUDY

2.1. Coeliac disease (CD)

CD develops by the ingestion of wheat gluten and related prolamines in rye, barley, and possibly oats leading to chronic inflammation and autoimmune damage of the small intestinal mucosa in genetically susceptible individuals.

During the last decades, it has become evident that CD occurs more frequently worldwide than previously thought, showing a prevalence of up to 3% in Western populations (Mäki et al 2003; Myleus et al 2009; Kang et al 2013; Lundin et al 2014). There has been a nearly two times increase in the prevalence of CD over two decades in Finland and more than four times increase in the United States over the past 50 years (Lohi et al 2007; Rubio-Tapia et al 2009).

For a long time, Estonia has been a country with a low prevalence of CD – screening studies carried out in 1990 and 1998 on the general population (in total 1939 children and adults) showed absence of CD cases (Uibo et al 1993; Metsküla et al 1998). In 1990–1994 an active search and screening for CD with immunoglobulin A (IgA) type antigliadin antibodies was carried out, leading to an increase in the incidence of CD (0.37 cases per 1000 live births), but the prevalence still remained low compared to other countries (Uibo 1994a; Uibo 1994b; Uibo et al 1996).

Genetic predisposition plays an important role in the pathogenesis of CD. Approximately 95% of patients carry the human leukocyte antigen (HLA) DR3DQ2 (DQA1*05:01 and DQB1*02:01 alleles), the remainder having the HLA DR4DQ8 haplotype (DQA1*03 and DQB1*03:02 alleles) (Fasano et al 2012). The frequency of relevant HLA haplotypes in the Estonian background population are comparable to the ones of neighbouring countries, including Finland – HLA DR3DQ2 haplotype has been found in 22.3% of Estonian and in 20.2% of Finnish population and DQB1*03:02 allele has been found in 17.8% and 19.3% respectively (Nejentsev et al 1998). As up to 40% of general population has the HLA DQ2 haplotype, other genes must be involved in the pathogenesis on CD (Kagnoff 2005; Heel et al 2006). In addition to HLA that account about 40% of CD predisposition, non-HLA genes have been found to determine about 14% of genetics of CD (Lundin et al 2014).

It has been established that both innate and adaptive immune responses have a role to play in the pathogenesis of CD (Heel et al 2006; Meresse et al 2012; Qiao et al 2012). The cascade of pathophysiological processes starts with impairment of the barrier in small intestinal mucosa and presentation of the toxic peptides to HLA DQ2 and/or DQ8 molecules expressed on antigen presenting cells. By the activation of antigen-specific helper T-cells (Th) several proinflammatory cytokines are released, for example interferon-γ (IFN-γ), interleukins (IL) 4 (IL-4) and 10 (IL-10), tumour necrosis factor α (TNF-α). This is followed by proliferation of autoreactive B-cells and production of

immunoglobulin A, G or M (IgA, IgG, IgM) type antibodies against a deamidated form of gliadin peptides (anti-DGP), endomysium (EmA) and tissue transglutaminase (anti-TG2) by plasma cells. Tissue transglutaminase (TG2) has been recognised as the target autoantigen for CD, as it has a role in deamidating the gliadin peptides (Heel et al 2006; Klöck et al 2012). The most characteristic finding of the multiple immune reactions in the pathogenesis is the presence of IgA-type antibodies to TG2 (IgA-anti-TG2). The activation of cytotoxic T-cells (Tc) and production of new cytokines leads to destruction of mucosal cells, crypt hyperplasia and villous atrophy. As a result of villous atrophy and crypt hyperplasia the classical symptom of CD is the malabsorption syndrome (Lundin et al 2014).

CD was first described by Samuel Gee in 1888 as a disease affecting both children and adults and presenting with periodic diarrhoea, weight loss and abdominal distension (Gee 1888). Today it is known that the clinical spectrum of CD is highly variable. Patients with classical clinical picture present with diarrhoea and failure to thrive and/or weight loss. Still most cases may be left undiagnosed as the majority of patients present with atypical symptoms – different gastrointestinal symptoms without failure to thrive or weight loss, extraintestinal symptoms or are asymptomatic (indentified as a consequence of screening) (Catassi et al 2002; Tikkakoski et al 2007; Giongo et al 2011; Husby et al 2012). Based on new diagnostic guideline for CD, it has been proposed to group the clinical symptoms of CD into gastrointestinal and extraintestinal symptoms and signs (Husby et al 2012). CD is defined as a silent form in case of positivity in CD-specific antibodies, HLA and small intestinal biopsy finding compatible with CD, despite insufficient symptoms and signs for clinical suspicion of CD. Latent CD is defined by the presence of HLA in the absence of enteropathy in a patient earlier diagnosed with CD. Potential CD can be defined by the presence of CD-specific antibodies and HLA in the absence of histological changes at the mucosal level (Husby et al 2012).

CD can be suspected based on clinical picture and serological testing results – anti-TG2, EmA and anti-DGP. However, definitive diagnosis requires documenting the histopathological changes in the small intestinal biopsy (Marsh 1992; Husby et al 2012). The only available effective treatment for CD patients is lifelong and strict gluten-free diet (Walker-Smith et al 1990; Husby et al 2012). As a rule an adequate diet results in complete resolution of serological and histological changes and complete disappearance of symptoms. Early identification and adequate treatment enables prevention of several important complications associated with CD, above all growth failure, disturbances of reproductive function, dental structural defects, iron-deficiency anaemia, osteoporosis and malignant tumours (Collin et al 1994; Haapalahti et al 2005; Kagnoff 2005; Heel et al 2006; Viljamaa et al 2006).

2.2. Atopic dermatitis (AD)

AD is a multifactorial chronic inflammatory skin disease, characterised by dry skin, intense pruritus, relapsing eczema with typical distribution of the skin lesions. Although AD does not have pathognomonic skin lesions or laboratory features, erythematous papules associated with excoriations, vesiculations and serous exudate are prevalent in acute AD. Subacute and chronic AD can be characterised by erythematous, excoriated, scaling papules and lichenification (Adkinson et al 2009). AD usually presents during infancy and childhood, but it can persist or start in adulthood as well (Bieber 2008). During infancy children have primarily eczema on the face and extensor surfaces of the extremities, but in older patients the flexural folds are predominantly involved (Adkinson et al 2009). The lifetime prevalence of AD is 10–20% in Estonian children (Voor et al 2005).

AD has a complex etiology with genetic predisposition, congenital skin barrier defects, environmental factors, microbial colonisation and immuno-regulatory mechanisms playing an important role (Silverberg et al 2012; Eyerich et al 2013; Leung 2013). A combination of genetic and acquired factors contributes to reduced epidermal differentiation and down-regulation of epidermal barrier function (Cork et al 2009; Kubo et al 2012; Wolf et al 2012). Following breakdown of the epidermal barrier both innate and adaptive immune mechanisms play a role. In the acute phase of the disease, after the allergen uptake and processing by Langerhans' cells, they are presented to naive Th cells, leading to induction of Th2 differentiation and cytokine release (IL-3, IL-4, IL-5, IL-13), allergen-specific immunoglobulin E (IgE) production by plasma cells and activation of eosinophils. The adaptive immune response contributes also to reduced epidermal differentiation and to reduced anti-microbial peptide expression (Cork et al 2009; Kubo et al 2012; Leung 2013). AD was previously considered a purely Th2 and IgE mediated inflammatory disease, but recent investigations have revealed an important role of Th1 type responses in the pathogenesis of the disease (Bieber 2008; Eyerich et al 2013). Mechanical trauma, microbial toxins, and secretion of proinflammatory cytokines (IL-12, IL-18) by inflammatory dendritic epidermal cells and eosinophils contribute to the switch from Th2 to Th1 and thereby lead to the chronic phase of disease (Bieber 2008; Leung 20s13).

It has been suggested that AD is the cutaneous manifestation of a systemic allergic disease that also gives rise to asthma, food allergy and allergic rhinitis (Spergel et al 2003; Dharmage et al 2014). Patients with AD and food allergy have been reported to have increased intestinal permeability, indicating a possible causative role for intestinal permeability changes in AD pathogenesis (Verkasalo et al 1983; Husby 1988; Räsänen et al 1994; Leung et al 2003). Increased permeability may be the primary abnormality of the gut or reflect intestinal mucosal damage caused by local immunological reactions to antigens (Pike et al 1986). Recently, Järvinen et al investigated a large cohort of children

with food allergy on a specific elimination diet and suggested increased permeability to be the intrinsic trait in a subset of patients (Järvinen et al 2013). Although in CD patients, the histological changes in small intestinal mucosa improve within 9–12 months after starting gluten-free diet (Selby et al 1999), repeated evaluation of food allergy patients showed stable intestinal permeability (Järvinen et al 2013). Therefore increased intestinal permeability could play important role in pathogenesis of food allergy as well as in AD.

The clinical phenotype of AD has been classified into extrinsic and intrinsic types, the latter comprising 10–45% of AD patients (Novak et al 2003; Tokura 2010). The intrinsic type reflects the non-allergic form of AD with normal IgE levels and absence of any detectable allergen sensitisation. Almost 2/3 of children that present with the clinical phenotype of AD have no identifiable allergen-specific sensitisation. On the other hand, in patients with extrinsic or allergic type of AD the serum IgE levels are increased together with sensitisation toward environmental allergens (Schmid-Grendelmeier et al 2001). Based on earlier studies sensitisation to food allergens frequently occurs in children with AD (10–50%), but food as a trigger of AD has long been a subject of debate (Ellman et al 2002; Fuiano et al 2012; Moghtaderi et al 2012).

2.3. Coexistence of CD and AD

During the last decades, the pathomechanism of AD has consistently been under investigation and the possible role for autoimmune mechanisms has been discussed (Valenta et al 1999; Rabin et al 2008; Tang et al 2012; Leung 2013). Various immune-mediated and non-immune mediated diseases may be associated with AD, including autoimmune diseases like CD (Simpson et al 2002; Rabin et al 2008; Silverberg et al 2012).

CD has been associated with an expanding list of diseases and conditions, autoimmune and non-autoimmune, and in most of these cases CD remains undiagnosed because of atypical or asymptomatic presentation (Catassi et al 2002; Green et al 2007). Some of the concomitant conditions are the manifestations of untreated malabsorption. However, in most cases, it is still a matter of discussion whether there is a pathogenetic association. Type 1 diabetes, autoimmune liver disease, autoimmune thyroid disease, Addison's disease, some chromosomal aberration disorders (Turner, Down and Williams syndromes), connective tissue disorders, IgA deficiency, IgA nephropathy and atopy are the most often reported concomitant disorders (Bottaro et al 1999; Husby et al 2012; Denham et al 2013).

Table 1. Overview of relevant studies on AD/atopy and CD published in PUBMED until January 2014*.

Reference	Country	Study subjects, n	No of cases found, (%)
Atopy in CD patients			
Bottaro et al, 1999	Italy	children and adults with CD, n=1026	8 (0.8%) with atopy
de Freitas et al, 2002	Brazil	adults with CD, n=48	3 (6%) with atopy
Kotze, 2009	Brazil	children and adults with CD, n=157	35 (22.3%) with atopy
AD in CD patients			
Cooper et al, 1978	UK	adults with CD, n=314	17 (5.4%) with AD
Verkasalo et al, 1983	Finland	children with CD, n=42	19 (45.2%) with AD
Williams, 1987	UK	adults with CD, n=76	5 (7%) with AD
Greco et al, 1990	Italy	children with CD, n=82	11 (13%) with AD
Ciacci et al, 2004	Italy	adults with CD, n=1044	40 (3.8%) with AD
Elli et al, 2012	Italy	children and adults with CD, n=1015	15 (1.5%) with AD
CD in atopy patients			
Zauli et al, 2000	Italy	children and adults with atopy, n=401	4 (1.0%) with CD
CD in AD patients			
Gallo et al, 1992	Italy	patients with AD, n=13	3 (23%) with CD
Uibo et al, 1996	Estonia	children with AD, n=105	1 (0.9%) with CD

* *Based on an electronic search conducted in PUBMED (US National Library of Medicine) for all publications reporting on atopic dermatitis and coeliac disease. Using keywords "atopic dermatitis", "atopic eczema", "atopy", "celiac disease", "coeliac disease" and "gluten sensitive enteropathy" the search resulted in 73 publications. Selection was made based on screening titles and abstracts.*

Only a few earlier reports refer to the possibility that CD and AD could coexist frequently (Table 1)(Cooper et al 1978; Verkasalo et al 1983; Williams 1987; Greco et al 1990; Gallo et al 1992; Uibo et al 1996; Bottaro et al 1999; Zauli et al 2000; Ciacci et al 2004; Kotze 2009; Elli et al 2012). In several reports patients with CD were observed more atopic than the general population. Verkasalo et al showed the 45% prevalence of AD among Finnish school-children with CD, compared to 25% prevalence of AD in the general population (Verkasalo et al 1983). Italian authors have shown, in a large group of CD patients that atopy is the most frequently associated disease after type 1 diabetes and that patients with CD have a 3-fold risk to have AD compared to the general population (Zauli et al 2000; Ciacci et al 2004).

On the other hand, CD has also been found more frequently in atopic patients than in the general population. Zauli et al have found 1% prevalence of CD in patients with different atopic diseases compared to 0.2% prevalence in the general population (Zauli et al 2000). In an earlier study, Uibo et al has found CD in 0.9% of Estonian children with AD, using antigliadin antibodies for screening (Uibo et al 1996). It is still not clear, whether the coexistence of CD and AD could be coincidental or whether there is a real association.

2.4. Antinuclear antibodies in AD

During the last decades, the possible role of autoimmunity in AD pathogenesis has been discussed. AD has a pattern of relapsing-remitting disease, similar to other chronic autoimmune diseases (Tokura 2010; Tang et al 2012). In general, autoimmune reactions have been reported in patients with AD more frequently than in the general population (Ohkouchi et al 1999; Higashi et al 2009; Tang et al 2012). Moreover, it has been shown that in chronic and severe forms of AD, high percentage of patients display IgE autoreactivity to a broad spectrum of human proteins, which are expressed in a variety of cell and tissue types (Valenta et al 2009). During exacerbation of skin manifestations, IgE auto-antibody levels have been shown to be increased (Mittermann et al 2004).

Antinuclear antibodies (ANA) are the most commonly detected auto-antibodies in various patient groups (Breda et al 2010). Several laboratories have reported the presence of ANA in 19–41% of patients with AD, which is higher than in the general population (Ohkouchi et al 1999; Higashi et al 2009; Tang et al 2012). Some studies have shown association with a presence of ANA in higher titers and especially in patients with severe facial lesions (Valenta et al 1999; Muro 2001; Higashi et al 2009). However, more recent studies have not confirmed the relationship between ANA positivity and facial lesions (Tang et al 2012).

