Tampere University

SAANA PAAVOLA

Presentation, Frequency, and Risk Factors of Celiac Disease in Relatives of Previously Diagnosed Patients

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Tampere, January 2023

Saana Paavola

ABSTRACT

Celiac disease is a chronic immune-mediated condition in which dietary gluten causes small-bowel mucosal damage in subjects at genetic risk for the disease. The disease affects approximately 1% of the population worldwide but remains heavily underdiagnosed. Even in Finland, where the diagnostic level of celiac disease is relatively good compared to many other countries, only about one third of those affected are currently diagnosed. A variable clinical picture hampers the recognition of patients and is one major reason for the suboptimal diagnostic yield. Celiac disease develops in genetically suspectible individuals and requires the presence of HLA (human leukocyte antigen) DQ2 and/or DQ8, without which the disease is highly unlikely.

An important risk group for celiac disease are the first-degree relatives (FDRs) of affected patients, who have an average 5-10 times increased risk of being affected compared to general population. The risk in more distant relatives has been much less studied, but may be also increased at least in second-degree relatives (SDRs). Several international guidelines and Finnish Current Care Guidelines recommend screening of FDRs, and some suggest extending screening to SDRs in cases where there already is more than one affected relative in the family. Exact recommendations on the implementation of family screening, however, are lacking, regarding, for example, the optimal age for screening and whether the screening should be repeated after one-time negative testing. The main reasons for this are that the individual risk factors for celiac disease among relatives are poorly understood and that systematic re-screening studies in family members are scarce.

The aim of the dissertation project was to assess the clinical picture of celiac disease within the same families, and further to evaluate the individual risk factors for screening positivity both at first screening and, among relatives with initially negative screening results, at later re-screening. The dissertation consists of three individual studies. Study populations were collected from family screening in 2006-2010 including approximately 1,000 celiac disease patients and their 3,000 previously non-celiac diseased relatives.

In Study **I**, the clinical picture of 200 siblings (100 sibling pairs) at diagnosis of celiac disease was evaluated, the first diagnosed sibling being an index patient. The

phenotype was categorized to gastrointestinal, malabsorption/anemia, extraintestinal and asymptomatic. Gastrointestinal symptoms were the most common among both index patients and later diagnosed siblings, but otherwise the symptoms were randomly distributed among siblings. Moreover, the results indicate that HLA genotypes do not explain the differences in the clinical picture.

In Study **II**, 2,714 at-risk relatives were screened for celiac disease and altogether 4.8% of them were affected. Although the percentage was highest among FDRs, it was also increased among SDRs and more distant relatives compared to general population. In addition, there were 229 relatives with previous diagnosis, giving an overall prevalence of celiac disease/screening positivity of 12.2% among all relatives. Age <18 years at diagnosis in index, age 41-60 years at screening in relative, being a sibling, and carrying high-risk HLA were risk factors for screening positivity. However, only high-risk HLA remained significant in multivariable analysis.

In Study **III**, all initially screening-negative relatives in Study **II** were invited to a follow-up study approximately ten years after the initial testing. Altogether 599 relatives participated. Eight relatives had received celiac disease diagnosis between the studies in normal clinical practice in healthcare and seven were screening-positive at the new screening, giving an incidence rate (IR) of 221/100,000 person-years. The IR was higher among subjects who were <30 years than those ≥30 years at initial screening and among carriers of high-risk HLA than among other HLA risk genotypes. In multivariable analysis, the effect of high-risk HLA overrode the effect of age.

This dissertation demonstrates that celiac disease may present with markedly different symptoms between siblings regardless of the HLA type, suggesting a significant role of environmental factors and/or non-HLA genes. Family screening revealed that a substantial part of the affected relatives was not detected in healthcare before the first screening and that new cases could also be found in all age groups at later re-screening. Furthermore, it was observed that the presence of high-risk HLA overides the effect of other risk factors, making determination of detailed HLA risk group an attractive idea for targeting of screening. Even "crude" assessment of HLA DQ2/8 could help to exclude follow-up screening from approximately 30% of relatives lacking these risk haplotypes. In light of these findings it seems reasonable to screen all FDRs after the index patient is diagnosed, but in the future studies on the cost-effectiveness of HLA in screening will be needed. In addition, although more studies on the risk of SDRs and more distant relatives are needed, the possibility of celiac disease should also be kept in mind in this subgroup.

TIIVISTELMÄ

Keliakia on immuunivälitteinen tauti, jossa ravinnon gluteeni aiheuttaa geneettisesti alttiille henkilölle suolen limakalvovaurion, villusatrofian. Taudin esiintyvyys on noin 1 % maailmanlaajuisesti, mutta se on vahvasti alidiagnosoitu. Jopa Suomessa, jossa tietoisuus keliakiasta ja diagnostinen valmius ovat hyvät verrattuna moniin muihin maihin, yksi kolmasosa potilaista on diagnosoitu. Vaihteleva taudinkuva tekee taudin tunnistamisen hankalaksi ja on yksi syy alidiagnostiikkaan. Keliakian kehittyminen vaatii perintötekijöiden määräämän HLA-DQ2 ja/tai -DQ8 molekyylien läsnäolon, joita ilman taudin kehittyminen on hyvin epätodennäköistä.

Tärkeän riskiryhmän muodostavat potilaiden ensimmäisen asteen sukulaiset, joiden riski on noin 5–10 kertainen väestöön verrattuna. Tätä kaukaisempien sukulaisten riskiä on paljon vähemmän tutkittu, mutta on jonkin verran näyttöä siitä, että riski on koholla myös toisen asteen sukulaisilla. Suurin osa kansainvälisistä suosituksista ja suomalainen Käypä Hoito -suositus suosittavat potilaiden ensimmäisten asteen sukulaisten seulontaa, ja osa suosituksista ulottaisi seulonnan myös toisen asteen sukulaisiin, joilla on perheessä enemmän kuin yksi aiemmin todettu potilas. Tarkemmat seulontasuositukset kuitenkin puuttuvat koskien muun muassa seulonnan aloitusikää ja sitä, onko seulontaa tarpeen toistaa kerran negatiivisen testaustuloksen jälkeen. Syy tähän on se, ettei yksilöllistä sairastumisriskiä perheenjäsenten kesken tunneta. Lisäksi systemaattisia tutkimuksia, joissa sukulaisia olisi seulottu säännöllisesti negatiivisen tuloksen jälkeen, on hyvin vähän.

Tämän väitöskirjatyön tavoitteena oli tutkia keliakian taudinkuvaa perheenjäsenten keskuudessa ja arvioida riskitekijöitä seulontapositiivisuudelle sekä ensimmäisessä seulonnassa että kerran seulontanegatiivisilla toistetussa seulonnassa. Väitöskirja koostuu kolmesta osatyöstä, joiden aineisto perustuu vuonna 2006-2010 tehtyyn perheenjäsenten seulontaan, johon osallistui noin 1000 aiempaa keliakiapotilasta ja heidän 3000 seulottua sukulaistaan.

Osatyössä **I** arvioitiin keliakian taudinkuvaa 200 sisaruksen (100 sisarusparin) kesken, joista ensin diagnosoitu keliaakikko oli indeksipotilas. Taudinkuva jaettiin vatsaoireisiin, imeytymishäiriöön ja anemiaan, suoliston ulkopuolisiin oireisiin sekä oireettomiin. Vatsaoireet olivat yleisimpiä sekä indeksipotilaiden että myöhemmin

diagnosoitujen sisarusten keskuudessa, mutta muuten oireet jakaantuivat sattumanvaraisesti sisarusten välillä, eikä HLA selittänyt näitä eroja.

Osatyössä **II** keliakia seulottiin vasta-aineilla 2714 sukulaiselta, joista 4,8 % oli seulontapositiivisia. Seulontapositiviisuus oli korkeinta ensimmäisen asteen sukulaisillla, mutta koholla myös toisen asteen ja sitä kaukaisemmilla sukulaisilla verrattuna normaaliväestöön. Lisäksi 229 sukulaisella oli aiemmin terveydenhuollossa diagnosoitu keliakia, jolloin keliakian/seulontapositiivisuuden kokonaisesiintyvyydeksi tuli 12,2 %. Seulontapositiivisuuden riskitekijöitä olivat indeksipotilaan diagnoosi-ikä alle 18 vuotta, seulotun ikä 41-60 vuotta, sisaruus verrattuna muihin sukulaissuhteisiin sekä seulotun korkean riskin HLA. Monitekijäanalyysissä ainoastaan korkean riskin HLA oli merkitsevä riskitekijä.

Osatyöhön **III** kutsuttiin ne sukulaiset, jotka saivat negatiivisen seulontatuloksen aiemmassa seulonnassa noin 10 vuotta aikaisemmin, ja yhteensä 599 osallistui. Kahdeksan sukulaista oli saanut keliakiadiagnoosin tutkimusten välissä ja seitsemän oli seulontapositiivisia uudessa seulonnassa. Ilmaantuvuustiheys oli 221/100,000 henkilövuotta ja se oli korkeampi niiden keskuudessa, jotka olivat olleet < 30-vuotiaita ensimmäisessä seulonnassa verrattuna heihin, jotka olivat olleet ≥ 30-vuotiaita, sekä heillä, joilla oli korkean riskin HLA verrattuna muihin riskigenotyyppeihin. Korkean riskin HLA ajoi riskitekijänä kuitenkin iän vaikutuksen ohi.