The role of autoimmune reactions in the development of AD is still unclear. Although, higher prevalence of autoimmune reactions might suggest a subtype of AD.

2.5. Transglutaminases as antibody targets

Transglutaminases are a family of calcium-dependent enzymes that have an important role in various biological processes, including cell structure organisation and apoptosis. As mentioned earlier, TG2 has been recognised as the target autoantigen for CD (Dieterich et al 1997), and IgA-anti-TG2 are often found in patients with CD and are used in the diagnosing of CD and dermatitis herpetiformis (DH) patients (Husby et al 2012; Salmi et al 2013). Data on the pathogenesis of DH has revealed epidermal transglutaminase (TG3), an enzyme very similar to TG2, as the major autoantigen in DH (Sárdy et al 2002; Hull et

al 2008). IgA type antibodies against TG3 (IgA-anti-TG3) develop in patients with DH and precipitate as immune complexes in the papillary dermis, playing a role in skin lesion pathogenesis (Sárdy et al 2002; Hull et al 2008). On the other hand, mutations in the coding gene of another epidermal transglutaminase, keratinocyte transglutaminase (TG1), have been reported to be deficient in lamellar ichthyosis, a disease with severely impaired epidermal barriers (Eckert et al 2005).

Both epidermal transglutaminases, TG3 and TG1, are expressed in the spinous and granular layers of the epidermis and are shown to have a role in epidermal barrier dysfunction as well as in barrier repair (Eckert et al 2005; Hitomi et al 2005). These proteins have been expressed in high concentrations in the skin of AD patients, especially in skin lesions (Cheng et al 2009; Liedén et al 2012). Also, humoral responses against TG3 have been shown in sera of CD patients as well as in DH patients (Borroni et al 2013).

As dysfunction of the epidermal barrier and autoimmunity are relevant in the pathogenesis of AD, and transglutaminases have been shown to have a role in epidermal barrier function, we could suspect that circulating IgA-anti-TG3 and IgA type antibodies specific for TG1 (IgA-anti-TG1) may play a role in childhood AD development.

3. AIMS OF THE STUDY

The general aim of the study was to investigate the incidence of CD in Estonian children and prevalence in children with AD and to find the clinical and immunological characteristics of coexistence of AD and CD.

Accordingly, the specific aims were:

1. To find the incidence of CD in children in Estonia and to analyse the trends in the incidence and clinical presentation.
2. To find the prevalence of CD in children with AD and to characterise the clinical features of concomitant manifestation of AD and CD.
3. To assess the general autoimmune background in children with AD by ANA determination and to evaluate clinical differences between ANA-positive and ANA-negative AD patients.
4. To detect the presence of IgA type antibodies against TG1 and TG3 in the serum of children with AD, children with CD and children without known autoimmune or inflammatory disease, and to assess the potential role of these antibodies in AD.

4. MATERIAL AND METHODS

4.1. Study subjects

The general characteristics of study groups are described in Table 2. All serum samples were stored at minus 20°C or lower degree until use.

Table 2. Characteristics of study groups.

Study group	Mean age (min; max)	Female n (%)	Institution (period of collection)	Study material	Paper, n
Random schoolchildren (n=1160)	14.5 years* (8.3; 17.8)	636 (54.8%)	Tartu County (1998–1999)	sera (n=1160)	I, II, IV
Random schoolchildren follow-up (n=891)	24.3 years* (16.9; 27.1)	503 (56.4%)	Tartu County (2007–2008)	sera (n=891)	II
35-year study group of CD patients (n=152)	4.9 years (0.4; 18.0)	84 (55.3%)	Estonia (1976–2010)	epidemiological data	III
AD (n=351)	5.8 years (0.5; 18.8)	149 (42.4%)	Tallinn Children's Hospital (2008–2012)	sera (n=351), small intestinal biopsy specimens (n=13)	IV, V, VI
CD (n=28)	6.2 years (1.0;16.5)	18 (64.3%)	Tallinn Children's Hospital (2008–2012) and Children's Clinic of Tartu University Hospital (1996–2012)	sera (n=28)	VI
Study controls (n=117)					
Study controls with small intestinal biopsy data (n=56)	9.1 years (0.5; 16.0)	28 (50.0%)	Tallinn Children's Hospital (2008–2012)	sera (n=56)	V, VI
Study controls without biopsy data (n=61)	6.8 years (1.5; 17.7)	24 (39.3%)	Children's Clinic of Tartu University Hospital (2008–2012)	sera (n=61)	V

** – median age used instead of mean age.*

Written informed consent was obtained from all studied patients or their parents. The studies were performed in conformance with the Declaration of Helsinki ethical guidelines and were approved by the Ethics Review Committee on Human Research of the University of Tartu, Estonia.

4.1.1. Study subjects for investigating prevalence of CD in schoolchildren in Estonia (Paper I, II)

In Paper I, the aim was to investigate the prevalence of CD in children population. Coded sera from 1160 randomly selected Estonian schoolchildren aged 9 and 15 years (median age 14.5 years, 54.8% females) were obtained from the European Youth Heart Study sample, collected in 1998–1999 in Tartu County (Harro et al 2001). In the original study, 25 schools were randomly selected using a probability proportional to the school size, and all children in grades III and IX were invited to participate based on the school registry.

In Paper II, rescreening for CD was carried out in the same population after a ten-year period. In 2007–2008, coded sera were obtained from follow-up sampling of the same sample from the European Youth Heart Study. Of the previously studied patients, 891 were located and agreed to participate (median age 24.3 years, 56.4% females), for a participation rate of 76.8%. The follow-up sample well-represented the entire original sample from 10 years prior, in age and sex distribution.

4.1.2. Study subjects for investigating incidence of CD in Estonia over a 35-year period (Paper III)

Paper III analysed epidemiological data of all children up to 19 years of age newly diagnosed with CD according to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) diagnostic criteria from 1976 to 2010 in all of Estonia were analysed (n=152, mean age 4.9 years, range 0.4–18.0 years, 55.3% females)(Walker-Smith et al 1990).

The earlier data collected by Oivi Uibo during 1976–2008 has been combined with the data collected during this study. In 1976–1989 data has been collected from hospital records retrospectively. Since 1990, all new CD cases have been recorded from two tertiary referral children's hospitals in Estonia – Children's Clinic of Tartu University Hospital and Tallinn Children's Hospital – where all childhood CD cases are diagnosed in Estonia.

Collected data included age at diagnoses, gender, symptoms at diagnosis, presence of associated conditions and data of histological investigations of small intestinal biopsy specimens. The clinical picture was defined as classical (diarrhoea with failure to thrive and/or weight loss), atypical gastrointestinal (any gastrointestinal symptom without failure to thrive or weight loss), extraintestinal (all other symptoms outside the gastrointestinal tract) or silent

presentation (asymptomatic, identified by testing because of associated condition, mainly type 1 diabetes, or by screening of first degree relatives) (McGowan et al 2009; Husby et al 2012).

4.1.3. Study subjects for investigating CD in children with AD (Paper IV)

Consecutive patients with active AD were recruited at Tallinn Children's Hospital in 2008–2012. Active AD was diagnosed in clinically symptomatic children based on clinical history, objective findings and in accordance with the UK Working Party's Diagnostic Criteria for Atopic Dermatitis (Williams et al 1994). In total, 351 patients with AD agreed to participate in the study (mean age 5.8 years, range 0.5–18.8 years, 42.4% females). Sera from children with AD were used for determining the prevalence of CD among AD patients.

Serum samples were collected from all patients for antibody analysis. Based on clinical suspicion and/or seropositivity for IgA-anti-TG2, a small intestinal biopsy was offered to categorise the intestinal mucosal state by Marsh classification and to confirm the diagnosis of CD (Marsh 1992).

Associated diseases and conditions were found in 121 AD patients: allergic rhinitis or rhinoconjunctivitis in 91 (25.9% of 351 AD patients), asthma in 57 (16.2%), urticaria in 36 (10.3%), juvenile arthritis in 2 (0.7%), and type 1 diabetes in one (0.3%).

Prevalence of CD in AD patients was compared to prevalence of CD in 1160 randomly selected Estonian schoolchildren, characterised in Paper I.

4.1.4. Study subjects for investigating antinuclear antibodies in AD (Paper V)

For assessing general autoimmune background in AD patients, serum samples from 346 patients with AD (excluding patients with concomitant CD) were analysed (mean age 5.8 years, range 0.5–18.8 years, 41.9% females). The patients were classified as having extrinsic or intrinsic types of AD based on clinical phenotype (defined by total IgE levels, allergen sensitisation and association with allergic asthma or rhinoconjunctivitis). The severity of AD was assessed based on patient medical files and was classified as mild (<3 points) or moderate/severe (3–9 points) based on Three Item Severity scoring (Wolkerstorfer et al 1999).

Sera in the control group (n=117, mean age 7.9 years, range 0.5–17.7 years, 44% females) were collected at Tallinn Children's Hospital (n=56) and at Children's Clinic of Tartu University Hospital (n=61) from children who had been hospitalized with various gastrointestinal, urological and other complaints for investigations but revealed to have no inflammatory or autoimmune diseases. Acute infection in study subjects was excluded using C-reactive protein and white blood cell count.

4.1.5. Study subjects for investigating antibodies against epidermal transglutaminases (Paper VI)

For assessing the prevalence and role of IgA-anti-TG1 and IgA-anti-TG3 in Paper VI, serum samples of four different study groups were analysed – children with AD, children with AD and concomitant CD, children with CD and group of children with biopsy confirmed normal small intestinal mucosa.

In this study, serum samples of 304 patients with only AD (mean age 5.5 years, 43% females) and 5 patients with AD and concomitant CD (mean age 5.6 years, 4 females) were analysed. Serum samples were collected as described in section 4.1.3.

Serum samples of 28 patients with CD (mean age 6.2 years, range 1.0–16.5 years, 64% females) were collected from patients diagnosed with CD according to ESPGHAN diagnostic criteria and in whom small intestinal biopsy specimens have been examined according to the Marsh classification (Walker-Smith et al 1990; Marsh 1992). Patients were recruited from Tallinn Children's Hospital (n=9) and Children's Clinic of Tartu University Hospital (n=19). The clinical presentation of CD was defined as classical, atypical gastrointestinal, extraintestinal or silent type (Marsh 1992). Sera were collected at the same time as or up to one month prior to the small intestinal biopsy.

For this study serum samples of 55 patients from study control groups with biopsy confirmed normal small intestinal mucosa, who had undergone gastroscopy for various functional gastrointestinal symptoms at Tallinn Children's Hospital, were available (mean age 9.2 years, range 0.5–16.0 years, 51% females). Patients presented predominantly with recurrent abdominal pain (58.9%), failure to gain weight (23.2%) or to thrive (17.8%), diarrhoea (17.8%) and abdominal bloating (16.1%). Asthma was found in 5 patients (8.9%). Acute infection in study subjects was excluded using C-reactive protein and white blood cell count determinations. Sera were collected at the same time as or up to one month prior to the small intestinal biopsy.

4.2. Methods used for antibody analysis

4.2.1. Total IgA, total IgE and IgE type antibodies to food allergen panels

For exclusion of IgA deficiency, all patients with AD, CD patients and study controls were tested for total IgA with chemiluminescence assay (Roche Diagnostics, Burgess Hill, England) and compared to age-specific reference values provided by the manufacturer.

All patients with AD, 9 patients with CD (recruited at Tallinn Children's Hospital) and 56 study controls with biopsy data were tested for total IgE with Roche Diagnostics, Burgess Hill, England; Siemens Healthcare Diagnostics Inc, Tarrytown, USA) and compared to age-specific reference values provided by

the manufacturer. In these patient groups, IgE type antibodies to food allergen panels fx5 (egg white, milk, fish, wheat, peanut, soybean) and fx20 (wheat, rye, barley, rice) were measured using ImmunoCAP Specific IgE assay (Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer's recommendations, values of 0.35 kU/l or more were considered positive.

4.2.2. Antibodies to tissue transglutaminase and deamidated gliadin peptides

IgA and IgG type anti-DGP (IgA-anti-DGP, IgG-anti-DGP) were measured by a fluoroenzyme immunoassay using the EliA Gliadin DP assay (Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer's recommendations, values of 10 EliA U/ml or more were considered positive and values less than 7 EliA U/ml were considered negative. Borderline values between 7 and 10 EliA U/ml were considered negative to make the results dichotomic for statistical analysis.

IgA-anti-TG2 and IgG type anti-TG2 (IgG-anti-TG2) were measured by a fluoroenzyme immunoassay using the EliA Celikey assay (Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer's recommendations, values of 10 EliA U/ml or more were considered positive, and values less than 7 EliA U/ml were considered negative. Borderline values between 7 and 10 EliA U/ml were considered negative to make the results dichotomic for statistical analysis.

4.2.3. Indirect immunofluorescence assay for detection of antinuclear antibodies

Serum samples of AD patients (n=351), control group patients (n=117) and CD patients (n=9) were tested for IgG ANA by indirect immunofluorescence on Hep-2 cells using commercial kit of ImmunoConcepts (Sacramento, CA, USA) and serum dilutions starting from 1:10 in PBS (phosphate-buffered saline) (Uibo et al 1998). This serum dilution was the lowest that has been reproducibly used at our laboratory for ANA screening among paediatric populations (data not shown). Sera with ANA at titer 1:160 were tested for autoantibodies against extractable nuclear antigens Sm, RNP, SSA/Ro, SSB/La, Scl-70, and Jo-1 with enzyme immunoassay (RELISA ENA, ImmunoConcept, Sacramento, CA) and for double stranded DNA (dsDNA) by indirect immunofluorescence using *Chritidia lucilia* for substrate (Fluorescent nDNA Test System, ImmunoConcept, Sacramento, CA).

4.2.4. IgA type antibodies to transglutaminase 1 and 3

IgA-anti-TG1 and IgA-anti-TG3 were measured by enzyme-linked immunosorbent assay (ELISA) as described earlier (Teesalu et al 2009) using recombinant TG1 and TG3 as target antigens (Zedira GmbH, Darmstadt, Germany). Universal binding 96-well microtiter plates (Thermo Fisher Scientific, Vantaa, Finland) were coated with 0.5 µg TG1 or TG3 per well in 100 µl TBS-Ca buffer containing 25 mmol/l Tris-HCl, 150 mmol/l NaCl, 5mmol/l CaCl2, (pH 7.4), and incubated overnight (16 h) at 4°C. Thereafter wells were washed five times with 300 µl TBS-TE buffer (25 mmol/l Tris-HCl, 150 mmol/l NaCl, 10 mmol/l EDTA, 1 ml/l Tween 20, pH 7.4), and once with a solution of 50 g/l sucrose, 0.5 g/l thimerosal in TBS. After discarding solution, plates were dried at 20°C for 3 h, sealed, and kept at 4°C until use. Serum samples were diluted 1:100 in TBS-T buffer (25 mmol/l Tris-HCl, 150 mmol/l NaCl, 1 ml/l Tween 20, pH 7.4) and incubated in duplicate on TG coated wells for 1h. Incubations were performed at 20°C and followed by washing step with 300 µl TBS-T five times. The wells were incubated with dilutions of alkaline phosphatase conjugated antibodies of goat anti-human IgA (Invitrogen) in TBS-T buffer for 30 min. Incubations was performed at 20°C and followed by washing step with 300 µl TBS-T five times. The color reaction was developed by incubating the wells with 100 µl substrate 4-p-nitrophenyl phosphate (1 g/l) in 1 mol/l diethanolamine, 0.5 mmol/l MgCl2 (pH 9.8) for 30 min and stopped by adding 50 µl 0.1 mol/l EDTA. The absorbances were read at 405 nm with 492 nm subtraction and the antibody levels expressed in arbitrary units (AU) as percentages of the reference serum optical density values. Reference values for IgA-anti-TG1 and IgA-anti-TG3 were calculated by determining the mean+2SD in control group patients with normal small intestinal mucosa (characterised in paper VI). Therefore IgA-anti-TG1 values of 37.3 AU or more and IgA-anti-TG3 values of 48.4 AU were considered positive.

4.3. Statistical analysis

For statistical analyses, R software for Windows (The R Foundation for Statistical Computing, Vienna, Austria) and MedCalc statistical software (MedCalc Software, Mariakerke, Belgium) were used. Data were expressed as absolute numbers or proportions for categorical variables and as means for continuous variables. Comparison between proportions and means was carried out using parametric and non-parametric tests as appropriate. A p-value less than 0.05 was used to reject the null hypothesis.

In Paper III, newly diagnosed cases were grouped according to their age at diagnosis in four groups: 0–4, 5–9, 10–14 and 15–19 years. Incidence rates (IR) for boys and girls of different age-groups and different calendar years at diagnosis are expressed as the number of new cases per 100 000 person-years using official mid-year population data for relevant age and sex groups from

Statistics Estonia. The 95% confidence interval (CI) for the incidence was estimated using the exact method based on the Poisson distribution. Bonferroni correction was used in multiple comparisons.

In Paper VI, the diagnostic performance of assays in terms of sensitivity and specificity expressed as a percentage was calculated based on the cut-off values described above.

5. RESULTS

5.1. CD in children in Estonia

5.1.1. Prevalence of CD in random population of schoolchildren in Estonia (Paper I, II)

Serum samples from 1160 Estonian schoolchildren aged 9 and 15 years were studied for IgA-anti-TG2. Five of the 1160 sample showed elevated IgA-anti-TG2 and were invited for follow-up studies. Four out of five, all non-relatives, agreed to participate and were again IgA-anti-TG2 positives and had the HLA-DQ2 allele characteristic of CD. In all four, CD was confirmed by biopsy, yielding a CD prevalence of 1:290 (0.34%; 95% CI: 0.09–0.88%) (Table 3). Three of them had complaints and symptoms that might be attributable to CD but were not considered by their family doctors – a 9-year-old girl presented with iron deficiency anaemia and recurrent abdominal pain, two 15-year-old girls presented with failure to thrive. One 15-year-old girl had neither complaints nor symptoms for suspecting CD, despite subtotal villous atrophy of the small intestinal mucosa.

Table 3. Data for subjects with positive IgA-anti-TG2 test results at screening and after follow-up.

		Baseline screening				Second screening		
No.	**Gender**	**Age (y)**	**Original IgA-anti-TG2 (U/ml)***	**Follow-up IgA-anti-TG2 (U/ml)***	**Histology**	**Age (y)**	**IgA-anti-TG2 (U/ml)***	**Gluten-free diet compliance**
1492	F	9	**15.0**	**55.0**	Marsh IIIc	18	1.8	Good
1511	F	15	**15.5**	NA	NA	NA	NA	NA
1691	F	15	**41.1**	**52.0**	Marsh IIIb	25	**23.7**	Poor
1873	F	15	**22.6**	**38.5**	Marsh IIIb	25	**51.7**	Poor
2027	F	15	**39.5**	**14.5**	Marsh IIIb	25	**14.0**	Poor

NA – not available for testing;
** positive test results typed in bold.*

In the second screening of the same population after a 10-year period, three subjects had positive IgA-anti-TG2 results (Table 3). All three were also IgA-anti-TG2 positive in the baseline screening and had biopsy-proven CD. Out of two other subjects who were initially seropositive at the first screening, one did not participate in the second study and the other with biopsy-verified CD has turned seronegative, as she had strictly followed the gluten-free diet during the follow-up years.

5.1.2. Incidence of CD in Estonia over a 35-year period (Paper III)

During 1976–2010, a total of 152 children (median age 2.3 years, 68 boys and 84 girls) were diagnosed with CD in Estonia. The overall incidence rate of CD was 1.12 per 100 000 person-years (95% CI: 0.94–1.31).

During the study period of 1976–1989 only 13 children were diagnosed with CD, compared to 139 children in the period of 1990–2010. Thus, the incidence rate increased from 0.21 per 100 000 person-years in the period of 1976–1989 to 1.85 per 100 000 person-years in the period of 1990–2010. A comparison of the general characteristics of the diagnosed cases is shown in Table 4.

Table 4. General characteristics of new CD cases in the two study periods.

	Period of 1976–1989	**Period of 1990–2010**
Cases	13	139
Male / female ratio	1 / 3.3	1 / 1.1
Incidence rate per 100 000 person years (95% CI)	0.21 (0.11–0.36)	1.85 (1.55–2.18)
Median age at diagnosis (years)	1.0	3.0
Age at diagnosis (n, %)		
0–4 years	13 (100%)	78 (56%)
5–9 years	0	28 (20%)
10–14 years	0	26 (19%)
15–19 years	0	7 (5%)
Clinical presentation of CD:		
classical symptoms (n, %)	10 (77%)	55 (39%)
atypical GI symptoms (n, %)	3 (23%)	42 (30%)
extraintestinal symptoms (n, %)	0	12 (9%)
silent CD (n, %)	0	30 (22%)

Our analyses shows that the annual incidence rate was 0.10 per 100 000 person-years (95% CI: 0.01–0.34) during 1976–1980 and showed remarkable increase to 3.14 per 100 000 person-years (95% CI: 2.29–4.18) in 2006–2010. The changes can be associated with the start of an active clinical search for CD, introduction of the gliadin antibody and/or EmA screening in 1990, introduction of anti-TG2 to routine clinical practice in 2003, and start of a CD screening among all children with type 1 diabetes in 2005 (Figure 1).

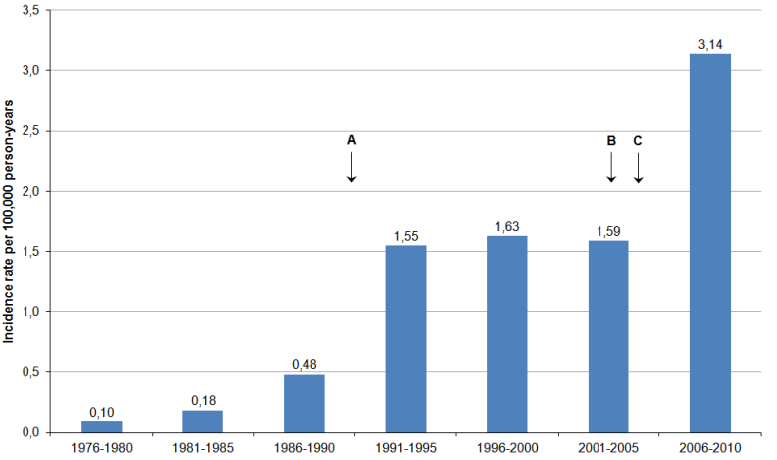


Figure 1. Distribution of CD incidence rate per 100 000 person years during 5 year periods. A – active clinical search for CD and introduction of gliadin antibody and/or EmA screening to clinical practice; B – introduction of anti-TG2 to routine clinical practice; C – introduction of CD screening among all children with type 1 diabetes.

In the period of 1990–2010, there was a significant increase in the median age of the patients from 1.0 in the earlier study period to 3.0 ($p < 0.001$; Figure 2). In 1976–2000 73.6% of cases were diagnosed before the age of five. The age-specific incidence rate in the older age groups showed largest increase during the period from 2006–2010. In the latter group, 71.7% of the cases were diagnosed after the age of five.

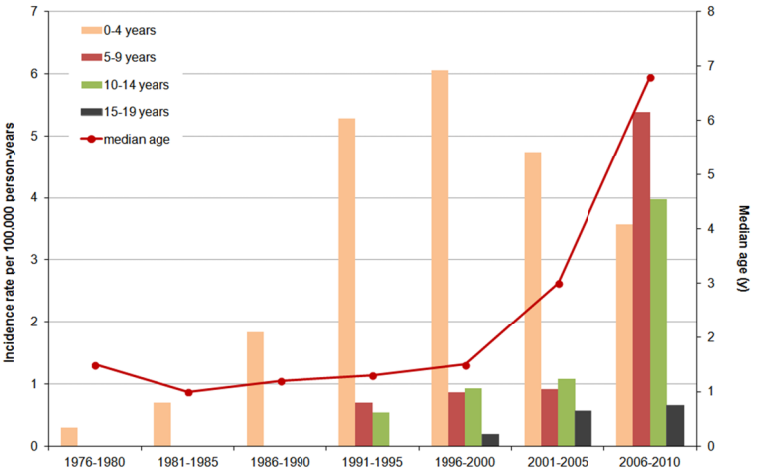
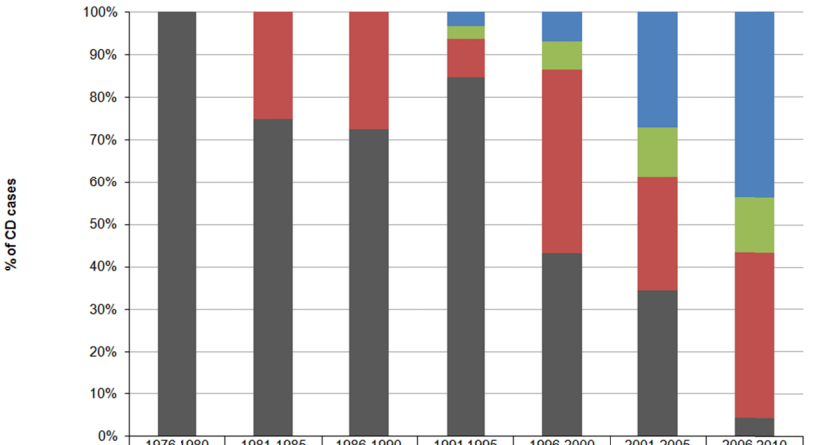


Figure 2. Distribution of incidence rate per 100 000 person-years according to the age-groups and the median ages of new CD cases.

During the study period, most of the cases (42.8%) were diagnosed in children with a classical clinical presentation (diarrhoea with failure to thrive or loss of weight). From 1976–1989, all new cases were diagnosed under the age of five years with either a classical (76.9%) or atypical gastrointestinal clinical presentation (23.1%). In contrast, during the years 2006–2010, silent clinical presentation was documented in 43.5% of the cases (Figure 3).



	1976-1980	1981-1985	1986-1990	1991-1995	1996-2000	2001-2005	2006-2010
silent (n)	0	0	0	1	2	7	20
extraintestinal (n)	0	0	0	1	2	3	6
atypical GI (n)	0	1	3	3	13	7	18
classical (n)	2	3	8	23	13	9	2

Figure 3. Distribution of new CD cases according to clinical presentation at the time of diagnosis.

Associated diseases and conditions were documented in 104 patients out of 152 CD patients, including predominantly type 1 diabetes (33 patients, 22%), rickets (13 patients, 9%), Down syndrome (9 patients, 6%) and AD (8 patients, 5.3%). Concomitant iron deficiency anaemia was found in 30 patients (20%) and elevated transaminases were noted in 10 patients (7%). Less than 3% of patients had other chromosomal diseases, DH, osteoporosis, epilepsy or herpetic stomatitis.

All diagnosed CD cases were regionally classified according to the place of residence at diagnosis. The incidence rate of CD for all regions of Estonia from 1976–2010 was 1.12 (95% CI: 0.94–1.31). The incidence rates for CD in Jõgeva County (IR 2.46; 95% CI: 1.18–4.53) and Järva County (IR 2.14; 95% CI: 0.98–4.07) were more than twice as high as the incidence rate for the whole country (Figure 4).

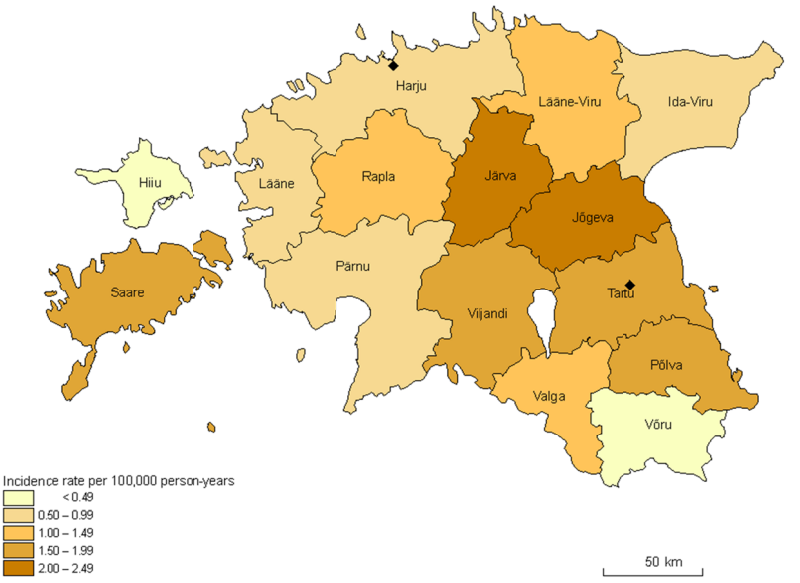


Figure 4. Incidence rate of childhood CD in Estonian counties from 1976–2010. The two referral hospitals are marked with diamonds.