Tämä väitöskirja osoitti keliakian taudinkuvan vaihtelevan merkittävästi sairastuneiden sisarusten välillä. Erot HLA-tyypissä eivät selittäneet näitä eroavaisuuksia, mikä saattaa kertoa siitä, että ympäristötekijöillä ja ei-HLA geeneillä on merkitystä taudinkuvaan. Lisäksi todettiin, että keliakia oli tunnistamatta merkittävällä osalla sairastuneista sukulaisista ennen seulontatutkimusta ja uusia potilaita kaikista ikäryhmistä todettiin niin ikään jatkoseulonnassa. Korkean riskin HLA oli tärkein riskitekijä molemmissa seulonnoissa ja sen käyttöä voitaisiin mahdollisesti hyödyntää tulevaisuudessa seulonnan kohdentamiseen. Lisäksi toistetusta seulonnasta voitaisiin kokonaan luopua noin 30 % sukulaisista, joilla ei ole keliakialle alstistavaa HLA-tyyppiä. Tämänhetkisen tiedon perusteella vaikuttaa kannattavalta seuloa ensimmäisen asteen sukulaiset sen jälkeen, kun indeksipotilas on todettu, mutta jatkotutkimuksia kustannustehokkuudesta HLA-määrityksen käytön suhteen tarvitaan. Lisäksi lisää tutkimuksia kaivataan kaukaisempien kuin ensimmäisen asteen sukulaisten sairastumisriskistä, joskin keliakian mahdollisuus on muistettava myös heillä.

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ABBREVIATIONS

AGA	Anti-gliadin antibodies
ARA	Anti-reticulin antibodies
BMD	Bone mineral density
DGP-ab	Antibodies against deamidated gliadin peptides
DH	Dermatitis herpetiformis
EATL	Enteropathy-associated T-cell lymphoma
EMA	Endomysial antibodies
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
ESsCD	European Society for the Study of Celiac Disease
FDR	First-degree relative
GFD	Gluten-free diet
HLA	Human leukocyte antigen
IBS	Irritable bowel syndrome
IEL	Intraepithelial lymphocytes
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL-15	Interleukin 15
IR	Incidence rate
IRR	Incidence rate ratio
NHL	Non-Hodgkin's lymphoma
POCTs	Point of care tests
PPV	Positive predictive value
RR	Risk ratio
SDR	Second-degree relative
TGA	Transglutaminase 2 antibodies
TG2	Transglutaminase 2
USA	United States of America
Vh/CrD	Villous height/crypt depth ratio

ORIGINAL PUBLICATIONS

I Kauma S, Kaukinen K, Huhtala H, Kivelä L, Pekki H, Salmi T, Saavalainen P, Lindfors K, Kurppa K (2019): The phenotype of celiac disease has low concordance between siblings, despite a similar distribution of HLA haplotypes. Nutrients 11:479.

II Paavola S, Lindfors K, Kivelä L, Cerqueira J, Huhtala H, Saavalainen P, Tauschi R, Kaukinen K, Kurppa K (2021): Presence of high-risk HLA genotype is the most important risk factor for coeliac disease among at-risk relatives. Aliment Pharmacol Ther 54:805-813.

III Paavola S, Kurppa K, Huhtala H, Saavalainen P, Lindfors K, Kaukinen K (2022): Coeliac disease re-screening among once seronegative at-risk relatives: A long-term follow-up study. United European Gastroenterol J 10:585-593.

 The former last name of the first writer was Kauma.

Author's contribution

I: Processing of data, statistical analysis, interpretation of the results, writing of the manuscript

II: Processing of data, statistical analysis, interpretation of the results, writing of the manuscript

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REVIEW OF THE LITERATURE

1 INTRODUCTION

Celiac disease is a chronic immune-mediated condition affecting ~1% of the population globally, but it is heavily underdiagnosed (Singh et al. 2018). In genetically susceptible individuals, ingestion of gluten - a protein component of wheat, barley, and rye – drives an immune reaction which leads to inflammation and eventually to morphological damage of the duodenal mucosa (Lindfors et al. 2019). Gluten-free diet (GFD) restores the mucosal damage and alleviates celiac disease related symptoms (Ilus et al. 2012; Murray et al. 2004). The first description of celiac disease is likely from the 1st century, when a chronic disturbance of digestion affecting adults with fatty stools and starvation was described (Adams 1856). Later Samuel Gee reported this "coeliac affection" to affect especially young children, causing them steatorrhea and distended abdomen (Gee 1888). The pathogenesis began to emerge in the 1950s, when it was observed that the symptoms could be relieved by excluding wheat and rye from the diet, indicating the connection between these cereals and disease development (Anderson et al. 1952; Dicke et al. 1953). Another milestone was the identification of the enzyme transglutaminase 2 (TG2) as the main autoantigen in 1997 (Dieterich et al. 1997).

As regards the diagnosis, intestinal biopsies were obtained for the first time in the 1950s, and the first official diagnostic criteria for celiac disease were published in the early 1970s (Meeuwisse 1970). The first celiac disease autoantibodies were identified in the 1970s (Seah et al. 1971), but these have been replaced by modern serological tests thereafter, particularly after the aforesaid discovery of TG2. In Finland, screening for celiac disease was already conducted in the 1980s with these first serological tests, but since the end of the 1990s modern TG2 tests have been used as a first-line screening method (Sulkanen et al. 1998a). The gold standard for celiac disease diagnosis has long been demonstration of small-bowel mucosal villous atrophy in duodenal biopsy, but according to recently updated guidelines the diagnosis can also be set with sufficiently high levels of the disease-related serum autoantibodies (Celiac disease, Current Care Guidelines 2018).

Nowadays celiac disease is known to cause variable, often mild, gastrointestinal and extraintestinal symptoms. In fact, a marked proportion of patients are

asymptomatic or have only subclinical phenotype and thus can only be identified by screening (Volta et al. 2014). The most important risk group consists of family members of affected patients with an estimated prevalence among first-degree relatives (FDRs) 5-10 times higher than in general population (Rubio-Tapia et al. 2008; Singh et al. 2015). Although active serological testing of family members is widely endorsed by various guidelines to decrease the underdiagnosis of celiac disease, there remain several open questions, such as the optimal starting age of screening and the need for repeated screening after a one-time negative test result (Al-Toma et al. 2019; Husby et al. 2012; Wessels et al. 2018).

The present dissertation project studies the presentation and screening of celiac disease among at-risk relatives. The study population was collected from 732 families comprising patients with an existing celiac disease diagnosis as well as previously healthy relatives who were screened for celiac disease. The clinical presentation among sibling pairs with celiac disease, individual risk factors for screen-detected disease and incidence of screening positivity after one-time negative screening result were assessed.

2 ETIOLOGY AND PATHOGENESIS OF CELIAC DISEASE

Gluten is the external antigen driving immunological reaction in celiac disease. Actually, gluten is a combination of the storage proteins (prolamins and glutelins) of cereals, but usually the term refers only to prolamins in wheat (gliadin), barley (hordein) and rye (secalin) (Cebolla et al. 2018; Lindfors et al. 2019). After ingestion, gluten is incompletely digested by gastric, pancreatic, and small intestinal brush border enzymes in all subjects. The remaining peptides may enter the lamina propria of the small intestine, where they are deamidated by TG2 (Dieterich et al. 1997; Lindfors et al. 2019).

Deamidation facilitates the binding of the gluten peptides to specific human leukocyte antigen (HLA) DQ2 and DQ8 molecules on antigen presenting cells (Lindfors et al. 2019). During adaptive immune response these deamidated gluten peptides are presented to gluten reactive CD4+ T cells in celiac disease patients (Lindfors et al. 2019). There is evidence that TG2 is present in enterocytes in gut lumen and that the antigen presentation to T cells also occurs by TG2-specific B-lymphocytes in Peyer's patches (Iversen et al. 2020). After antigen presentation, the activated T cells secrete various cytokines and induce differentiation of B cells to plasma cells, which in turn produce antibodies against TG2 (TGA) and also against deamidated gliadin peptides (DGP-ab) (Catassi et al. 2022). Of note, TGA may also have an active role in the pathogenesis, for example by increasing the permeability of the small bowel epithelial barrier and in the development of extraintestinal symptoms (Lindfors et al. 2019).

Innate immunity is also believed to contribute to the development of celiac disease at mucosal level (Setty et al. 2015). Stressed enterocytes express interleukin 15 (IL-15) and other cytokines, and are expected to be activated, for example, by gluten-derived peptides, although the detailed mechanisms remain somewhat unclear (Lindfors et al. 2019). Subsequently, this leads to reprogramming of intraepithelial CD8+ T cells to cytotoxic cells, eventually resulting in intestinal epithelial cell apoptosis (Lindfors et al. 2019). Interactions between the adaptive and innate immune responses remain incompletely understood, but it has been hypothesized, for example, that cytokines from activated CD4+ cells promote expression of IL-15

and apoptosis of epithelial cells (van Bergen et al. 2015). Il-15 can also inhibit regulatory T cells, possibly contributing to loss of oral tolerance (Lindfors et al. 2019).

2.1 Environmental factors

Only a minority of genetically predisposed subjects develop celiac disease (Chapter 2.2), indicating that environmental factors play a role in the pathogenesis. Further support for this hypothesis is seen in the so-called celiac disease epidemic among Swedish infants in the mid-1980s, when the incidence rate (IR) quadrupled in a short period of time. Once this "epidemic" was noticed, feeding instructions were modified and the amount of gluten was decreased in industrially produced infant foods. Subsequently, IR declined concomitantly with an increase in the proportion of breastfed children (Ivarsson et al. 2000). The most important environmental factor necessary for celiac disease development is dietary gluten, but there are also other, mostly yet unidentified environmental factors affecting pathogenesis (Catassi et al. 2022). Observations supporting the role of these additional environmental exposures are marked differences in the prevalence among people with similar HLA distribution and gluten consumption (Kondrashova et al. 2008), as well as the reported rapid increase in the true prevalence in certain geographical areas (Lohi et al. 2007).

Later, however, randomized birth cohort studies have not confirmed the role of either age of gluten introduction, duration of breastfeeding or presence of breastfeeding during gluten introduction to affect celiac disease risk (Lionetti et al. 2014; Vriezinga et al. 2014). Nevertheless, higher gluten intake before the age of two years may contribute to increased risk (Aronsson et al. 2016; Aronsson et al. 2019), although there are also contradictory findings (Crespo-Escobar et al. 2017). In addition, according to a few studies, several microbes may contribute to disease development (Stordal et al. 2021). For example, an association between enterovirus exposures at 1-2 years of age and celiac disease autoimmunity has been reported, with a cumulative effect of higher gluten intake (Lindfors et al. 2020). There are controversial findings as to whether gastrointestinal infections increase the risk (Kemppainen et al. 2017; Vriezinga et al. 2014). The suggested mechanism behind the viral infections and celiac disease pathogenesis may involve the loss of oral tolerance, since reoviruses and possibly also enteroviruses lead to type 1 interferon-induced activation of gluten reactive CD4+ T cells and inhibition of regulatory T

cells (Brown et al. 2018; Lindfors et al. 2020). Furthermore, dysbiosis in intestinal microbiota has been reported in celiac disease patients (Verdu & Schuppan 2021), probiotics may modulate the immune response in children with celiac autoimmunity (Håkansson et al. 2019), and exposure to systemic antibiotics in early life could be a risk factor for celiac disease (Dydensborg Sander et al. 2019).

2.2 Genetics

The HLA class II heterodimers DQ2 and DQ8 are the most important genetic risk factors for celiac disease (Karell et al. 2003). These HLA molecules are composed of α and β chains encoded by HLA-DQA1 and HLA-DQB1 genes on chromosome 6p21.3 (Sollid 2002) (Figure 1). These molecules bind the gluten peptides and present them to CD4+ T cells, which is necessary for disease development (Sollid 2002).

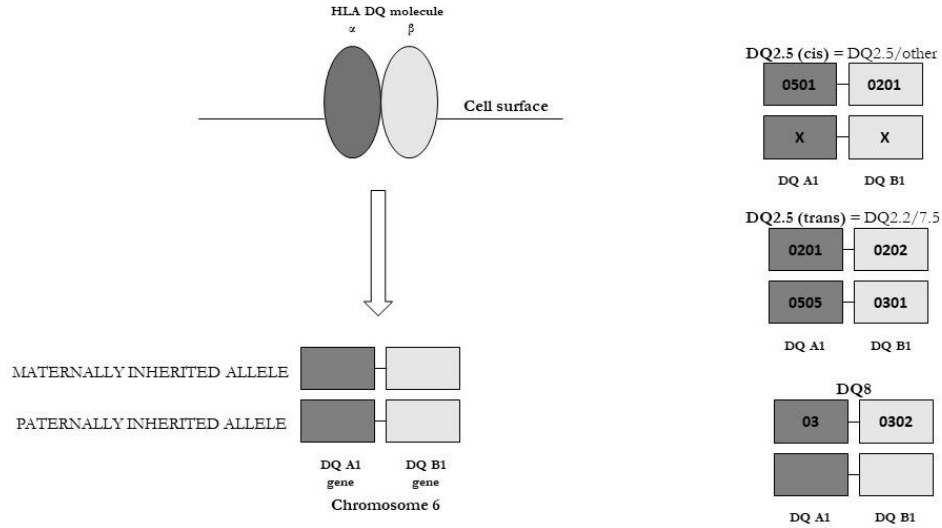

Figure 1. Composition of human leukocyte antigen (HLA) molecule and HLA-DQ2/8 haplotypes. Adapted from Sollid 2002 and Sollid & Lie 2005.

More specifically, the HLA-DQ2 heterodimer is presented in two different haplotypes, DQ2.5 and DQ2.2, and celiac disease risk is related particularly to the HLA-DQ2.5 haplotype (Choung et al. 2020a). It is found in over 90% of patients in

cis (DQA1*0501-DQB1*0201 in the same chromosome) or in trans (HLA-DQ2.2/DQ7.5) position (Sollid & Lie 2005; Karinen et al. 2006a) (Figure 1). The rest of the patients usually carry the HLA-DQ8 haplotype (Karell et al. 2003; Karinen et al. 2006a). HLA-DQ2.2 haplotype is related to celiac disease risk mainly when combined with DQ2.5 or DQ8 (Choung et al. 2020a). Between 0 and 6% of the patients carry neither DQ2.5 nor DQ8, but most of these subjects have at least half a heterodimer of DQ2.5 haplotype (either DQA1*05 or DQB1*02 allele); without any of these alleles celiac disease is highly unlikely (Karell et al. 2003).

The disease risk is highest in patients homozygous for DQB1*02 allele, i.e., in those carrying DQ2.5/2.5 or DQ2.5/2.2 (Pietzak et al. 2009). The seroprevalence has ranged from 15% to 28% among subjects in risk groups carrying these haplotypes (Choung et al. 2020a; Pietzak et al. 2009). Seropositivity figures of as high as nearly 40% by the age of ten years have been reported in children with affected FDR and the high-risk HLA (Lionetti et al. 2014). The risk decreases gradually from DQ2.5 heterozygous to DQ8 homozygous and those subjects carrying both DQ8 and DQ2.2, to DQ2.2. homozygous and finally to DQ8 heterozygous carriers (Sharp et al. 2020). It has been reported that less than 1% of DQ2.2 heterozygous carriers develop celiac disease (Choung et al. 2020a; Pietzak et al. 2009).

HLA-DQ2 and DQ8 heterodimers are nevertheless present in up to ~50% of Western population, and in an even greater part of FDRs of celiac disease patients (Kårhus et al. 2018; Vriezinga et al. 2014) (Figure 2). Approximately 25% of the population carry HLA DQ2.5 and 20% DQ8 (Figure 2). However, as mentioned, only a fraction of individuals with the risk genetics will eventually develop celiac disease (Liu et al. 2017). Additionally, several non-HLA gene regions associated with celiac disease have been found, these accounting for ~15% of the genetic risk (Dubois et al. 2010; Trynka et al. 2011; van Heel et al. 2007). The disease risk can be predicted more accurately by using the non-HLA risk variants in addition to HLA testing, especially among subjects with the highest genetic risk (Romanos et al. 2014; Sharp et al. 2020). All variants identified so far explain on average 50% of the genetic heterogeneity in celiac disease (Trynka et al. 2011).

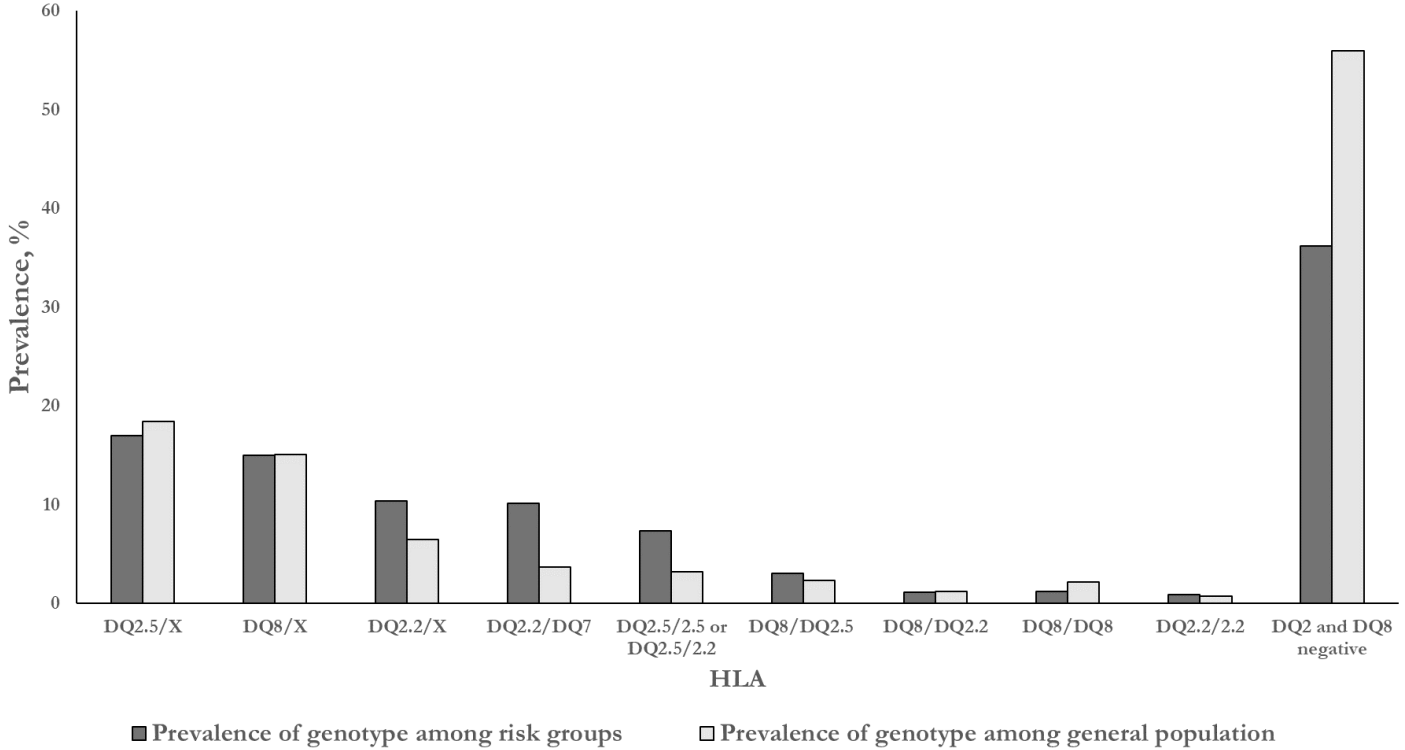

Figure 2. Distribution of celiac disease-associated human leukocyte antigen (HLA) DQ2 and DQ8 haplotypes in at-risk groups, such as relatives of celiac disease patients and subjects with another autoimmune condition, and in general Western population. Mean prevalences are given in case of the reported frequencies varied between the publications. Adapted from Bourgey et al. 2007, Liu et al. 2017, Kårhus et al. 2018, Pietzak et al. 2009 and Vriezinga et al. 2014.