5.2. CD in children with AD (Paper IV)

Altogether 351 children with AD were screened for CD, and CD was diagnosed in 5 of them (1.4%; 95% CI: 0.46–3.32) (Table 5).

For exclusion of IgA deficiency, all patients were tested for total IgA. Deficiency of IgA was diagnosed in 9 (2.6%) patients with AD, but none of them had seropositivity for CD-associated antibodies, IgG-anti-TG2 or IgG-anti-DGP.

IgA-anti-DGP were found in 11 (3.1%) AD patients (3 of them also IgA-anti-TG2 positive), and IgG-anti-DGP were found in 10 AD patients (2.8%) (4 of them also IgA-anti-TG2 positive). IgA-anti-TG2 positivity was found in 4 (1.1%) of AD patients, and IgG-anti-TG2 positivity in 2 (0.6%) of AD patients (both positive for IgA-anti-TG2).

Among the four female IgA-anti-TG2 positive patients, all were positive for IgG-anti-DGP, while 3 were positive for IgA-anti-DGP and 2 were positive for IgG-anti-TG2. Based on clinical suspicion or seropositivity, small intestinal biopsy was performed in 13 patients. CD was confirmed in 4 patients with IgA-anti-TG2 positivity, showing villous atrophy and severe crypt hyperplasia. CD was also diagnosed in a 1.2 years old boy with high clinical suspicion based on pronounced chronic intermittent diarrhoea but no reactivity for any tested autoantibody. Histological analysis of small intestinal mucosa showed villous atrophy and increased crypt hyperplasia (Marsh IIIa).

Table 5. Characteristics of AD patients studied by small intestinal biopsy.

Subject no	Age (y)	Gender	IgA-anti-TG2* (U/ml)	IgG-anti-TG2* (U/ml)	IgA-anti-DGP* (U/ml)	IgG-anti-DGP* (U/ml)	Clinical picture for CD	Histology	Diagnosis
AT018	6.9	F	**>128**	7.0	**11.5**	**16.3**	none	**Marsh IIIb**	AD+CD
AT091	3.5	F	**>128**	**>128**	**142.0**	**134.0**	none	**Marsh IIIc**	AD+CD
TT001	1.2	M	0.1	0.1	0.5	1.4	chronic diarrhoea	**Marsh IIIa**	AD+CD
TT069	5.5	F	**>128**	**24.0**	2.6	**14.0**	chronic diarrhoea, constipation, dental enamel defects	**Marsh IIIa**	AD+CD
TT060	10.8	F	**60.0**	8.1	**35.7**	**58.1**	chronic diarrhoea, abdominal pain, iron deficiency anaemia, dental enamel defects	**Marsh IIIc**	AD+CD
AT005	5.1	M	1.0	5.0	0.4	1.5	none	normal mucosa	AD
AT010	10.8	F	0.3	0.3	1.3	4.4	failure to thrive	normal mucosa	AD
TT003	3.5	F	0.1	0.8	0.7	1.3	failure to thrive and gain weight	normal mucosa	AD
TT033	8.0	F	0.3	0.5	0.9	2.1	episodic diarrhoea, bloating, abdominal pain, dental enamel defects	normal mucosa	AD
TT044	1.3	M	0	0.2	0.7	4.4	gastroesophageal reflux	normal mucosa	AD
TT051	10.7	F	0.1	0.3	0.4	2.5	abdominal pain	normal mucosa	AD
TT061	8.9	F	0.6	1.6	**23.7**	6.9	abdominal pain	normal mucosa	AD
TT067	8.0	M	0.1	0.0	2.5	0.7	abdominal pain	normal mucosa	AD

* *positive test results typed in bold.*

In the rest of IgA-anti-DGP and IgG-anti-DGP positive patients, CD was not diagnosed, because of lack of clinical suspicion, normalisation of analysis in follow-up sampling or normal small intestinal histology. One patient (Table 5, TT061) with IgA-anti-DGP underwent small intestinal biopsy because of abdominal pain, but revealed normal intestinal histology. Five patients showed normalisation of IgA-anti-DGP and/or IgG-anti-DGP on follow-up visitation with their treating physician and in six patients the only slightly elevated antibody levels were considered clinically non-relevant, therefore small intestinal biopsy was not performed.

5.3. Antinuclear antibodies in AD (Paper V)

For assessing general autoimmune background 346 patients with AD (excluding patients with AD and concomitant CD) and 117 study control group patients were studied.

Total-IgE levels were elevated in 59.5% of patients with AD, and 44.2% had allergen-specific IgE to food allergens. No statistically significant difference in ANA-positivity was noted between patients with elevated and normal total-IgE levels (15.0% and 11.4% respectively). Also, despite significant differences in sensitisation rate to different allergen panels in AD patients ($p<0.05$), no remarkable differences were observed in ANA-positivity between sensitisation to different allergen panels.

From studied AD patients, 29.8% could be classified as having intrinsic type of AD (normal IgE levels, no allergen-specific IgE and no association with allergic respiratory diseases), and 70.2% had extrinsic type of AD (Table 6). ANA was found to be positive in 10 patients with intrinsic AD (9.7%) at titers up to 1:80 and in 37 patients with extrinsic AD (15.2%) at titers up to 1:160. Although the rate of ANA-positivity at titer 1:10 was slightly higher in extrinsic type of AD patients than in intrinsic type, the difference was not significant (OR 1.7; 95% CI 0.80–3.50).

Based on severity of AD symptoms, 69 (19.9%) of the patients with AD could be classified as having moderate or severe form of AD. ANA positivity was found more frequently in mild form of AD (14.8%) when compared to moderate or severe form (8.7%), but the difference was not statistically significant.

In total, 47 patients with AD (13.6%) and 15 patients (12.8%) from the control group were positive for ANA, with mostly homogenous or speckled fluorescent pattern (Table 7). Compared to the control group, ANA was found at slightly higher titres in AD patients. Only AD patients had ANA at titre 1:160, all of them presented with extrinsic type of AD and with no clinical signs or symptoms for systemic autoimmune diseases.

Table 6. Characteristics of AD patients (n=346).

	Extrinsic type of AD	Intrinsic type of AD
Number (%)	243 (70.2%)	103 (29.8%)
Mean age	6.2 years	4.8 years
Male gender	145 (59.7%)	56 (54.4%)
Elevated total IgE (>age related reference)	206 (84.8%)	0
Mean total IgE	420.7 kU/l	22.3 kU/l
Specific IgE to panel fx5 (>0.35 kU/l) (egg white, milk, fish, wheat, peanut, soybean)	140 (40.5%)	
Specific IgE to panel fx20 (>0.35 kU/l) (wheat, rye, barley, rice)	67 (19.4%)	
ANA 1:10 positive	37 (15.2%)	10 (9.7%)

Table 7. ANA pattern in AD patients and study controls.

	ANA titer			
	1:10	**1:40**	**1:80**	**1:160**
AD, in total (n=346)	**47 (13.6%)**	**28 (8.1%)**	**17 (4.9%)**	**3 (0.9%)**
homogenous, n	33	19	11	1*
speckled, n	8	4	3	1*
few nuclear dots, n	5	4	3	1**
nucleolar, n	1	1	0	0
Study control subjects, in total (n=117)	**15 (12.8%)**	**9 (7.7%)**	**4 (3.4%)**	**0**
homogenous, n	10	5	2	0
speckled, n	3	2	0	0
few nuclear dots, n	2	2	2	0

* *additionally tested for extractable nuclear antigens and dsDNA, no positivity;*
** *additionally tested for extractable nuclear antigens and dsDNA, weak positive reaction to Scl-70 antigen, without any known symptoms for systemic connective tissue disease.*

ANA was found significantly more often in female than in male AD patients (OR 3.5; 95% CI: 1.8–6.8). On the other hand, there was no significant difference in odds for ANA-positivity based on gender in control subjects.

In AD patients, ANA was noted already at the age of 2 years, whereas in controls, ANA was not found before the age of 4.6 years. Although the mean age of ANA-positive patients in the AD group was slightly younger than in the control group (8.2 vs 9.1 years), the difference was not statistically significant. However, the mean age in the AD group differed significantly between ANA-positive and ANA-negative AD patients at titres up to 1:40 (8.2 vs 5.6 years; $p<0.005$). No significant difference in mean age was noted based on ANA-positivity in control subjects (Figure 5)

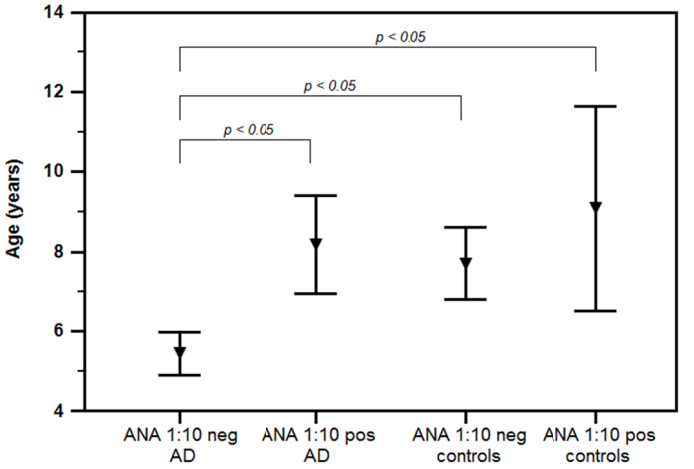


Figure 5. Mean age in AD and control patients based on ANA-positivity at titre 1:10. The mean age differed significantly between ANA-negative and ANA-positive patients in AD group (5.4 vs 8.2 years; $p<0.005$), but not in control subjects (7.7 vs 9.1).

5.4. Antibodies against epidermal transglutaminases (Paper VI)

For assessing the prevalence and role of IgA-anti-TG1 and IgA-anti-TG3, serum of children with AD (n=304), children with AD and concomitant CD (n=5), children with CD (n=28) and study control children with normal small intestinal mucosa were investigated (n=55).

Due to IgA deficiency in the current study groups, 7 (2%) of the studied AD patients and 3 (5%) of study control patients were excluded from further antibody and statistical analysis, leaving 297 AD patients and 52 study controls for the statistical analysis of this study. Antibody responses to TG2 and epidermal transglutaminases (TG1 and TG3), together with IgA-anti-DGP in different study groups are shown in Table 8.

Table 8. Seropositivity rates in different patient groups (IgA deficient cases excluded).

	IgA-anti-TG1		IgA-anti-TG2		IgA-anti-TG3		IgA-anti-DGP	
	+	–	+	–	+	-	+	-
AD (n=297)	6 (2%)	291 (98%)	0	297 (100%)	9 (3%)	288 (97%)	7 (2%)	290 (98%)
AD+CD (n=5)	2 (40%)	3 (60%)	4 (80%)	1 (20%)	0	5 (100%)	3 (60%)	2 (40%)
CD (n=28)	10 (36%)	18 (64%)	27 (96%)	1 (4%)	5 (18%)	23 (82%)	24 (86%)	4 (14%)
Study controls (n=52)	2 (4%)	50 (96%)	1 (2%)	51 (98%)	2 (4%)	50 (96%)	2 (4%)	50 (96%)
	AD vs controls p=0.340		AD vs controls p=0.149		AD vs controls p=0.671		AD vs controls p=0.628	
	AD vs CD **p=0***		AD vs CD **p=0**		AD vs CD **p<0.005**		AD vs CD **p=0**	
	AD vs AD+CD **p=0.006**		AD vs ADCD **p=0**		AD vs AD+CD p=1		AD vs AD+CD **p<0.005**	
	AD+CD vs controls **p=0.035**		AD+CD vs controls **p<0.005**		AD+CD vs controls p=1		AD+CD vs controls **p<0.005**	
	AD+CD vs CD p=1		AD+CD vs CD p=0.284		AD+CD vs CD p=0.569		AD+CD vs CD p=0.216	
	CD vs controls **p<0.005**		CD vs controls **p=0**		CD vs controls **p=0.048**		CD vs controls **p=0**	

* *p values marked in bold are statistically significant.*

In the AD group, 7 patients (2%) had slight IgA-anti-DGP reactivity, all were >2 years of age and none were seropositive for other markers. Six patients with AD (2%) had elevated IgA-anti-TG1 and 9 patients with AD (3%) had elevated IgA-anti-TG3, all of them were IgA-anti-TG2 negative.

Of the 5 AD patients with concomitant CD, 4 had IgA-anti-TG2 and 3 had IgA-anti-DGP antibodies. Two AD and concomitant CD patients with positive IgA-anti-TG1 responses also had IgA-anti-TG2 and IgA-anti-DGP responses (Table 5, AT018 and AT091).

IgA-anti-TG2 was identified in 27 (96%) and IgA-anti-DGP identified in 24 (86%) CD patients (all these 24 were also positive for IgA-anti-TG2). Out of 28 CD patients 10 (36%) showed IgA-anti-TG1 and 5 (18%) showed IgA-anti-TG3 responses. All of them were also IgA-anti-TG2 positive and most of them also IgA-anti-DGP positive.

In the control group, only 1 patient presented with borderline IgA-anti-TG2 values without any accompanying seropositivity. Elevated IgA-anti-DGP values were found in the sera of 2 control group patients, both <2 years of age and without other detectable autoantibodies. No changes to the small intestine mucosa were identified in these 3 patients (Marsh 0). Elevated IgA-anti-TG1 and IgA-anti-TG3 levels were also found in 3 control group patients (4%), none of whom had elevated IgA-anti-TG2 levels. One patient presented with long-lasting diarrhoea and cystic fibrosis, and two patients with acute gastritis resulting from a *Helicobacter pylori* infection; all of them presented with normal small intestinal mucosal histology (Marsh 0).

Seropositivity rate and mean antibody levels of IgA-anti-TG1 and IgA-anti-TG3 were higher in CD patients (mean antibody levels 48.3 and 67.0 AU respectively) than among controls (mean antibody levels 14.5 and 18.7 AU) or AD patients (mean antibody levels 9.5 and 13.7 AU) (Figure 6). In AD patients with concomitant CD, IgA-anti-TG1 responses were as prevalent as in the CD patients but more common compared to controls ($p<0.05$) or AD patients ($p<0.005$), indicating that IgA-anti-TG1 and IgA-anti-TG3 responses were associated with CD and not with AD.