3 CLINICAL PICTURE

3.1 Gastrointestinal symptoms

Approximately 50% of celiac disease patients present with various gastrointestinal symptoms, including, for example, abdominal pain, diarrhea, constipation, vomiting and abdominal distention (Kivelä et al. 2017; McGowan et al. 2009; Schosler et al. 2015; Volta et al. 2014). Sometimes patients suffer also from more ambiguous intestinal symptoms such as heartburn and regurgitation (Nachman et al. 2011; Volta et al. 2014). Gastrointestinal symptoms may resemble irritable bowel syndrome (IBS); in fact, celiac disease has been reported to be markedly overrepresented among IBS patients (Sanders et al. 2001).

3.2 Extraintestinal symptoms

Up to 60% of celiac disease patients have been reported to suffer from one or more extraintestinal symptoms, but in only 10-20% of cases are these the sole clinical presentation and/or the main reason for disease suspicion (Jericho et al. 2017; Nurminen et al. 2019).

A particularly common extraintestinal manifestation is anemia, present in 11-27% of untreated pediatric celiac disease patients (Mubarak et al. 2013; Nurminen et al. 2019; Rajalahti et al. 2017; Roma et al. 2009) and 23-48% of adult patients (Abu Daya et al. 2013; Jericho et al. 2017; Volta et al. 2014), although figures as high as 70-90% have been reported (Berry et al. 2018; Pulido et al. 2013). Iron deficiency is the most commonly reported cause of anemia, but anemia due to inflammation and vitamin B12 and folic acid decifiencies may contribute (Berry et al. 2018; di Sabatino et al. 2006; Repo et al. 2017; Volta et al. 2014).

The best-defined dermatological manifestation of celiac disease is dermatitis herpetiformis (DH), occurring in approximately 2% of pediatric (Jericho et al. 2017; Nurminen et al. 2019) and 10-13% of adult patients (Salmi et al. 2011; West et al. 2014). The condition is characterized by blistering rash typically on the elbows, knees and buttocks, and it is slightly more common among men than women (Salmi et al.

2011). It has been proposed that DH develops as a dermatological complication of long-term untreated celiac disease (Salmi et al. 2015).

Estimated prevalence for osteoporosis or osteopenia among newly diagnosed untreated adult patients may be up to 62-72% (Kurppa et al. 2010a; Sategna-Guidetti et al. 2000; Vilppula et al. 2011), and reduced bone mineral density (BMD) is also possible among pediatric patients (Kavak et al. 2003). Among children, growth failure has been reported in 13-71% of patients (Nurminen et al. 2019; Rashid et al. 2005; Savilahti et al. 2010).

Elevated liver enzymes have been reported in 3-14% of pediatric (Jericho et al. 2017; Nurminen et al. 2019; Äärelä et al. 2016) and 9-40% of adult patients (Castillo et al. 2015; Jericho et al. 2017). Strict adherence to GFD in most cases normalizes the liver values (Äärelä et al. 2016). Although hypertransaminasemia is usually mild, untreated celiac disease may even lead to liver failure (Kaukinen et al. 2002).

Celiac disease has been reported to manifest with different joint symptoms in 6-17% of pediatric and adult patients (Jericho et al. 2017; Nurminen et al. 2019). These symptoms are typically described as arthralgia and myalgia, but arthritis and joint effusions resembling the clinical picture of enteropathic arthritis have also been reported (Iagnocco et al. 2014; Lubrano et al. 1996).

In addition to the above-mentioned symptoms and signs, various other extraintestinal manifestations may occur in both children and adults. These include adverse pregnancy outcomes in adults and delayed puberty in children (Grode et al. 2018a; Jericho et al. 2017), as well as recurrent aphthous ulcers and dental enamel defects (Campisi et al. 2007). Furthermore, neurological symptoms such as headache and gluten ataxia have been reported (Hadjivassiliou et al. 2010; Jericho et al. 2017). Moreover, psychiatric disorders likely to be gluten related are frequently seen (Therrien et al. 2020).

3.3 Malabsorption

The so-called "classical celiac disease" refers to patients with signs of malabsorption combined particularly with diarrhea (Ludvigsson et al. 2013a). Signs of malabsorption include weight loss or failure to thrive in children and steatorrhea, as well as hypoalbuminemia and other laboratory abnormalities indicative of malnutrition (Ludvigsson et al. 2013a).

Malabsorption has been reported in 13-48% of adult patients (Dominguez Castro et al. 2017; Volta et al. 2014). According to a recent meta-analysis, iron deficiency is

present in 6-82%, folic acid deficiency in 11-75%, and vitamin B12 deficiency in 5-19% of untreated celiac disease patients (Kreutz et al. 2020). Besides malabsorption of nutrients, there are likely other mechanisms behind anemia, osteoporosis and failure to thrive (Street et al. 2008). This is supported by the fact that iron deficiency and decreased BMD may already be present in seropositive subjects with normal small-bowel mucosal structure (Kurppa et al. 2010a; Repo et al. 2017).

3.4 Changing clinical picture

The clinical picture of celiac disease has become milder concurrently with the increased prevalence (Chapter 5.1). In fact, severity of symptoms may already have gradually started to abate in the 1970s, this phenomenon being seen in both children and adults (Mäki et al. 1988; Rampertab et al. 2006).

Particularly severe gastrointestinal symptoms and malabsorption have decreased among subjects diagnosed in the 21st century compared to those diagnosed earlier (Kivelä et al. 2015; Rampertab et al. 2006). For example, up to 91% of patients diagnosed before the 1980s suffered from diarrhea, while the corresponding figure after 2000 was only 37% (Rampertab et al. 2006). Nowadays most celiac disease patients suffer from fairly mild gastrointestinal and extraintestinal symptoms (Volta et al. 2014). In fact, 12-35% of children and adults are currently screen-detected, compared to less than 5% before the 21st century (Kivelä et al. 2015; Rampertab et al. 2006; Volta et al. 2014). At the same time, median age at diagnosis has increased from under five years to seven to nine years in children (Kivelä et al. 2015; McGowan et al. 2009) and from ~30 years to ~40 years in adults (Dominguez Castro et al. 2017; Rampertab et al. 2006).

3.5 Factors affecting the clinical picture

The reason for the above-described highly variable phenotype of celiac disease remains obscure. Age at diagnosis may be one factor, since there is evidence of more advanced disease among infants and toddlers than among older children (Koskimaa et al. 2020). Among adults, patients aged >65 years at diagnosis may suffer more often from extraintestinal manifestations than younger patients (Kalkan et al. 2017). More severe villous atrophy and higher TGA levels have been associated with

presence of anemia in both children and adults (Abu Daya et al. 2013; Rajalahti et al. 2017).

Individual HLA genotype may moreover affect both age at diagnosis and clinical severity of celiac disease, but the results are controversial between studies. In a study by Liu et al., children homozygous for HLA-DQ2 developed the disease more often under five years of age than did those with other risk alleles (Liu et al. 2014). Similar trends of lower median age at diagnosis among those with high-risk HLA (double dose of HLA-DQB1*0201 allele) have also been reported in adults (Karinen et al. 2006b). Karinen et al. (Karinen et al. 2006b) reported high-risk HLA also to be an independent risk factor for anemia and diarrhea and for more severe villous atrophy. However, the effect of the number of HLA-DQB1*02 alleles on age at diagnosis, presence of diarrhea, and histological picture has not been confirmed by a meta-analysis (Bajor et al. 2019) or a recent study from Finland (Airaksinen et al. 2020). Interestingly, a recent Finnish study reported that non-HLA genes may also contribute to the phenotype at diagnosis (Cerqueira et al. 2021).

Another factor possibly affecting presentation of celiac disease is gut microbiome (Viitasalo et al. 2018; Wacklin et al. 2013). For example, the diversity of the microbiome has been shown to be higher among patients with DH than among patients with other phenotypes (Wacklin et al. 2013).

4 DIAGNOSIS OF CELIAC DISEASE

4.1 Duodenal biopsy

The diagnosis of celiac disease has long been based on the demonstration of pathological changes in small-bowel mucosal biopsy (Meeuwisse 1970). In healthy individuals, the biopsy typically shows long mucosal villi projecting into the lumen, their length being approximately 2-3x the length of the adjacent crypts (Dai et al. 2019), a relation reported as villous height/crypt depth ratio (Vh/CrD) (Kuitunen et al. 1982). Besides the morphological damage including elongated crypts and shortened villi, the disease is characterized by mucosal inflammation; i.e., increased number of intraepithelial lymphocytes (IEL) (Dai et al. 2019). In 1992, Michael Marsh categorized mucosal lesions into four categories to simplify histological classification (Marsh 1992), and later Oberhuber et al. presented the so-called Marsh-Oberhuber classification (Oberhuber et al 1999). In the Marsh classification, type I lesion consists of normal villous architecture with increased number of IEL, type II lesion signifies hyperplastic crypts, and type III lesion presence of villous atrophy. Long before the Marsh classification, villous atrophy was subdivided into partial, subtotal, and total villus atrophy, these corresponding approximately to Marsh-Oberhuber classification 3a, 3b and 3c respectively (Kuitunen et al. 1982).