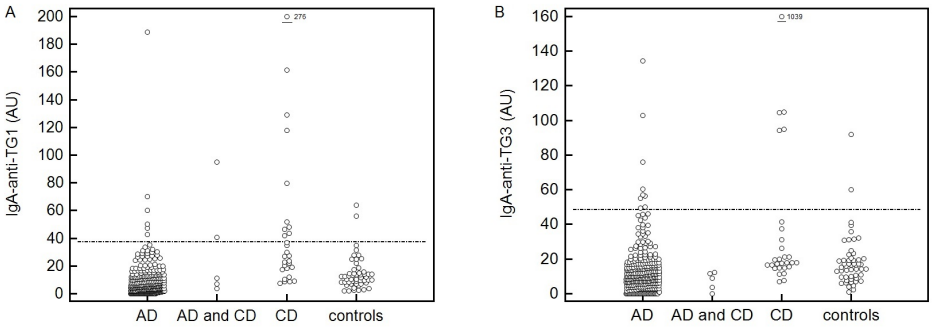


Figure 6. IgA-anti-TG1 (A) and IgA-anti-TG3 (B) values in the different patient groups.

AD, atopic dermatitis; CD, coeliac disease;
** Horizontal line – reference value based on the mean +2SD of the control subjects.*

Elevated IgA-anti-TG1 and IgA-anti-TG3 levels were found in 6 and 9 AD patients, respectively, whereas only 1 patient with AD had both elevated IgA-anti-TG1 and IgA-anti-TG3 antibody levels. On the other hand, patients presenting with CD or with AD and concomitant CD tended to be seropositive for IgA-anti-TG1 and/or IgA-anti-TG3 together with IgA-anti-TG2 and IgA-anti-DGP seropositivity.

6. DISCUSSION

6.1. CD in children in Estonia

6.1.1. Prevalence of CD

Until 1990 CD was considered a very rare disease in Estonia. Retrospective analysis of all childhood CD cases in Estonia by Uibo et al revealed 0.04 new CD cases per 1000 live births in 1976–1990. In 1990–1994, Uibo et al carried out an active clinical search and serological screening for CD that in conjunction with regular educational activities (e.g., seminars and lectures, postgraduate courses, publications) for physicians has led to a marked increase in the detection of new CD cases in Estonia (0.37 cases per 1000 live births). Still, the rate remained approximately ten times lower compared to other countries (Uibo 1994a; Uibo 1994b; Uibo et al 1996). In addition, Uibo et al have screened 1939 persons from the general population including 1461 adults in 1990 and 478 schoolchildren in 1998, finding no new cases of CD (Uibo et al 1993; Metsküla et al 1998).

In present study we have expanded these investigations by screening 1160 randomly selected schoolchildren and have found the prevalence of CD at least 1 case per 290, i.e. 0.34% (95% CI 0.09–0.88) for Estonia, showing significant increase in prevalence. We believe that the increase in CD prevalence represents the real prevalence of CD and is not caused by difference of the screening method. The latter reason is unlikely as the combination of antigliadin and antireticulin antibody assays (used by Uibo et al) has been shown to be highly effective in detecting CD, although somewhat inferior to IgA-TGA assay (used in this study) regarding sensitivity (Rubio-Tapia et al 2009; Kagnoff 2005).

In recent years, several population screening studies have shown upward trends, especially among the younger age groups (Heel et al 2006; Lohi et al 2007; Olsson et al 2008; Vilppula et al 2009). Therefore we expected to find new CD cases after the 10-year interval, however follow-up sampling of the same population revealed no new antibody-positive cases in the rescreening of 891 children. The relatively high median age may have influenced the study outcome as CD tends to develop more frequently in children. In support, Mariné et al has found five times higher prevalence in children when compared to adults (Mariné et al 2011). On the other hand, the size of our study sample and length of follow-up period could have been insufficient for finding actual rate of new cases.

Our findings about the prevalence of CD in Estonia differ noticeably from results from Finland and Sweden, where CD was detected in up to 3% of the children, indicating the possibility of a major environmental difference between these countries (Ivarsson et al 2000; Mäki et al 2003; Kondrashova et al 2008; Olsson et al 2008; Myléus et al 2009). Underlying causes for the difference in CD incidence these neighbouring countries might include previously shown

different microbial load in Estonian and Swedish populations, increase in the intake of disease-inducing gluten and other prolamines, together with awareness of CD among physicians (Björkstén et al 1999; Böttcher et al 2003; Kagnoff 2005; Rubio-Tapia et al 2009; Vilppula et al 2009; Ivarsson et al 2013). In addition, the influence of genetic and environmental factors on the incidence of CD in the Estonian population compared to other European populations and other age groups requires further investigation.

6.1.2. Trends in the incidence and the clinical presentation of CD

In this study, analysing the hospital data, we have demonstrated a dramatic nationwide increase in the incidence of CD among children in Estonia during a 35-year period. Up to 1990, no remarkable changes were noted in the incidence rate of CD. On average, one new case per year was noted in Estonia. After the introduction of gliadin antibody and/or EmA screening methods into routine clinical practice in 1990 and active clinical searches for CD, especially in high risk groups, the incidence rate increased substantially. Several studies have shown high rate of undiagnosed CD in different countries, indicating the need for raising awareness of variable clinical patterns of CD and probably indicating the need for screening special risk groups (Myleus et al 2009; Norström et al 2011). Therefore, the increase in the prevalence of clinically detected CD in Norway and South Wales is probably related to high awareness of the disease in these countries (Størdal et al 2013; Whyte and Jenkins 2013).

In addition to the impact of introducing routine CD screening methods and active clinical search for CD, activities directed to increase the physicians' awareness (started in 1990) may have had strong effect on increasing the incidence of CD in Estonia. Unfortunately, during some intervening periods, the detection rate of CD decreased, emphasising the need for continuous and active awareness programs.

During 1976–1989, all newly diagnosed cases presented either with the classical clinical symptoms of CD or with atypical gastrointestinal symptoms, and the median age at diagnosis was 1.0 year. The introduction of active clinical searches for CD and introduction of the gliadin antibody and/or EmA screening in 1990 and introduction of anti-TG2 to routine clinical practice in 2003 together with anti-TG2 screening among all children with type 1 diabetes in 2005 led to a significant increase in the prevalence of CD together with more than a 6-fold increase in the median age of patients diagnosed with CD. Our results confirm the shift towards atypical symptoms and older age diagnosis, as reported previously for other countries (Roma et al 2009).

Associated disease and conditions were found in 68% of patients with CD – type 1 diabetes, iron deficiency anaemia, chromosomal diseases (including Down syndrome), rickets, AD and concomitant elevated transaminases were frequently observed. Rickets was associated with classical gastrointestinal

symptoms and was diagnosed only during the first period of study (1976–1989). Most commonly CD was associated with type 1 diabetes (22%), but AD was revealed in 5.3% of all registered CD patients. Our findings are in accordance with other reports, as CD has been often associated with other autoimmune and inflammatory diseases as well as with atopy (Elli et al 2012; Reilly et al 2012; Iwańczak et al 2013).

In addition to the rising awareness of CD, a real rise in the incidence rate of CD could have taken place, with difference in microbial load as one of the possible reason. In western industrialised countries, people mainly consume industrially processed food, but the Estonian diet is still largely composed of locally produced foods and various lactic acid fermented products. However, over the last decades (after regaining national independence in 1991), there have been remarkable changes in the state of the economy and dietary habits in Estonia. Increased consumption of wheat and use of gluten in food processing, together with the introduction of wheat varieties with increased CD toxicity may have an impact on the incidence of CD (van den Broeck et al 2010). Still, based on Eurostat database (http://epp.eurostat.ec.europa.eu/), during 2000–2009 the apparent average wheat consumption per capita was three times lower in Estonia (51 kg/head) than in Italy (157 kg/head), probably leading to a lower incidence of CD in Estonia compared to Italy (Volta et al 2001).

Additionally, infant feeding practices (introducing wheat containing products at an earlier time and in higher amounts), together with socioeconomic factors may attribute to the differences in the CD incidence rate between Estonia and other countries. In 1998, when Estonia belonged to the group of countries with very low prevalence of CD, Mitt and Uibo demonstrated that there is a low cereal intake in Estonian infants compared to Finnish and Swedish infants, and the mean wheat intakes in infants at the age of 12 months were 23 g, 23.3 g, and 49 g per day, respectively (Mitt et al 1998). Unfortunately, we have no later comparative investigations on this important topic.

There are studies showing increased risk for CD among people of a lower socioeconomic status (Ivarsson 2005; Wingren et al 2012) and also studies showing increased risk among those with higher socioeconomic position (Olén et al 2012; Whyte and Kotecha et al 2014). According to data from Statistics Estonia, Jõgeva and Järva counties belong to the group of counties with higher unemployment rates during the last decade. A lower income together with other socioeconomic factors might dispose people to an earlier introduction and higher consumption of complementary cereals (i.e., wheat) due to their low cost. The relatively higher incidence rates of CD in these counties could therefore be at least partly explained by their lower socioeconomic status.

Therefore, introduction of serological screening methods, active clinical search and raising awareness among physicians have increased the detection rate of CD in Estonia. However, true increase in incidence may have also occurred together with a shift towards older age and atypical clinical presentation.

6.2. CD in children with AD

Previous studies have shown increased prevalence of CD in different atopic disorders (Zauli et al 2000). However, results about CD prevalence in AD patients have been controversial, probably due to small cohort sizes and different inclusion criteria (Gallo et al 1992; Uibo et al 1996). We have studied patients with AD with active clinical cutaneous manifestations, as in chronic eczematous lesions Th1 type responses play a major role in addition to the earlier phase of Th2-mediated responses (Bieber 2008). As exogenous allergens play a role in AD and in case of chronic active allergic inflammation, it might be speculated that chronic ingestion of allergens may lead to tissue damage of the gut epithelial cells and therefore expose possible autoallergens.

Screening of 351 children with AD led to diagnosing CD in 5 (1.4%; 95% CI: 0.46–3.32) children. Three patients (1.2-year-old boy, 10.8-year-old girl and 5.5-year-old girl) presented with pronounced (typical) gastrointestinal symptoms (chronic/episodic diarrhoea, abdominal distension, recurrent abdominal pain), with small intestinal histological studies showing at least partial villous atrophy (Marsh IIIa–IIIc).

However, two female AD patients (6.9 and 3.5 years old) newly diagnosed with CD were totally asymptomatic for CD, but histological studies showed subtotal/total villous atrophy at the small intestinal mucosa level (Marsh IIIb and IIIc respectively). These cases clearly illustrate challenges of diagnosing the atypical or silent forms of CD in AD.

When screening the randomly selected population of schoolchildren in our earlier study (Paper I), we found CD in 0.34% of the general children population (95% CI: 0.09–0.88). Based on our results, the risk for developing CD is more than four times higher in AD patients when compared to the general children population (OR=4.18; 95% CI: 1.12–15.64).

Difference in mean ages of AD patients and schoolchildren population could have biased our comparison, as the mean age of randomly selected schoolchildren is remarkably higher than that of AD patients (14.5 vs 4.3 years respectively). However, as CD incidence tends to increase with age, one could expect higher prevalence of CD in older study group (i.e. random schoolchildren population) instead.

Deficiency of local immune response and low IgA levels have been associated with higher prevalence of autoimmune and allergic diseases (Hodgson et al 1976; Aghamohammadi et al 2009). In this study of 351 AD children, IgA deficiency was found in 2.6% patients: that is twelve times higher prevalence than in the Estonian general population (0.2%)(Velbri et al 2005). As IgA has major role in providing immune responses at the mucosal level, IgA deficiency, together with recurrent infections, may lead to inflammation at the mucosal level. On the other hand, permeability of intestinal mucosa has shown to increase both in CD and AD (Williams 1987; Walker-Smith et al 1990; Marsh 1992). Therefore, IgA deficiency, inflammation at the mucosal level and

increased permeability of intestinal mucosa may play a role in determining the passage of autoantigens through the intestine and channel the comanifestation of CD and AD. However, in our study, CD was not detected in any of the AD patients with IgA deficiency.

In autoimmune and allergic diseases, a major role for pathogenesis has been admitted to dysregulation of immune responses, suggesting a defect in the function of regulatory T cells (Treg) (Banham et al 2006; Taams et al 2006). Evidence suggests a role for Treg in the pathogenesis of both diseases – CD and AD. In several autoimmune diseases $CD4^+CD25^+$ Treg from peripheral blood were found to have reduced capacity to suppress T-cell proliferation and IFN-γ production. A study by Vorobjova et al showed increased FOXP3 expression in small intestinal mucosa of children with CD and type 1 diabetes (Vorobjova et al 2009). Ito et al have shown that percentages of circulating $FOXP3^+CD25^+$ Treg are higher in AD patients than in controls and correlate with the activity of the disease (Ito et al 2009). However, these cells are unable to leave the circulation and enter the skin, therefore resulting in the exaggerated cutaneous inflammatory response characteristic of AD (Verhagen et al 2006; Ito et al 2009). Hence, the defect of mucosal barrier together with dysregulated immune response may play a role in the coexistence of AD and CD. Future studies are needed to evaluate the role of $FOXP3^+CD25^+$ Treg in the intestinal mucosa of patients with concomitant AD and CD.

6.3. Autoimmune reactions in children with AD

Several studies have suggested that autoimmune mechanisms play a role in the development and progression of AD and IgE-mediated autoreactivity is significantly associated with persistence of eczema and the presence of impaired skin barrier and food allergy (Valenta et al 1999; Mothes et al 2005). High percentages of AD patients have been reported to have autoreactivity towards a broad spectrum of human proteins, and that has been shown to increase during exacerbations of skin inflammation (Wolkerstorfer et al 1999; Mittermann et al 2004; Szakos et al 2004). Autoimmune processes may play a pathogenic role in severe and chronic forms of atopy (Valenta et al 2009).

6.3.1. General autoimmune background of AD

Among autoimmune markers, antibodies against different common components of cells have been widely used for detection of general autoimmune propensity and background in various diseases. Among many candidates, autoantibodies to nuclear proteins and protein complexes, collectively called ANA, have received considerable attention in many diseases (Agmon-Levin et al 2014). ANA-positivity in AD patients has varied widely in earlier reports, some of them showing remarkable difference and others, no significant difference in the

prevalence of ANA between AD and control patients (Williams et al 1994; Ohkouchi et al 1999; Higashi et al 2009; Tang et al 2012). We found low titer ANA in children with AD at the frequency (13.6%) comparable to the control group (12.8%).

Although most of our studied patients with AD belonged to extrinsic form of AD (70.2%) and the rate for ANA-positivity was somewhat higher in this group of patients, there was no statistical difference of ANA-positivity between patients with intrinsic and extrinsic type of AD. Although we found ANA-positivity slightly more frequently in AD patients with elevated serum IgE levels (15.0%) when compared to patients with normal total-IgE levels (11.4%), there was no statistically significant difference. However, increased total-IgE levels have been associated with increased prevalence of IgE-autoreactivity, and the latter may perpetuate allergic inflammation (Tang et al 2012).