Traditionally, presence of villous atrophy (Marsh III) has confirmed the diagnosis for celiac disease (Al-Toma et al. 2019; Ludvigsson et al. 2014; Rubio-Tapia et al. 2013). However, interpreting villous shortening and distinguishing between Marsh II and III lesions is challenging as the mucosal damage develops gradually and may be patchy (Kaukinen et al. 2001; Marsh 1992; Ravelli et al. 2010). Therefore, in some criteria Marsh II lesions are also considered diagnostic (Husby et al. 2019; Husby et al. 2020). To improve diagnostic accuracy, duodenal biopsies should be taken on gluten-containing diet. Moreover, several biopsies are required, including at least four from the distal duodenum and 1-2 from the so-called anatomical duodenal bulb (Al-Toma et al. 2019; Husby et al. 2020; Rubio-Tapia et al. 2013). The samples should be well-orientated (Taavela et al. 2013), and especially the results on bulb biopsies should be considered with caution, since they may lead to false-positive findings especially among seronegative subjects (Taavela et al. 2016). Serological tests are used

to recognize subjects who need to be referred to upper endoscopy (Chapter 4.2), but in case of strong clinical suspicion duodenal biopsy is indicated even among seronegative subjects (Al-Toma et al. 2019; Husby et al. 2012; Ludvigsson et al. 2014; Rubio-Tapia et al. 2013).

Lymphocytic duodenosis (Marsh I) has a fairly low specificity for celiac disease and may be present, for example, in *H.pylori* infection, food hypersensitivity, and non-steroidal anti-inflammatory drug use (Al-Toma et al. 2019). Reasons for seronegative villous atrophy other than celiac disease include, for example, inflammatory bowel disease, giardiasis, common variable immunodeficiency, and autoimmune enteropathy (Gustafsson et al. 2020; Volta et al. 2016a).

If endoscopied, villous atrophy is present in approximately 75% of the patients with DH, but the diagnosis is based on the detection of granular IgA deposits in the dermal papillae by direct immunofluorescence from samples taken from the normal looking skin next to the lesion. Routine upper endoscopy does not influence the long-term prognosis and is not required in the diagnosis of DH. (Reunala et al. 2021)

4.2 Serology

Antibodies against reticular fibers of the endomysium, anti-reticulin antibodies (ARA) (Seah et al. 1971), and gliadin-part of gluten, anti-gliadin antibodies (AGA) (Kelly et al. 1983), were the first discovered serological tests for celiac disease, but have recently been replaced by more accurate serological tests (Hill et al. 2005). The sensitivity and specificity of AGA vary 80-90% in most studies (Rostom et al. 2005). Although more specific than AGA and fairly sensitive among children, ARA have suboptimal sensitivity of 60-78% among untreated adult celiac disease patients, and are therefore no longer used in clinical practice (Seah et al. 1973; Sulkanen et al. 1998a; Sulkanen et al. 1998b).

IgA-class antibodies against TG2 in endomysium, a connective tissue layer covering muscle fibers, endomysial antibodies (EMA), were established in 1983 (Chorzelski et al. 1983). They are detected by indirect immunofluorescence using either human umbilical cord or monkey esophagus as an antigen substrate; the technique requires expertise and is operator dependent (Dieterich et al. 1997; Ladinser et al. 1994). EMA have a sensitivity of approximately 90% and specificity of nearly 100% for untreated celiac disease (Table 1).

Sensitive IgA-class TGA tests have been used since the end of the 1990s (Dieterich et al. 1997; Sulkanen et al. 1998a) and are widely recommended as a first

step diagnostic study both in clinical case-finding and in screening of risk groups (Al-Toma et al. 2019; Hill et al. 2016; Husby et al. 2020; Rubio-Tapia et al. 2013). Confirmatory EMA could be used at least with borderline TGA values or if there are contradictory findings between serology and histology (Al-Toma et al. 2019; Husby et al. 2012). TGAs are usually detected by immunoassay, either by enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immune assay (EliA) (Sulkanen et al. 1998c; Werkstetter et al. 2017), or by radiobinding assay (Candon et al. 2012). Low false-positive results of unknown significance may be found, for example, in patients with liver cirrhosis (Villalta et al. 2005) and viral infections (Ferrara et al. 2010).

DGP-ab may detect some patients who are TGA and EMA negative, and the sensitivity may be better, especially in early-stage celiac disease, when villous atrophy has not yet developed (Kurppa et al. 2011). On the other hand, positive predictive value (PPV) for celiac disease is estimated to be only 15.5% among subjects with isolated DGP-ab positivity (Hoerter et al. 2017). It has been suggested that the sensitivity of TGA and EMA is inferior in young children compared to AGA and DGP-ab, but the results are controversial (Baudon et al. 2004; Holding et al. 2009; Maglio et al. 2010; Åberg & Olcén 2009). Use of AGA is discouraged by all present guidelines, and the recent European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines stated that neither does determination of DGP-ab significantly increase the sensitivity for diagnosis among IgA-competent young children (Husby et al 2020).

IgG-class TGA and EMA have low sensitivity among IgA-competent patients (12-70%), but show excellent accuracy (sensitivity 99-100%, specificity 97-100%) among IgA-deficient patients (Korponay-Szabó et al. 2003; Sblattero et al. 2000; Sulkanen et al. 1998b). Determination of total IgA is recommended as a first-line step together with IgA-class TGA to identify subjects with IgA deficiency (Al-Toma et al. 2019; Hill et al. 2016, Husby et al. 2020), or alternatively including IgG class DGP-ab in testing is advised (Ludvigsson et al. 2014; Rubio-Tapia et al. 2013).

In addition, several so-called point of care tests (POCTs) detecting TGA and/or DGP-ab are available. However, the sensitivities of these tests have varied and further studies on their clinical use are needed (Singh et al. 2019).

Table 1. Diagnostic accuracy of celiac disease serology in studies including both children and adults. The sensitivity and specificity are set according to the manufacturer's lowest cut-off value.

	TGA from serum		TGA from whole blood			EMA	DGP-ab	
Test	QUANTA Lite INOVA h-tTG IgA, ELISA[1]	hr-tTG IgA, ELISA[2]	Celiac IgA EIA, Self tTG ELISA[3]	Biocard Celiac test, tTG rapid test[3, 4]	Nunc Immunostick, tTG rapid test[3, 5]	EMA-HU	QUANTA Lite INOVA	Simtomax Blood Drop, CD-LFIA rapid test[4, 6]
Sensitivity, %	90	98	91	90	97	96	90	79
Specificity, %	98	99	98	94	98	100	94	96

ELISA, enzyme-linked immunosorbent assay; EMA, endomysial antibodies; EMA-HU, endomysial antibodies human umbilical cord; DGP-ab, antibodies against deamidated gliadin peptides; IgA, immunoglobulin A; LFIA, lateral flow immunochromatography assay; TGA, transglutaminase 2 antibodies; tTG, tissue transglutaminase. [1] Native human transglutaminase 2 (TG2) isolated from red blood cells as an antigen; [2] Human recombinant TG2 as an antigen, for example Celikey Phadia test; [3] Patients' own TG2 liberated from red blood cells as an antigen; [4] Immunochromatographic assay; [5] Detects IgA TGA, and total IgA; [6] Detects IgA, IgG class DGP-ab, and total IgA. Adapted from Agardh 2007 (children only), Benkebil et al. 2013 (Simtomax Blood Drop-test, mixed population), Korponay-Szabó et al. 2005 (mixed population), Raivio et al. 2008 (mixed population), Rostom et al. 2005 (pooled numbers for children and adults), Singh et al. 2019 (pooled number for Biocard-test, mixed population) and Sugai et al. 2010 (adults only).

4.2.1 Serology-based diagnosis

Celiac disease diagnosis has traditionally been based on duodenal biopsy showing villous atrophy (Al-Toma et al. 2019; Ludvigsson et al. 2014; Rubio-Tapia et al., 2013). In 2012, new serology-based pediatric criteria were introduced, stating that the diagnosis could be established based on high (>10x upper limit of normal, ULN) IgA class TGA levels and positive EMA, presence of HLA DQ2 and/or DQ8, and celiac-disease related symptoms (Husby et al. 2012). In 2020 the requirements of symptoms and HLA were removed, and in the revised guidelines the diagnosis can set in children based solely on the aforesaid serological criteria (Husby et al. 2020). This approach has been shown to have PPV of 99-100% (Werkstetter et al. 2017; Wolf et al. 2017).

The revised Finnish Current Care Guidelines allowing a first-time equal serology-based diagnosis also in patients >18 years of age were published in 2018 (Celiac disease, Current Care Guidelines 2018) and these new criteria were reported to be accurate in Finnish adult population (Fuchs et al. 2019). In almost all other countries, no-biopsy criteria for adults remain to be established (Losurdo et al. 2021). Even in the Finnish guidelines, endoscopy is still indicated in cases with TGA >10x ULN but "redflag" symptoms, such as bloody stool or dysphagia (Celiac disease, Current Care Guidelines 2018). Determination of HLA is not required for the diagnosis, although it may be utilized in excluding celiac disease in inconclusive cases (Fuchs et al. 2019; Werkstetter et al. 2017).

4.2.2 Seronegative celiac disease

Seronegative celiac disease, signifying the presence of villous atrophy and HLA DQ2/8 but negative TGA and EMA, is a rather rare condition (Gustafsson et al. 2020; Schiepatti et al. 2020). As a first step, other conditions causing villous atrophy should be excluded (Chapter 4.1). In addition, negative serology may be caused by self-initiated GFD, immunosuppressive medication or DH (Schiepatti et al. 2020).

Approximately 1-6% of adult celiac disease patients have been reported to have a true seronegative disease, characterized by older age at diagnosis and presence of malabsorption (Salmi et al. 2006; Schiepatti et al. 2017; Volta et al. 2016a). These

patients may have increased risk for enteropathy-associated T-cell lymphoma (EATL) and refractory celiac disease (RCD) (Salmi et al. 2006; Schiepatti et al. 2021).

4.2.3 Performance of serology in screening studies

In the majority of screening studies among FDRs of celiac disease patients, positivity for both TGA and EMA have predicted Marsh III lesions in >90% of cases, although the PPVs have varied from 77% to 100% (Bonamico et al. 2006; Bourgey et al. 2007; Rubio-Tapia et al. 2008; Uenishi et al. 2014). Of note, in the study reporting the lowest PPV, the value increased up to 100% when Marsh I-II lesions were approved as diagnostic (Bourgey et al. 2007). In addition, Fasano et al. reported a PPV of 100% even in general population in individuals with positive EMA and HLA DQ2/8 (Fasano et al. 2003), whereas Katz et al. observed a value of 94% in those with TGA and EMA (Katz et al. 2011). In another study, PPV was slightly lower (86%) among asymptomatic type 1 diabetes patients (Gould et al. 2021).

In contrast, it has been reported that TGA as a single positive antibody test has low PPV among subjects with low pretest probability (Sugai et al. 2010), but there are also contradictory results and the performance may depend on the assay used (Ylönen et al. 2020). It has been proposed that positive EMA in the presence of HLA DQ2/8 would offer a specific definition for celiac disease (Fasano et al. 2003; Pietzak et al. 2009), particularly as screen-detected subjects refuse endoscopy relatively often (Fasano et al. 2003; Rubio-Tapia et al. 2008).

4.3 Potential celiac disease

Potential celiac disease is defined as positive serology (TGA and/or EMA) with either completely normal small intestinal mucosa or only non-diagnostic (Marsh I) histological changes (Ludvigsson et al. 2013a). Inclusion of Marsh II lesions is debatable (Husby et al. 2019; Ludvigsson et al. 2014). Initial EMA positivity has been reported to be a strong indicator for disease progression, up to 88% of Finnish children with Marsh 0-I and 96% of adults with Marsh 0-II having been reported to either develop villous atrophy or show a positive clinical, serological, and histological response to GFD during later follow-up (Kurppa et al. 2010b; Kurppa et al. 2012a). On the other hand, much lower incidences (6-15%) for development of villous atrophy were found in Italian asymptomatic seropositive subjects having Marsh 0-I

lesion (Auricchio et al. 2014; Volta et al. 2016b), likely reflecting differences in the diagnostic definitions and study designs.

It is thus unclear whether GFD benefits asymptomatic subjects with potential celiac disease and, on the other hand, how many of them will eventually develop celiac disease related symptoms (Auricchio et al. 2014; Volta et al. 2016b). A trial on GFD might be considered and other causes of lymphocytic duodenosis should be excluded in subjects with potential celiac disease (Husby et al. 2020; Ludvigsson et al. 2014).

5 EPIDEMIOLOGY

5.1 Prevalence of celiac disease

The global prevalence of celiac disease is estimated to be ~1%, making it one of the most common food-related chronic diseases (Singh et al. 2018). There are, however, major differences between geographical areas, seroprevalences varying from 0.1-5.6% (Table 2). Celiac disease affects 1-2% of the population in Europe, the Middle East, North and South America, Northern Africa, and Oceania, but there is also diversity within Europe (Table 2). Probably due to differences in wheat consumption and distribution of at-risk HLA alleles, the disease is rare in Eastern and Southeast Asia, while there are substantial geographical differences in India and China (Lionett & Catassi 2014; Yuan et al. 2017). The figures also differ depending on whether the prevalence is based on wide-scale population screening or solely on clinically-detected cases (Table 2). Despite the increased detection rate in recent decades (Chapter 5.2), celiac disease remains markedly underdiagnosed (Table 2).

The true prevalence of celiac disease in Finland is approximately 2% (Lohi et al. 2007) (Table 2). Among the elderly, positive TGA have been found to be present in almost 3% of the population (Vilppula et al. 2009). Even though the prevalence of clinically-detected celiac disease (0.5-0.9%) is one of the highest worldwide, celiac disease thus remains underdiagnosed in approximately two-thirds of patients even in Finland (Lohi et al. 2007; Vilppula et al. 2008; Virta et al. 2009). According to the study by Lohi et al. on Finnish adult population (Lohi et al. 2007), the true prevalence has increased from 1.05% to 1.99% in a twenty-year period. A similar phenomenon has later been reported from the United States (USA) (Rubio-Tapia et al. 2009).

Table 2. Examples of population-based screening studies reporting seroprevalence for celiac autoantibodies and possibly given prevalences of biopsy-proven celiac disease and previously clinically-detected patients.

Country	Reference	Serology used	Subjects	Time of screening	Prevalence, %		
					Serology[1]	Biopsy-proven[2]	Clinically-detected[3]
Africa							
Algeria	Catassi et al. 1999	EMA	989 children	1998	5.6	ND	ND
Tunisia	Ben Hariz et al. 2007	TGA and EMA	6,286 children	2003-2004	0.7	0.4	0.03
Asia							
India	Ramakrishna et al. 2016	TGA	23,311 adults	2001	0.1-1.2 [4]	ND	ND
Israel	Shamir et al. 2002	TGA, EMA or AGA[5]	1,571 adults	2000-2001	3.8	0.6	ND
Japan	Fukunaga et al. 2018	TGA or EMA	2,008 adults	2014-2016	0.2 [6]	0.05	ND
Russia	Kondrashova et al. 2008	TGA and EMA	1,988 children	1997-2001	0.5	0.2	0.05
Australia and Oceania							
Australia	Chin et al. 2009	TGA	3,011 adults	1994-1995	1.6	0.5 [7]	ND
New Zealand	Cook et al. 2000	EMA	1,064 adults	1996	1.2	1.2	0.3
Europe							
Finland	Lohi et al. 2007	TGA and EMA	6,993 adults	1978-1980	1.1	ND	0.03
Finland	Mäki et al. 2003	TGA and EMA	3,654 children	1994	1.5	1.0	0.3 [8]
Finland	Lohi et al. 2007	TGA and EMA	6,402 adults	2000-2001	2.0	ND	0.5
Finland	Vilppula et al. 2008	TGA and EMA	2,815 elderly adults	2002	2.5	2.1	0.9
Germany	Mustalahti et al. 2010	TGA and EMA	4,173 adults	1999-2001	0.3	0.1 [7,9]	0.02

Germany	Laass et al. 2015	TGA	12,741 children	2003-2006	0.9	ND	0.07
Hungary	Korponay-Szabó et al. 2007	TGA and EMA	2,690 children	2005	1.7	1.4	0.2
Italy	Mustalahti et al. 2010	TGA and EMA	2,645 children	1997-2002	1.1	0.7 [9]	0
Italy	Mustalahti et al. 2010	TGA and EMA	4,781 adults	2000-2002	0.7	0.5 [9]	0.02
Latvia	Leja et al. 2015	TGA and EMA	1,444 adults	2008-2009	0.4	ND	ND
Sweden	Ivarsson et al. 1999	EMA	1,894 adults	1994	0.6	0.5	0.1
Sweden	Myléus et al. 2009	TGA[10]	7,567 children[11]	2005	3.6	2.9	0.9
United Kingdom	Mustalahti et al. 2010	TGA and EMA	4,656 adults	1986-1987	1.5	0.3 [7,9]	0.3
United Kingdom	Mustalahti et al. 2010	TGA and EMA	1,975 children	2000	0.7	0.2 [7,9]	0.05
North and South America							
Argentina	Mora et al. 2012	TGA and EMA	2,219 children	2008-2009	1.6	1.3	0.3
Brazil	Oliveira et al. 2007	TGA	3,000 adults	2003-2004	1.5	0.5 [7]	ND
USA	Fasano et al. 2003	EMA	4,126 all ages	1996-2001	0.8	0.2 [7]	ND
USA	Rubio-Tapia et al. 2012	TGA and EMA	7,798 all ages	2009-2010	0.7	ND	0.08 [12]

EMA, endomysial antibodies; HLA, human leukocyte antigen; ND, no data, TGA, transglutaminase 2 antibodies; UK, United Kingdom; USA, United States of America. [1] Screened seropositive cases and possible previously clinically-detected cases; [2] Biopsy-proven screened cases and possible previously clinically - detected cases; [3] Prevalence detected before the screening study in clinical practise; [4] Regional differences; [5] Anti-gliadin antibodies (AGA) was verified with EMA and AGA IgG was used for subjects with IgA deficiency; [6] All seropositive subjects were EMA negative, but had TGA >5x upper limit of normal (ULN); [7] Biopsy performed on <50% of seropositive cases; [8] Diagnosis in 1994-2001; [9] Small-bowel biopsy offered to all TGA-positive or borderline positive + EMA-positive subjects; [10] Borderline TGA confirmed with EMA; [11] 7,207 children screened, 67 previously clinically-detected cases found among 7,567 children; [12] Self-reported diagnosis

5.2 Incidence of celiac disease

The occurence of clinically-detected celiac disease over a specific period of time, i.e. the incidence, has increased markedly, up to 10-fold, from the 1970s to the 21st century in many industrialized countries (Collin et al. 1997; Cook et al. 2000; Hawkes et al. 2000; Whyte & Jenkins 2013). The main reasons for this are presumably first the development of endoscopic methods and later the active use of non-invasive serological tests (Chapter 4.2), as well as improved recognition of the disease by physicians (Collin et al. 1997; Lohi et al. 2007; Murray et al. 2003). The reported IRs for clinically-detected celiac disease in the 21st century have been 8.2-54.0/100,000 person-years in European and North American children and 2.6-39.3 in adults (Table 3).