We found ANA more often in female than male patients, and the difference could probably be explained by the overall tendency for autoimmunity in females, but also by the higher age of females in our study group. As the prevalence of ANA generally tends to increase with older age, prevalence of ANA-positivity could be lower in younger patient groups. On the contrary, we found ANA-positivity in AD patients already at the age of 2 years, whereas in the control group, ANA was found at a remarkably older age.

Although some earlier reports have shown higher prevalence and higher titres of ANA in more severe forms of AD, we did not find significant differences in relation to the overall severity of AD (Mothes et al 2005; Higashi et al 2009). We have found ANA at slightly higher titres in AD patients when compared to controls, with the fluorescent pattern being mostly homogenous or speckled. ANA is often present many years before the clinical manifestation of an autoimmune disease. As we have found ANA in remarkably younger age in AD patients, we could suggest higher risk for autoimmune diseases in patients with AD, especially those with AD since early infancy. Therefore patients with active and severe AD should be considered for screening for ANA, and ANA-positive (especially in high titer) patients should be regularly followed-up, as these patients could be disposed to earlier development of systemic autoreactivity.

6.3.2. IgA type antibodies to transglutaminase 1 and 3 and their potential role in pathogenesis of AD

In recent years, characterisation of skin-related immune processes and involvement of autoimmune reactions associated with the pathogenesis of AD have received much attention (Liedén et al 2012; Tang et al 2012). So far, there is no reliable biomarker or autoantigen that can distinguish AD from other diseases (Eichenfield et al 2014). As skin barrier defects play an important role in pathogenesis of AD, and epidermal transglutaminases have been suggested as playing a role in skin barrier repair (Cheng et al 2009; de Koning et al 2012;

Liedén et al 2012), we hypothesised that IgA-anti-TG1 and IgA-anti-TG3 could play a role in AD development.

We found significantly higher levels of IgA-anti-TG1 and IgA-anti-TG3 in CD patients, but not in patients with AD, suggesting that circulating IgA antibodies specific for TG isoenzymes expressed in the dermis are not characteristic of AD.

In accordance with earlier studies (Marietta et al 2008; Jaskowski et al 2009), we found IgA-anti-TG3 in 18% of CD patients. Considering IgA-anti-TG3 as a marker for DH, the finding may indicate the clinical development of the CD skin phenotype later in life (Hull et al 2008). However, the high prevalence (36%) of IgA-anti-TG1 responses in CD patients has not been reported before and is therefore difficult to explain.

We found moderate correlation between IgA-anti-TG2 and IgA-anti-TG1 or IgA-anti-TG3, indicating possible cross-reactivity between tested TGs. Nevertheless, we claim that antigen-specific IgA reactions exist against separate TGs, as none of the controls or AD patients had significantly elevated IgA-anti-TG1 and/or IgA-anti-TG3 in association with IgA-anti-TG2.

Based on our results, circulating antibodies to TG1 and TG3 might not be related to AD. Whether these antibodies have a role in AD pathogenesis will require further studies focusing on IgA-anti-TG1 and IgA-anti-TG3 measurement in the skin biopsies of AD patients.

7. CONCLUSIONS

1. Our nationwide study including all diagnosed children with CD up to 19 years of age showed more than a 30-fold increase in the incidence of CD together with a shift towards older age and atypical presentation over a 35-year period. However, prevalence of childhood CD in Estonia is at least 0.34% in a random sample of schoolchildren aged 9 and 15 years and it has not changed during the 10 years of follow-up.
2. Prevalence of CD in children with AD is more than four times higher than in the general children population in Estonia. In AD patients, CD tends to manifest with atypical gastrointestinal symptoms or asymptomatically, therefore, children with active AD should be considered for screening for CD in order to prevent serious long-term complications due to small intestinal mucosal damage. Special attention should be paid to AD patients with gastrointestinal symptoms.
3. The frequency of ANA positivity does not differ between children with AD and the control group. No difference in ANA-positivity was found between intrinsic and extrinsic subtypes of AD or between different sensitisation patterns. In AD patients, ANA was found in younger patients and in slightly higher titres, but the difference was not significant compared to controls.
4. IgA-anti-TG1 and IgA-anti-TG3 were more common and in higher levels among patients with CD when compared to controls or AD patients. In CD patients, the antibodies tended to appear simultaneously, but not in AD patients. The higher prevalence of IgA-anti-TG3 in CD patients may indicate the development of skin phenotype of CD in later life. The level of circulating antibodies might not be related with the skin lesions and the future work should focus on measuring IgA-anti-TG1 and IgA-anti-TG3 in the skin biopsies of AD patients.

8. FUTURE PROSPECTS

The present study shows more than a 30-fold increase in the incidence of CD together with a shift towards atypical clinical presentation and older age of presentation during a 35-year period in Estonia. As recent publications have reported increasing prevalence of CD in older age groups, it would be interesting to follow-up with the random schoolchildren population to find out the rate of new CD cases in adulthood. Also, the recent works put the focus back on the microbial load and the potential protective measures (infant feeding practices, use of pre- and probiotics, etc) that would provide us with valuable information for preventing CD in high-risk populations.

The more than four times higher prevalence of CD in AD patients compared to the general population emphasizes the need of larger international multicentered study for evaluating the (cost)effectiveness of screening AD patients for CD (as a risk group), as it has been applied for type 1 diabetes.

Autoimmune reactions in AD pathogenesis should deserve more attention in future studies, concentrating on finding autoantigen target for AD and specific biomarkers for differentiating phenotypes of AD. As our results indicate that circulating antibodies to TG1 and TG3 might not be related to AD, future studies should focus on assessing the role of IgA-anti-TG1 and IgA-anti-TG3 in AD pathogenesis at the skin level. Based on the associations found between AD and CD – two chronic inflammatory diseases with dysregulated immune responses, the role of $FOXP3^{+}CD25^{+}$ Treg and dendritic cell subgroups in intestinal mucosa remains to be evaluated.

SUMMARY IN ESTONIAN

Lapseea tsöliaakia Eestis, esinemine atoopilise dermatiidiga lastel ja koosesinemise immunoloogiline iseloomustus

Tsöliaakia on multifaktoriaalne krooniline haigus, mille korral nisu-, rukki-, odra- ja võimalik, et ka kaeravalkude poolt vallandatavad ning koe- ehk 2. tüüpi transglutaminaasi vastu tekkivad autoimmuunreaktsioonid põhjustavad päriliku eelsoodumusega isikutel peensoole limaskesta kahjustuse – hattude atroofia ja krüptide hüpertroofia. Tsöliaakia tekkes on oluline roll nii geneetilisel eelsoodumusel (ligikaudu 95%-l haigetest on leitud HLA DR3DQ2 haplotüüp) kui ka loomulikul ja omandatud immuunvastusel. Patofüsioloogiliste protsesside kaskaad saab tõenäoliselt alguse peensoole limaskesta barjäärifunktsiooni häirumisest, sellele järgneb toksiliste peptiidide esitlemine antigeeni esitlevate rakkude pinnal olevate HLA DQ2 või DQ8 molekulide kaudu T-helper lümfotsüütidele, mille tulemusena vabanevad mitmed proinflammatoorsed tsütokiinid. Järgneb autoreaktiivsete B-rakkude proliferatsioon ning plasmarakkude poolt deamideeritud gliadiinipeptiidide-, endomüüsiumi- ja koetransglutaminaasivastaste antikehade produktsioon. Põletikulise reaktsiooni tõttu suureneb intraepiteliaalsete lümfotsüütide hulk peensoole limaskestas ning tsütotoksiliste mediaatorite vabanemine viib enterotsüütide kahjustuse ja hattude atroofia tekkeni. Põletikust tingitud kahjustus kutsub aga esile koetransglutaminaasi vabanemise, suurendades seeläbi ka T-lümfotsüütide reaktiivsust.

Tsöliaakia varajane avastamine ja seega õigeaegne ravi on iseäranis oluline, sest see võimaldab vältida ulatuslikust peensoolelimaskesta kahjustusest tulenevaid tüsistusi – kasvupeetust, jäävhammaste struktuuri defekte, rauavaegusaneemiat, osteoporoosi, reproduktiivse funktsiooni häireid, pahaloomulisi kasvajaid. Tsöliaakia ainus ravi on range ja eluaegne gluteenivaba dieet, mille toimel peensoole limaskest paraneb ning vaevused ja sümptomid taanduvad.

Uutele rahvusvahelistele tsöliaakia diagnostika ja ravijuhistele tuginedes on klassikalise tsöliaakia korral peamiseks ilminguks malabsorptsioonisündroom (korduv või krooniline kõhulahtisus koos kaalu- ja kasvupeetuse või kõhnumisega), seevastu atüüpiliste seedetrakti vaevuste korral kaalu- ja kasvupeetust ega kõhnumist ei kaasu. Lisaks võib tsöliaakia väljenduda seedetraktiväliste vaevustega või hoopis asümptomaatilisena.

Kuni 1990 aastateni peeti tsöliaakiat harvaesinevaks lastehaiguseks, sest peamiselt kliinilisele kahtlusele tuginenud diagnoosimise alusel leiti erinevates riikides haigust vähem kui 0,1%-l lastest. Kuid viimastel aastatel läbiviidud uuringutele (sh sõeluuringutele) tuginedes esineb tsöliaakiat palju sagedamini kui senini on arvatud ja avastatud, kuni 3% rahvastikust, ning üha sagedamini ka vanemas eas. Seejuures on ligikaudu pooled haigusjuhud varjatud või atüüpilise kliinilise pildiga, jäädes seetõttu sageli õigeaegselt diagnoosimata.

Ka Eestis on tsöliaakiat peetud kaua väga harva esinevaks haiguseks – aastatel 1976–1989 diagnoositi Eestis vaid 0,04 juhtu 1000 elusalt sündinu kohta. Ajavahemikul 1990–1994 läbiviidud aktiivse kliinilise otsingu ja kogu Eestis rakendatud sõeluuringute abil sagenes tsöliaakia diagnoosimine küll oluliselt (0,37 uut juhtu 1000 elusalt sündinu kohta), kuid võrreldes teiste riikidega jäi erinevus siiski umbes kümnekordseks.

Tsöliaakiat on kirjeldatud koos ligi 100 erineva haigusega, kõige sagedamini koos I tüüpi suhkurtõve, erinevate autoimmuunhaiguste või kromosomaalsete häiretega. Kaasuva haiguse foonil avaldub tsöliaakia sageli varjatud või atüüpilises vormis ning seetõttu jääb sageli õigeaegselt avastamata. Atoopilise dermatiidi ja tsöliaakia, kui kahe lapseea kõige sagedamini esineva kroonilise immuunsüsteemi haiguse omavaheline seos on senini täpselt teadmata.

Atoopiline dermatiit on multifaktoriaalne krooniline põletikuline nahahaigus, mida iseloomustab naha sügelus, eksematoosne lööve näo piirkonnas, käte ja jalgade sirutus- (imikutel ning väikelastel) ning painutuspindadel (vanematel lastel). Kirjanduse andmetel esineb atoopiline dermatiit 10–20% lastest. Atoopilise dermatiidi tekkes on oluline roll nii geneetilisel eelsoodumusel, väliskeskkonna faktoritel, naha barjäärifunktsiooni häirumisel kui ka immunoregulatoorsetel mehhanismidel. Haigust võib klassifitseerida väliste ja sisemiste faktorite poolt vahendatud alavormidesse. Varasemalt peeti atoopilist dermatiiti puhtalt T-helper 2 raku poolt vahendatud haiguseks, kuid viimaste aastate uuringutega on selgunud, et ka autoimmuunreaktsioonid etendavad antud haiguse patogeneesis olulist rolli. Kuigi valdaval osas atoopilise dermatiidi patsientidest ei esine kõrgenenud üldist IgE taset ega allergeenide suhtes sensibiliseerumist, on lastel sageli leitud sensibiliseerumist toiduallergeenide suhtes, kuid nähtuse põhjuslikkuse osas ei ole jõutud ühisele seisukohale. Kuna toiduallergia korral on leitud, et haigete peensoolelimaskesta permeaabelsus on märkimisväärselt suurenenud, siis võib seedetrakti limaskesta permeaabelsuse muutustel ning limaskesta kahjustusel olla teatav roll ka atoopilise dermatiidi tekkes.

Üksikud varasemad uuringud viitavad, et tsöliaakia ja allergilised haigused võivad esineda samaaegselt. Kirjanduse andmetel esineb tsöliaakiahaigetel allergiat sagedamini kui üldpopulatsioonis. Samuti on leitud, et tsöliaakiahaigetel on atoopia 1. tüüpi diabeedi järel tsöliaakiaga kõige sagedamini kaasuv haigus ning tsöliaakiahaigetel on atoopilise dermatiidi tekkeks võrreldes üldpopulatsiooniga kolmekordne risk. Samas on ka atoopilise dermatiidi patsientide hulgas sagedamini leitud autoimmuunnähte ning ka tsöliaakiat. Senini on erialakirjanduses diskuteeritav, kas tsöliaakia ja atoopilise dermatiidi koosesinemine on juhuslik või on tegemist tõelise assotsiatsiooni ja ühiste patogeneetiliste mehhanismidega. Kuigi nii tsöliaakia kui atoopilise dermatiidi patogneesis osalevad sarnased immunoloogilised komponendid, ei ole teada kas need mõjutavad ka nende haiguste koosesinemist.

Käesoleva uuringu eesmärgiks oli:

– hinnata tsöliaakia haigestumust Eesti lastel ja iseloomustada haigestumuses ja kliinilises avaldumises esinevaid muutusi,

– hinnata tsöliaakia levimust aktiivse atoopilise dermatiidiga lastel ja iseloomustada atoopilise dermatiidi ja tsöliaakia koosesinemise kliinilisi eripärasid,
– hinnata atoopilise dermatiidiga laste üldist autoimmuunset tausta ja iseloomustada patsientide erinevusi tulenevalt tuumavastaste antikehade olemasolust,
– määrata IgA tüüpi autoantikehad epidermaalsete transglutaminaaside (transglutaminaas 1 ja 3) vastu vereseerumis ning hinnata nende antikehade potentsiaalset rolli atoopilise dermatiidi patogeneesis.

Tsöliaakia levimuse hindamiseks viidi läbi tsöliaakia sõeluuringud juhusliku valimi alusel 1998–1999 aastal kogutud 1160 Tartumaa 9 ja 15 aastasel koolilapsel. Sama gruppi uuriti korduvalt 10-aastase perioodi möödudes 2007–2008 aastal.