There are some suggestions that the peak in the incidence has been reached in the past decade at least in Western countries (Bergman et al. 2021; Kim et al. 2016; Ludvigsson et al. 2013b). In Finland, the incidence increased from the early 2000s to mid-2000's from 31/100,000 person-years to 39/100,000 in adults and from 31/100,000 to 58 in children (Collin et al. 2007; Kivelä et al. 2015, Virta et al. 2009). During the last decade, the published IRs have been approximately 30/100,000 person-years among adults and 40 among children (Kivelä et al. 2015; Virta et al. 2017) (Table 3). At least a partial explanation for the observed plateau may be unchanged diagnostics, since, for example in Finland, the first nationwide recommendations for the detection and treatment of celiac disease were published as early as in 1998 (Celiac disease, Current Care Guidelines 2018). Other possible explanations include the unchanged environmental factors and genetic background and increased initiation of self-prescibed GFD (Kim et al. 2016).

There has been a higher incidence for at least clinically-detected celiac disease among women compared to men, the difference speculated to be explained by differences in health care behaviour between women and men (Lebwohl et al. 2012; Ludvigsson et al. 2013b; Schøsler et al. 2015). However, according to a recent meta-analysis, there is an increased risk for celiac disease among women compared to men (RR 1.4, 95% CI 1.27-1.57) and among girls compared to boys (RR 1.8, 1.4-2.2) even when looking at patients found by screening general population (Jansson-Knodell et al. 2019).

Celiac disease can develop at any age and *de novo* cases appear even among the elderly (Vilppula et al. 2009). The estimated IRs for celiac autoantibody positivity

after one-time seronegative test have been reported to vary 16-36/100,000 person-years (Catassi et al. 2010; Choung et al. 2020b). Population-based screening studies suggest that approximately half of cases develop in childhood and the rest later in life (Lohi et al. 2007; Mäki et al. 2003). Furthermore, based on a birth cohort study among genetically at-risk children, there may be a peak in the incidence of celiac disease before the age of ten years, particularly between two to four years of age (Hagopian et al. 2017), but it is unclear if this finding can be generalized to population level.

Table 3. Reported incidence rate (IR) of celiac disease in children and adults in the 21st century.

Country	Reference	Diagnostic criteria	Number of new cases	Study period	IR per 100,000 person-years
Children					
Finland	Kivelä et al. 2015	Serology[1]	ND	2001-2013	44.0
Italy	Zingone et al. 2015	ESPGHAN criteria	1,059	2011-2013	27.2
Netherlands	Van Kalleveen et al. 2018	ESPGHAN criteria	105	2007-2016	21.1
Norway	Beitnes et al. 2017	Biopsy[2]	400	2000-2010	31.4
Spain	Cilleruelo et al. 2014	ESPGHAN criteria	659	2006-2007	54.0
UK	Whyte and Jenkins et al. 2013	ESPGHAN criteria	163	2005-2011	8.2
USA	Almallouhi et al. 2017	ESPGHAN and NASPGHAN criteria	100	2000-2014	17.4
Adults					
Bosnia and Herzegovina	Tosic et al. 2013	Serology[3] and biopsy	42	2007-2009	2.6
Denmark	Schøsler et al. 2016	Serology and biopsy[4]	93	2008-2013	6.3
Finland	Virta et al. 2009	Established guidelines[5]	5,020	2004-2006	39.3
Finland	Virta et al. 2017	Established guidelines[5]	12,847	2005-2014	33.1
Italy	Zingone et al. 2015	Serology[1] and biopsy or HLA or biopsy and HLA	990	2011-2013	7.3
USA	Ludvigsson et al. 2013b	Diagnosis from data bases[6]	249	2000-2010	17.4

ESPGHAN, European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; IR, incidence rate ND, no data; HLA, human leukocyte antigen; NASPGHAN, North American Society for Pediatric Gastroenterology, Hepatology and Nutrition; UK, United Kingdom; USA, United States of America.

[1] Transglutaminase 2 antibodies and/or endomysial antibodies (EMA); [2] Among children referred for upper endoscopy; [3] Anti-gliadin antibodies (AGA) or EMA, 98% EMA-positive; [4] HLA determination and special methods if necessary; [5] Celiac patients entitled to dietary reimbursement; [6] For example International Classification of Diseases codes (ICD) for celiac disease

6 RISK GROUPS AND COMORBIDITIES

6.1 Relatives of celiac disease patients

Family members of affected patients have an increased risk for celiac disease. The prevalence of celiac disease among FDRs has varied markedly from as low as 1.3% to as high as 44.1% (Kotze et al. 2009; Tursi et al. 2003). In studies involving larger numbers of FDRs and using modern serological tests, somewhat lower prevalence figures (2.2%-17.2%) have been reported (Table 4). Accordingly, a recent meta-analysis showed pooled prevalence of 7.5% (Singh et al. 2015). The wide variability of the results is at least partly explained by inconsistent study designs. For example, age of screened subjects may have differed markedly between studies, the number of relatives has often been small and the proportion of previously detected family members has usually not been reported. Moreover, particularly in earlier studies, subjects with e.g. only positive AGA and Marsh I lesion may have been considered to have celiac disease despite the low PPV of these findings (Tursi et al. 2003).

The risk of celiac disease among second-degree relatives (SDRs) of patients has hardly been studied, but the current evidence suggests somewhat increased risk – although smaller compared to FDRs (Fasano et al. 2003; Singh et al. 2015) (Table 4). In addition, being a member of a multiple case family may further increase the risk among SDRs (Fraser et al. 2006). In one study, a prevalence as high as 19.5% was reported among SDRs belonging to families with two affected siblings (Book et al. 2003).

Table 4. Prevalence of celiac disease in screening studies conducted in at-risk family members and published from 2000 onwards.

Reference	Relatives*	Time and country	Diagnostic criteria	Prevalence, %
Petaros et al. 2002	354 FDR of all ages	ND, Italy	EMA + biopsy	5.6
Book et al. 2003	163 FDR, 82 SDR and 47 first-degree cousins of the sibling pairs with celiac disease, all ages	ND, USA	EMA + confirmatory TGA and HLA	17.2 in FDR 19.5 in SDR 17.0 in cousins
Cataldo & Marino 2003	225 FDR, children and adults	ND, Italy	EMA and TGA and IgA + biopsy (Marsh III)	4.8
Fasano et al. 2003	4,508 FDR, 1,275 SDR, children and adults	1996-2001, USA[1]	EMA and HLA or EMA[2] + biopsy (Marsh II-III)	4.5 in FDR 2.6 in SDR
Bonamico et al. 2006	441 FDR, children and adults	ND; Italy	EMA and/or TGA, total IgA[3] + biopsy (Marsh III)	9.5
Fraser et al. 2006	585 FDR, 139 SDR and 24 more distant relatives, children and adults	ND, UK	IgA and IgG TGA + confirmatory EMA	5.6 in FDR 1.4 in SDR 0 in more distant
Bourgey et al. 2007	360 parents, adults and 239 siblings, children	2001-2003, Italy	EMA and TGA + biopsy (Marsh II-III or Marsh I-II intermediate)	2.2 in parents[4] 7.1 in siblings[5]
Rubio-Tapia et al. 2008	344 FDR, adults	ND, USA	EMA and TGA + biopsy (Marsh I-III) or TGA + biopsy (Marsh I-III) or EMA and TGA and HLA DQ2/8 or HLA DQ2/8 and symptoms + biopsy (Marsh III)	11.3 [6]
Martins et al. 2010	207 FDR, children and adults	2004-2008, Brazil	EMA and/or TGA and total IgA + biopsy compatible with celiac disease	6.8
Dogan et al. 2012	484 FDR, children and adults	ND, Turkey	TGA and total IgA + biopsy (Marsh I-IV)	4.8
Oliveira et al. 2012	268 parents and siblings, children and adults	2009-2010, Portugal	TGA[7]+ biopsy (Marsh III)	2.6

Uenishi et al. 2014	450 FDR, children and adults	2000-2012, Brazil	TGA + confirmatory EMA, HLA DQ/8 and biopsy compatible with celiac disease	4.2
Wessels et al. 2018	427 FDR, children and adults	1994-2016, Netherlands[1]	ESPGHAN criteria for children[8], EMA and/or TGA + biopsy (Marsh II-III) for adults[9]	15.0

AGA, anti-gliadin antibodies; EMA, endomysial antibodies; FDR, first-degree relatives; ND, no data; SDR, second-degree relatives; TGA, transglutaminase 2 antibodies *Only studies comprising >200 relatives are included. [1] Retrospective study; [2] AGA IgG if IgA deficiency; [3] EMA IgG and TGA IgG if IgA deficiency or immunodeficiency; [4] 4.3% with previously detected relatives; [5] 9.8% with previously detected relatives; [6] 16.4% with previously detected relatives; [7] Biocard-rapid test with qualitative detection of total IgA, confirmed with serum TGA; [8] Repeated testing was offered for the first-time screening-negative human leukocyte antigen (HLA) DQ2/8 positive children; [9] One adult was diagnosed based on positive TGA and presence of HLA-DQ2 and resolution of symptoms after starting gluten-free diet.

The individual risk among family members and the detailed risk factors such as the role of HLA haplotype remain poorly studied. In general, the prevalence has been suggested to be higher among siblings (8.9%) compared to offspring (7.9%) and parents (3.0%) (Singh et al. 2015). Bourgey et al. studied the effect of HLA haplotype among siblings and found that 28% of siblings who were homozygous for DQB1*02 allele had celiac disease (Bourgey et al. 2007), while Rubio-Tapia et al. found a 16-times higher risk among DQ2-positive FDRs compared to general population (Rubio-Tapia et al. 2008). The highest incidences so far have been reported in prospective birth cohort studies among FDRs homozygous for DQ2 (Chapter 2.2) (Lionetti et al. 2014).