Tsöliaakia haigestumuse hindamiseks Eestis analüüsiti perioodil 1976–2010 kõiki Eesti lastel diagnoositud tsöliaakia esmasjuhte. Andmed on kogutud Tartu Ülikooli Kliinikumi Lastekliinikust ja Tallinna Lastehaiglast.

Lisaks kaasati uuringusse 351 järjestikust aktiivse atoopilise dermatiidiga last Tallinna Lastehaiglast ning 28 histoloogiliselt kinnitatud tsöliaakiaga last Tallinna Lastehaiglast ja Tartu Ülikooli Kliinikumi Lastekliinikust. Kontrollgrupi moodustasid muudel põhjustel hospitaliseeritud 56 last Tallinna Lastehaiglast, kellel muudel põhjustel teostatud gastroskoopia abil on kinnitatud histoloogiliselt normaalne peensoole limaskest, ning 61 last Tartu Ülikooli Kliinikumi Lastekliinikust. Kontrollgruppi kaasatud lastel eelnevalt teostatud uuringute tulemusena autoimmuunset ega ägedat põletikulist haigust ei leitud.

Koolilastel läbiviidud uuringute alusel leidsime tsöliaakia levimuseks Eesti lastel vähemalt 0,34%. Sama grupi korduval uurimisel kümne aastase perioodi järel uusi tsöliaakiajuhte ei lisandunud. Samas hinnates tsöliaakia haigestumust kogu Eestis diagnoositud lastel, selgub enam kui 30-kordne haigestumuskordaja tõus 35-aastase uuringuperioodi jooksul – aastatel 1976–1980 diagnoositi vaid 0,10 juhtu 100 000 inimaasta kohta, seevastu aastatel 2006–2010 diagnoositi 3,14 juhtu 100 000 inimaasta kohta. Samas on aastate lõikes täheldatav nii märkimisväärne keskmise vanuse tõus diagnoosimisel kui ka haiguse kliinilise pildi varieeruvus – kui uuringu algaastatel diagnoositi kõik tsöliaakiajuhud alla kaheaastastel tüüpiliste seedetrakti kaebustega lastel, siis uuringu viimastel aastatel diagnoositi enamus juhte atüüpilise või asümptomaatilise väljendusega lastel, kellel vanust üle viie aasta.

Atoopilise dermatiidiga laste grupis diagnoositi tsöliaakia viiel lapsel 351-st (1,4%). Viiest diagnoositud lapsest kolmel esinesid väljendunud seedetrakti vaevused ning peensoole histoloogilisel uuringul leiti vähemalt osaline hattude atroofia (Marsh IIIa–IIIc). Ülejäänud kahel diagnoositud lapsel tsöliaakiale viitavaid kaebusi ei esinenud, kuigi vereanalüüsis leiti kõrges tiitris IgA tüüpi koetransglutaminaasivastased antikehad ning peensoole histoloogilisel uuringul esines väljendunud hattude atroofia (Marsh IIIb ja IIIc).

Kui võrrelda tsöliaakia levimust koolilastel ja atoopilise dermatiidiga lastel, siis on risk tsöliaakia tekkeks atoopilise dermatiidiga laste hulgas üle nelja korra kõrgem. Tulenevalt tsöliaakia sagedasest atüüpilisest või asümptomaatilisest avaldumisest ning atoopilise dermatiidiga patsientide suurenenud riskist, oleks soovitatav kaaluda atoopilise dermatiidiga patsientidel plaaniliste tsöliaakia sõeluuringute teostamist, vältimaks peensoole kahjustusest tingitud tüsistusi. Kindlasti soovitame võimaliku tsöliaakia suhtes uurida atoopilise dermatiidi patsiente, kellel esinevad seedetrakti vaevused.

Valdaval osal uuritud atoopilise dermatiidi patsientidest (70,2%) esines väliste faktorite poolt vahendatud atoopilise dermatiidi vorm ning enamikul oli täheldatava kõrgenenud üldine IgE tase (59,5%) ja sensibiliseerumine toiduallergeenide suhtes (44,2%). Mitmed uuringud on atoopilise dermatiidi tekkes ja progressioonis täheldanud teatavat rolli autoimmuunreaktsioonidel, kuigi haigusspetsiifilist autoantigeeni ega biomarkerit seni leitud ei ole. Teostatud uuringutega leidsime tuumavastaseid antikehi võrdselt nii kontrollgrupi patsientidel kui ka atoopilise dermatiidiga patsientidel. Kuigi need antikehad esinesid atoopilise dermatiidiga patsientidel veidi kõrgemas tiitris ja nooremas vanuses võrreldes kontrollgrupiga, ei olnud erinevused statistiliselt olulised. Tuumavastaseid antikehi leiti sagedamini kõrgenenud IgE tasemega patsientidel (15,0% vs 1,4) ja väliste faktorite poolt vahendatud atoopilise dermatiidiga vormiga patsientidel (15,2% vs 9,7%), kuid erinevused ei olnud statistiliselt olulised.

Kuna autoantikehad ilmuvad sageli aastaid enne autoimmuunhaiguse väljakujunemist, siis võivad saadud tulemused viidata suurenenud autoimmuunreaktsioonide riskile varajase algusega aktiivse atoopilise dermatiidi korral. Seetõttu peaks atoopilise dermatiidiga patsiente uurima tuumavastaste antikehade suhtes ning jälgima võimaliku autoimmuunhaiguse väljakujunemise osas.

Atoopilise dermatiidi patogeneesis on viimase aja uuringute põhjal oluline roll naha kaitsebarjääri häirumisel ja selle taastamisel. Tulenevalt epidermaalsete transglutaminaaside võimalikust rollist epidermaalse barjääri taastamises, võiks neil olla ka teatav roll atoopilise dermatiidi patogeneesis. Uurides aga atoopilise dermatiidi, tsöliaakia ja kontrollgrupi lapsi transglutaminaas 1 ja 3 vastaste antikehade suhtes, leidsime antud antikehi tsöliaakiapatsientidel oluliselt suuremas hulgas kui atoopilise dermatiidi või kontrollgrupi patsientidel. Seega ei ole alust tsirkuleerivaid epidermaalsete transglutaminaaside vastaseid antikehi seostada atoopilise dermatiidi korral esineva nahakahjustusega. Nende võimaliku rolli väljaselgitamiseks atoopilise dermatiidi patogeneesis oleks vajalik hinnata antud antikehi atoopilise dermatiidiga patsientide nahabiopsias.

Lähtudes antud uurimuse tulemustest võib väita, et tsöliaakia esineb Eestis oluliselt sagedamini kui varasemalt arvatud (vähemalt 0,34%) ning väljendub üha sagedamini atüüpiliste kaebustega või hoopis asümptomaatiliselt ning enamasti (71,7%) üle viie aasta vanustel lastel.

Atoopilise dermatiidiga patsientide hulgas esineb tsöliaakiat üle nelja korra sagedamini kui üldrahvastikus (1,4% vs 0,34%), mistõttu vältimaks peensoole limaskesta kahjustusest tulenevaid tüsistusi, tuleks kaaluda atoopilise dermatiidi lisamist tsöliaakia riskigruppide hulka (nagu seda on tehtud 1. tüüpi diabeediga).

Atoopilise dermatiidi korral võivad tuumavastased autoantikehad esineda juba varases eas ning seetõttu tuleks eeskätt raskekululise atoopilise dermatiidiga patsiente uurida nende antikehade suhtes ning antikeha-positiivseid patsiente jälgida regulaarselt võimaliku autoimmuunhaiguse väljakujunemise suhtes.

Tsirkuleerivad epidermaalsete transglutaminaaside (transglutaminaas 1 ja 3) vastased antikehad ei assotsieeru atoopilise dermatiidi puhuse nahakahjustusega. Samas on transglutaminaas 1 ja 3 vastased antikehad sageli leitavad tsöliaakiaga patsientidel (vastavalt 36% ja 18%), samaaegselt koetransglutaminaasi vastaste antikehadega. Transglutaminaas 3 vastaste antikehade leid võib viidata herpetiformse dermatiidi kõrgenenud tekke riskile, kuid transglutaminaas 1 vastaste antikehade esinemine vajab edasisi täpsustavaid uuringuid. Transglutaminaaside võimaliku rolli hindamiseks atoopilise dermatiidi patogeneesis oleks vajalikud edasised täpsustavad uuringud atoopilise dermatiidi patsientide nahabioptaadist.

REFERENCES

Adkinson NF, Busse WW, Bochner BS, et al. (2009) Atopic dermatitis. *Middleton's Allergy: Principles and Practice, 7th edition*. Elsevier Inc

Aghamohammadi A, Cheraghi T, Gharagozlou M, et al. (2009) IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol* 29:130–6

Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. (2014) International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 73:17–23

Banham AH, Powrie FM, Suri-Payer E. (2006) FOXP3+ regulatory T cells: current controversies and future perspectives. *Eur J Immunol* 36:2832–6

Barker JM, Liu E. (2008) Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. *Adv Pediatr* 55:349–65

Bieber T. (2008) Atopic dermatitis. *N Engl J Med* 358:1483–94

Björkstén B, Naaber P, Sepp E, et al. (1999) The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 29:342–6

Borroni G, Biagi F, Ciocca O, et al. (2013) IgA anti-epidermal transglutaminase autoantibodies: a sensible and sensitive marker for diagnosis of dermatitis herpetiformis in adult patients. *J Europ Acad Dermatol Venereol* 27:836–41

Bottaro G, Cataldo F, Rotolo N, et al. (1999) The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. *Am J Gastroenterol* 94:691–6

Breda L, Nozzi M, De Sanctis S, et al. (2010) Laboratory tests in the diagnosis and follow-up of pediatric rheumatic diseases: an update. *Semin Arthritis Rheum* 40:53–72

Böttcher MF, Björkstén B, Gustafson S, et al. (2003) Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 33:295–300

Catassi C, Fornaroli F, Fasano A. (2002) Celiac disease: From basic immunology to bedside practice. *Clin Applied Immunol Rev* 3:61–71

Cheng T, Tjabringa GS, van Vlijmen-Willems IMJJ, et al. (2009) The cystatin M/E-controlled pathway of skin barrier formation: expression of its key components in psoriasis and atopic dermatitis. *Brit J Dermatol* 161:253–64

Ciacci C, Cavallaro R, Iovino P, et al. (2004) Allergy prevalence in adult celiac disease. *J Allergy Clin Immunol* 113:1199–203

Collin P, Reunala T, Pukkala E, et al. (1994) Coeliac disease – associated disorders and survival. *Gut* 35:1215–8

Cooper BT, Holmes GKT, Cooke WT. (1978) Coeliac disease and immunological disorders. *Br Med J* 1:537–9

Cork MJ, Danby SG, Vasilopoulos Y, et al. (2009) Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 129:1892–908

de Freitas IN, Sipahi AM, Damião AO, et al. (2002) Celiac disease in Brazilian adults. *J Clin Gastroenterol* 34:430–4

de Koning HD, van den Bogaard EH, Bergboer JGM, et al. (2012) Expression profile of cornified envelope structural proteins and keratinocyte differentiation-regulating proteins during skin barrier repair. *Brit J Dermatol* 166:1245–54

Denham JM, Hill ID. (2013) Celiac disease and autoimmunity: review and controversies. *Curr Allergy Asthma Rep* 13:347–53

Dharmage SC, Lowe AJ, Matheson MC, et al. (2014) Atopic dermatitis and the atopic march revisited. *Allergy* Allergy 69:17–27
Dieterich W, Ehnis T, Bauer M, et al. (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 3:797–801
Eckert RL, Sturniolol MT, Broome A-M, et al. (2005) Transglutaminase function in epidermis. *J Invest Dermatol* 124:481–92
Eichenfield LF, Tom WL, Chamlin SL, et al. (2014) Guidelines of care for the management of atopic dermatitis: Section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol* 70:338–51
Elli L, Bonura A, Garavaglia D, et al. (2012) Immunological comorbidity in coeliac disease: associations, risk factors and clinical implications. *J Clin Immunol* 32:984–90
Ellman LK, Chatchatee P, Sicherer SH, et al. (2002) Food hypersensitivity in two groups of children and young adults with atopic dermatitis evaluated a decade apart. *Pediatr Allergy Immunol* 13:295–8
Eyerich K, Novak N. (2013) Immunology of atopic eczema: overcoming the Th1/Th2 paradigm. *Allergy* 68: 974–82
Fasano A, Catassi C. (2012) Clinical practice. Celiac disease. *N Engl J Med* 367:2419–26
Fuiano N, Incorvaia C. (2012) Dissecting the cause of atopic dermatitis in children: less foods, more mites. *Allergol Int* 61:231–43
Gallo C, Vighi G, Schroeder J, et al. (1992) Chronic urticaria atopic dermatitis and celiac disease. *Am J Gastroenterol* 87:1684
Gee SJ. (1888) On the coeliac affection. St. *Bartholomew's Hospital Reports* 24:17–20
Giongo A, Gano KA, Crabb DB, et al. (2011) Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 5:82–91
Greco L, De Seta L, D'Adamo G, et al. (1990) Atopy and Coeliac disease: bias or true relation? *Acta Pædiatr Scand* 79:670–4
Green PH, Cellier C. (2007) Celiac disease. *N Engl J Med* 357:1731–43
Haapalahti M, Kulmala P, Karttunen TJ, et al. (2005) Nutritional status in adolescents and young adults with screen-detected celiac disease. *J Pediatr Gastroenterol Nutr* 40:566–70
Harro M, Eensoo D, Kiive E, et al. (2001) Platelet monoamine oxidase in healthy 9- and 15-years old children: the effect of gender, smoking and puberty. *Prog Neuropsychopharmacol Biol Psychiatry* 25:1497–511
Heel DA, West J. (2006) Recent advances in coeliac disease. *Gut* 55:1037–46
Higashi N, Niimi Y, Aoki M, et al. (2009) Clinical features of antinuclear antibody-positive patients with atopic dermatitis. *J Nippon Med Sch* 76:300–7
Hitomi K. (2005) Transglutaminases in skin epidermis. *Eur J Dermatol* 15:313–9
Hodgson HJF, Davies RJ. (1976) Atopic disorders and adult coeliac disease. *Lancet* 1:115–7
Holmes GK. (2001) Coeliac disease and Type 1 diabetes mellitus – the case for screening. *Diabet Med* 18:169–77
Hull CM, Liddle M, Hansen N, et al. (2008) Elevation of IgA anti-epidermal transglutaminase antibodies in dermatitis herpetiformis. *Brit J Dermatol* 159:120–4
Husby S. (1988) Dietary antigens: uptake and humoral immunity in man. *APMIS Suppl* 1:1–40

Husby S, Koletzko S, Korponay-Szabó IR, et al. (2012) European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 54:136–60

Ito Y, Adachi Y, Makino T, et al. (2009) Expansion of FOXP3-positive CD4+CD25+ T cells associated with disease activity in atopic dermatitis. *Ann Allergy Asthma Immunol* 103:160–5

Ivarsson A. (2005) The Swedish epidemic of coeliac disease explored using an epidemiological approach – some lessons to be learnt. *Best Pract Res Clin Gastroenterol* 19:425–40

Ivarsson A, Myléus A, Norström F, et al. (2013) Prevalence of childhood celiac disease and changes in infant feeding. *Pediatrics* 131:e687–94

Ivarsson A, Persson LA, Nystrom L, et al. (2000) Epidemic of coeliac disease in Swedish children. *Acta Paediatr* 89:165–71

Iwańczak B, Matusiewicz K, Iwańczak F. (2013) Clinical picture of classical, atypical and silent celiac disease in children and adolescents. *Adv Clin Exp Med* 22:667–73

Jaskowski TD, Hamblin T, Wilson A, et al. (2009) IgA anti-epidermal transglutaminase antibodies in dermatitis herpetiformis and pediatric celiac disease. *J Invest Dermatol* 129:2728–30

Järvinen KM, Konstantinou GN, Pilapil M, et al. (2013) Intestinal permeability in children with food allergy on specific elimination diets. *Pediatr Allergy Immunol* 24:589–95

Kagnoff MF. (2005) Overview and pathogenesis of celiac disease. *Gastroenterology* 128:S10–8

Kang JY, Kang AHY, Green A, et al. (2013) Systematic review: worldwide variation in the frequency of coeliac disease and changes over time. *Aliment Pharmacol Ther* 38:226–45

Klöck C, DiRaimondo T, Khosla C. (2012) Role of transglutaminase 2 in celiac disease pathogenesis. *Semin Immunopathol* 34:513–22

Kneepkens CMF, von Blomberg BME. (2012) Clinical practice. Coeliac disease. *Eur J Pediatr* 171:1011–21

Kondrashova A, Mustalahti K, Kaukinen K, et al. (2008) Lower economic status and inferior hygienic environment may protect against celiac disease. *Ann Med* 40:223–31

Kotze LMS. (2009) Celiac disease in Brazilian patients: associations, complications and causes of death. Fourty years of clinical experience. *Arq Gastroenterol* 46:261–9

Kubo A, Nagao K, Amagai M. (2012) Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *Clin Invest* 122:440–7

Kupfer SS, Jabri B. (2012) Pathophysiology of celiac disease. *Gastrointest Endoscopy Clin N Am* 22:639–60

Leung DYM. (2013) New insights into atopic dermatitis: role of skin barrier and immune dysregulation. *Allergol Int* 62:151–61

Leung DYM, Bieber T. (2003) Atopic dermatitis. *Lancet* 361:151–60

Liedén A, Winge MCG, Sääf A, et al. (2012) Genetic variation in the epidermal transglutaminase genes is not associated with atopic dermatitis. PLOS *ONE* 7:e49694

Lohi S, Mustalahti K, Kaukinen K, et al. (2007) Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther* 26:1217–25

Lundin KE, Sollid LM. (2014) Advances in coeliac disease. *Curr Opin Gastroenterol* 30:154–62

Marietta EV, Camilleri MJ, Castro LA, et al. (2008) Transglutaminase autoantibodies in dermatitis herpetiformis and celiac sprue. *J Invest Dermatol* 128:332–5
Mariné M, Farre C, Alsina M, et al. (2011) The prevalence of coeliac disease is significantly higher in children compared with adults. *Aliment Pharmacol Ther* 33:477–86
Marsh MN. (1992) Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ("celiac sprue"). *Gastroenterology* 102:330–54
McGowan KE, Castiglione DA, Butzner JD. (2009) The changing face of childhood celiac disease in North America: impact of serological testing. *Pediatrics* 124:1572–8
Meresse B, Malamut G, Cerf-Bensussan N. (2012) Celiac disease: an immunological jigsaw. *Immunity* 36:907–19
Metsküla K, Grünberg H, Uibo O, et al. (1998) Antigliadin antibodies and autoantibodies among 9, 12 and 15 year-old schoolchildren. *Central-Europ J Immunol* 23:197–202
Mitt K, Uibo O. (1998) Low cereal intake in Estonian infants: the possible explanation for the low frequency of coeliac disease in Estonia. *Eur J Clin Nutr* 52:85–8
Mittermann I, Aichberger KJ, Bünder R, et al. (2004) Autoimmunity and atopic dermatitis. *Curr Opin Allergy Clin Immunol* 4:367–71
Moghtaderi M, Farjadian S, Kashef S, et al. (2012) Specific IgE to common food allergens in children with atopic dermatitis. *Iran J Immunol* 9:32–8
Mothes N, Niggemann B, Jenneck C, et al. (2005) The cradle of IgE autoreactivity in atopic eczema lies in early infancy. *J Allergy Clin Immunol* 116:706–9
Muro Y. (2001) Autoantibodies in atopic dermatitis. *J Dermatol Sci* 25:171–8
Mustalahti K, Catassi C, Reunanen A, et al. (2010) The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann Med* 42:587–95
Mäki M, Mustalahti K, Kokkonen J, et al. (2003) Prevalence of celiac disease among children in Finland. *N Engl J Med* 348:2517–24
Myléus A, Ivarsson A, Webb C, et al. (2009) Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. *J Pediatr Gastroenterol Nutr* 49:170–6
Nejentsev S, Koskinen S, Sjöroos M, et al. (1998) Distribution of insulin-dependent diabetes mellitus (IDDM)-related HLA alleles correlates with the difference in IDDM incidence in four populations of the Eastern Baltic region. *Tissue Antigens* 52:473–7
Norstsröm F, Lindholm L, Sandström O, et al. (2011) Delay to celiac disease diagnosis and its implications for health-related quality of life. *BMC Gastroenterol* 11:118
Novak N, Bieber T. (2003) Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 112:252–62
Ohkouchi K, Mizutani H, Tanaka M, et al. (1999) Anti-elongation factor-1alpha autoantibody in adult atopic dermatitis patients. *Int Immunol* 11:1635–40
Olén O, Bihagen E, Rasmussen F, et al. (2012) Socioeconomicposition and education in patients with coeliac disease. *Dig Liver Dis* 44:471–6
Olsson C, Hernell O, Hörnell A, et al. (2008) Difference in celiac disease risk between swedish birth cohorts suggests an opportunity for primary prevention. *Pediatrics* 122;528–34
Pike MG, Heddle RJ, Boulton P, et al. (1986) Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 86:101–4

Qiao S-W, Iversen R, Ráki M, et al. (2012) The adaptive immune response in celiac disease. *Semin Immunopathol* 34:523–40

Rabin RL, Levinson AI. (2008) The nexus between atopic disease and autoimmunity: a review of the epidemiological and mechanistic literature. *Clin Exp Immunol* 153:19–30

Reilly NR, Green PHR. (2012) Epidemiology and clinical presentation of celiac disease. *Semin Immunopathol* 34:473–8

Roma E, Panayiotou J, Karantana H, et al. (2009) Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. *Digestion* 80:185–91

Rubio-Tapia A, Kyle RA, Kaplan EL, et al. (2009) Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* ;137:88–93

Räsänen L, Lehto M, Turjanmaa K, et al. (1994) Allergy to ingested cereals in atopic children. *Allergy* 49:871–6

Salmi TT, Hervonen K, Laurila K, et al. (2013) Small Bowel Transglutaminase 2-specific IgA Deposits in Dermatitis Herpetiformis. *Acta Derm Venereol* doi:10.2340/00015555-1764 (E-pub ahead of print)

Sárdy M, Kárpáti S, Merkl B, et al. (2002) Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J Exp Med* 195:747–57

Schmid-Grendelmeier P, Simon D, Simon HU, et al. (2001) Epidemiology, clinical features, and immunology of the "intrinsic" (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 56:841–9

Selby WS, Painter D, Collins A, et al. (1999) Persistent mucosal abnormalities in coeliac disease are not related to the ingestion of trace amounts of gluten. *Scand J Gastroenterol* 34:909–14

Silverberg JI, Silverberg NB. (2012) Atopic dermatitis: update on pathogenesis and comorbidities. *Curr Derm Rep* 1:168–78

Simpson CR, Anderson WJA, Helms PJ, et al. (2002) Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized General Practice data. *Clin Exp All* 32:37–42

Spergel JM, Paller AS. (2003) Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 112:S118–27

Szakos E, Lakos G, Aleksza M, et al. (2004) Relationship between skin bacterial colonization and the occurrence of allergen-specific and non-allergen-specific antibodies in sera of children with atopic eczema/dermatitis syndrome. *Acta Derm Venereol* 84:32–6

Størdal K, Bakken IJ, Surén P, et al. (2013) Epidemiology of coeliac disease and comorbidity in Norwegian children. *J Pediatr Gastroenterol Nutr* 57:467–71

Taams LS, Palmer DB, Akbar AN, et al. (2006) Regulatory T cells in human disease and their potential for therapeutic manipulation. *Immunology* 118:1–9

Tang TS, Bieber T, Williams HC. (2012) Does “autoreactivity” play a role in atopic dermatitis? *J Allergy Clin Immunol* 129:1209–15

Teesalu K, Agardh D, Panarina M, et al. (2009) A modified ELISA for improved detection of IgA, IgG, and IgM anti-tissue transglutaminase antibodies in celiac disease. *Clin Chim Acta* 403:37–41

Tikkakoski S, Savilahti E, Kolho KL. (2007) Undiagnosed coeliac disease and nutritional deficencies in adults screened in primary health care. *Scand J Gastroenterol* 42:60–5

Tokura Y. (2010) Extrinsic and intrinsic types of atopic dermatitis. *J Dermatol Sci* 58:1–7

Uibo O. (1994a) Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Academic dissertation (Doctor of medical sciences). University Press. University of Tartu, 1–84

Uibo O. (1994b) Childhood celiac disease in Estonia: Efficacy of the IgA-class antigliadin antibody Test in the search for new cases. *J Pediatr Gastroenterol Nutr* 13:53–5

Uibo O, Metsküla K, Kukk T, et al. (1996) Results of coeliac disease screening in Estonia in 1990–1994. *Acta Paediatr* 412:39–41

Uibo O, Uibo R, Kleimola V, et al. (1993) Serum IgA anti-gliadin antibodies in an adult population sample. High prevalence without celiac disease. *Dig Dis Sci* 38:2034–7

Uibo R, Talja I, Jõgi R, et al. (1998) Autoantibodies in Estonia and Sweden, populations with different responses to allergens. *Int Arch Allergy Immunol* 117:126–30

Valenta R, Mittermann I, Werfel T, et al. (2009) Linking allergy to autoimmune disease. *Trends Immunol* 30:109–16

Valenta R, Natter S, Seiberler S, et al. (1999) Autoallergy: a pathogenetic factor in atopic dermatitis? *Curr Probl Dermatol* 28:45–50

van den Broeck HC, de Jong HC, Salentijn EM, et al. (2010) Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease. *Theor Appl Genet* 121:1527–39

Velbri S, Pärlist M, Leito K, et al. (2005) Primaarne immuunpuudulikkus Eestis. *Eesti Arst* 84:310–4

Verhagen J, Akdis M, Traidl-Hoffmann C, et al. (2006) Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol* 117:176–83

Verkasalo M, Tiilikainen A, Kuitunen P, et al. (1983) HLA antigens and atopy in children with coeliac disease. *Gut* 24:306–10

Viljamaa M, Kaukinen K, Pukkala E, et al. (2006) Malignancies and mortality in patients with coeliac disease and dermatitis herpetiformis: 30-year population-based study. *Dig Liver Dis* 38:374–80

Vilppula A, Kaukinen K, Luostarinen L, et al. (2009) Increasing prevalence and high incidence of celiac disease in elderly people: A population-based study. *BMC Gastroenterol* 9:49

Volta U, Bellentani S, Bianchi FB, et al. (2001) High prevalence of celiac disease in Italian general population. *Dig Dis Sci* 46:1500–5

Volta U, Tovoli F, Caio G. (2011) Clinical and immunological features of celiac disease in patients with Type 1 diabetes mellitus. *Expert Rev Gastroenterol Hepatol* 5:479–87

Voor T, Julge K, Böttcher MF, et al. (2005) Atopic sensitization and atopic dermatitis in Estonian and Swedish infants. *Clin Exp Allergy* 35:153–9

Vorobjova T, Uibo O, Heilman K, et al. (2009) Increased FOXP3 expression in small-bowel mucosa of children with coeliac disease and type I diabetes mellitus. *Scand J Gastroenterol* 44:422–30

Walker-Smith JA, Guandalini S, Schmitz J, et al. (1990) Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Child* 65:909–11

Whyte LA, Jenkins HR. (2013) The epidemiology of coeliac disease in South Wales: a 28-year perspective. *Arch Dis Child* 98:405–7

Whyte LA, Kotecha S, Watkins WJ, et al. (2014) Coeliac disease is more common in children with high socio-economic status. *Acta Paediatr* 103:289–94

Williams AJ. (1987) Coeliac disease and allergic manifestations. *Lancet* 1:808

Williams HC, Burney PGJ, Pembroke AC, et al. (1994) The U.K. Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Brit J Dermatol* 131:406–16

Wingren CJ, Björck S, Lynch KF, et al. (2012) Coeliac disease in children: a social epidemiological study in Sweden. *Acta Paediatr* 1010:185–9

Wolf R, Wolf D. (2012) Abnormal epidermal barrier in the pathogenesis of atopic dermatitis. *Clin Dermatol* 30:329–34

Wolkerstorfer A, de Waard van der Spek FB, Glazenburg EJ, et al. (1999) Scoring the severity of atopic dermatitis: three item severity score as a rough system for daily practice and as a pre-screening tool for studies. *Acta Derm Venereol* 79:356–9

Zauli D, Grassi A, Granito A, et al. (2000) Prevalence of silent coeliac disease in atopics. *Dig Liver Dis* 32:775–9

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Ilmunud on kaheksa teadusartiklit, millest viis rahvusvahelistes eelretsenseeritavates teadusajakirjades ning kolm ajakirjas Eesti Arst. Kaksteist ettekannet rahvusvahelistel erialakonverentsidel.

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