6.2 Autoimmune diseases and other comorbidities

Approximately 20% of celiac disease patients have at least one coexisting autoimmune disease (Cosnes et al. 2008; Viljamaa et al. 2005a). For example, prevalence of the disease among children with type 1 diabetes is 4.8-9.3% and, conversely, celiac disease patients are estimated to have an approximately 2.4-fold risk for developing diabetes by the age of 20 years (Kurppa et al. 2018; Ludvigsson et al. 2006). Another well-studied risk group is patients with autoimmune thyroid disease, in whom prevalence of celiac disease has been reported to be 4.9-7.6% (Larizza et al. 2001; Sari et al. 2009). There is also evidence that celiac disease is associated with Addison's' disease (Myhre et al. 2003), IgA nephropathy (Nurmi et al. 2018; Nurmi et al. 2021), and autoimmune hepatitis (di Biase et al. 2010; van Gerven et al. 2014). Autoimmune skin diseases may sporadically co-occur in celiac

disease patients (Rodrigo et al. 2018). Large population-based studies on the risk of rheumatological conditions among celiac disease patients are lacking, although there is a probable association with Sjögren's syndrome and juvenile idiopathic arthritis (Zylberberg et al. 2018). There are conflicting results on a possible association with systemic lupus erythematosus, while no association with rheumatoid arthritis and seronegative spondylarthropaties with celiac disease has so far been proven (Zylberberg et al. 2018).

As regards other co-morbities, patients with Down's syndrome and Turner's syndrome have had reported prevalences for celiac disease of 9.8% (Liu et al. 2020) and 9.4% (Nadeem and Roche 2013) respectively. Furthermore, up to 7.7% of IgA-deficient subjects are reported to have celiac disease (Meini et al. 1996) and, conversely, 1.9-2.6% of celiac disease patients have selective IgA deficiency (Cataldo et al. 1998; Chow et al. 2012). In addition, a 1.5-3-fold increased risk for psoriasis, urticaria, and atopic dermatitis has been reported in celiac disease patients compared to non-celiac disease controls (Rodrigo et al. 2018). There is also some evidence that celiac disease is associated with an increased risk for thromboembolic manifestations and cardiovascular events (Fousekis et al. 2020), asthma (Canova et al. 2015), and epilepsy (Canova et al. 2020), but more studies are needed.

7 TREATMENT AND FOLLOW-UP

7.1 Gluten-free diet and other possible treatments

The only officially accepted treatment for celiac disease at present is a lifelong GFD, meaning strict elimination of storage proteins of wheat, barley, and rye from the diet (Bascuñan et al. 2017). An average Western diet contains approximately 10 grams of gluten per day (Hoppe et al. 2017). The term 'gluten-free' can be used to refer to content with a maximum of 20 mg/kg of gluten and "very low gluten" for 100mg/kg of gluten (European Commission 2014). Of note, GFD consists of ingredients that are processed to be gluten-free in addition to naturally gluten-free products. The use of purified gluten-free oat is considered to be safe for most patients since the composition of storage proteins differs from those in the three aforementioned cereals (Aaltonen et al. 2017). Despite some observations on a possible association of increased intraepithelial lymphocytosis (Ilus et al. 2012), oat consumption has not been associated with impaired mucosal healing in the short term nor with the development of complications or presence of symptoms in the long term (Aaltonen et al. 2017; Janatuinen et al. 1995).

Gastrointestinal symptoms in the majority of the patients diminish after days to weeks after initiation of GFD (Murray et al. 2004), and usually the diet also alleviates extraintestinal symptoms (Jericho et al. 2017) and reduces the risk of complications (Chapter 8.3). In contrast, histological recovery of the duodenal mucosa may take several years, approximately 30-40% of adult celiac patients having been reported to have persistent villous atrophy after one year on strict GFD (Pekki et al. 2015; Sharkey et al. 2013). However, slow histological recovery does not appear to be associated with poorer prognosis or development of complications in long-term follow-up among strictly adherent patients (Pekki et al. 2015). Children tend to generally have a faster and more complete response than adults (Wahab et al. 2002). Slower recovery is related especially to more severe mucosal damage at diagnosis (Pekki et al. 2015; Pekki et al. 2017; Rubio-Tapia et al. 2010). An excellent long-term outcome on GFD is in any case possible, as full recovery of villous atrophy was detected in 96% of Finnish adults after a median of nine years on a strict diet (Ilus et al. 2012). Of note, approximately half of these patients still presented with low-

level intraepithelial lymphocytosis, but this did not seem to be of any clinical relevance (Ilus 2012). Normalization of serum antibodies is often faster than mucosal recovery, as in a study by Pekki et al. (Pekki et al. 2015) approximately 15% of the patients remained TGA-positive after one year on GFD.

Among clinically-detected patients, the quality of life improves and is comparable to that in general population when celiac disease is diagnosed and treated appropriately (Kurppa et al. 2010; Norström et al. 2011; Pasternack et al. 2015). However, following the GFD has also other aspects, such as adverse effects on social life and increased costs, which may decrease commitment to the demanding treatment (Kivelä et al. 2022; See et al. 2015). Accordingly, strict adherence has ranged 42-91% in earlier studies (Hall et al. 2009), while in Finland figures of 79-96% have been reported during the past decade (Ilus et al. 2014; Kinos et al. 2012; Kivelä et al. 2017; Kivelä et al. 2022; Pekki et al. 2017).

As additional possible downsides of the dietary treatment, there have been concerns about increased fat and sugar ingestion and decreased fiber intake in celiac patients on GFD, possibly due to the replacement of healthier gluten-containing carbohydrates with less healthier choices (Barone et al. 2016). In addition, deficiencies of iron and B vitamins have been reported (See et al. 2015). However, deficiencies of vitamins and trace elements are also common in general population and a recent study from Spain detected no significant nutritional differences between treated celiac disease patients and controls (Ballestero-Fernández et al. 2021).

The unresponsiveness to GFD in some celiac disease patients and the burden of the dietary treatment call for additional therapies. Examples of novel investigative therapies include anti-IL-15 monoclonal antibody AMG 714, epithelial permeability decreasing agent Larazotide, glutenase ALV003, and a transglutaminase inhibitor ZED1227 (Kivelä et al. 2021; Schuppan et al. 2021). At present, however, none of these have been accepted as an official treatment for celiac disease.

7.2 Follow-up

Particularly during the first few years after diagnosis, it is essential to provide appropriate information about and guidance on GFD to optimize dietary adherence and coping with the demanding treatment (Al-Toma et al. 2019; Ludvigsson et al. 2014; Rubio-Tapia et al. 2013). The timing of the first control visit could be individualized depending on age and clinical presentation (Celiac disease, Current Care Guidelines 2018). Annual or biennial follow-up visits are recommended

thereafter (Al-Toma et al. 2019, Celiac disease, Current Care Guidelines 2018). Adherence to GFD, decrease/normalization of serum antibody levels, correction of possible other abnormal laboratory values, and presence of persistent symptoms or co-morbidities should be evaluated at each visit (Al-Toma et al. 2019; Ludvigsson et al. 2014). Follow-up endoscopy is usually not indicated either in children or adults, except in case of non-responsive celiac disease or in initially seronegative – especially elderly – patients (Al-Toma et al. 2019; Celiac disease, Current Care Guidelines 2018; Ludvigsson et al. 2014; Rubio-Tapia et al. 2013).

It seems that most patients are not followed-up according to the recommendations, but it is unclear whether this lack of follow-up affects the long-term prognosis and success of treament (Kivelä et al. 2022; Pekki et al. 2018). In fact, adherence to GFD may depend more on factors other than regular follow-up, for example on availability of gluten-free products, age, and current symptoms (Kurppa et al. 2012b; White et al. 2016).

8 PROGNOSIS

8.1 Persistent symptoms

Up to 20-30% of celiac disease patients have reported persistent gastrointestinal complaints even after long-term GFD (Laurikka et al. 2016; Murray et al. 2004; Paavola et al. 2012), although usually with reduced severity (Laurikka et al. 2016; Murray et al. 2004). Persistent extraintestinal symptoms have been studied less than gastrointestinal symptoms, but low improvement rates (40-50%) on a long-term GFD have been reported in connection with psychiatric disorders in children and with headache, psychiatric disorders, arthralgia, myalgia, and fatigue in adults (Jericho et al. 2017). In addition, up to 35-44% of pediatric patients with growth disturbance may fail to reach appropriate catch-up growth on GFD, but this percentage is lower when other co-existing conditions are excluded (Jericho et al. 2017; Sansotta et al. 2018).

Gluten contamination and non-adherence to GFD are the most important causes of persistent symptoms, but ongoing symptoms have also been reported among patients on a long-term strict diet (Laurikka et al. 2016; Sansotta et al. 2018). It is somewhat debatable whether strictness of diet has an association with persistent symptoms (Paavola et al. 2012, Sansotta et al. 2018). Besides non-adherence, other risk factors for persistent symptoms are comorbidities such as IBS and duration of symptoms over 5-10 years before the celiac disease diagnosis (Murray et al. 2004; Paarlahti et al. 2013; Sansotta et al. 2018). Persistent gastrointestinal symptoms on GFD, especially constipation, may respond positively to an increase in fiber intake (Laurikka et al. 2019; Murray et al. 2004).

8.2 Refractory celiac disease

RCD is defined as presence of malabsorptive symptoms and mucosal atrophy despite a strict GFD for at least 12 months in patients in whom other causes of non-recovery have been excluded. It is considered a rare complication (Rubio-Tapia & Murray 2010) and in Finland affects only 0.3% of celiac disease patients (Ilus et al.

2014b). In type 1 RCD, the IELs present with normal phenotype, but in type 2 RCD, they lack surface expression of CD3 and CD8 and have clonal rearrangement of the γ chain of T-cell receptor. These abnormal lymphocytes predispose to lymphoma, which occurs in 30-46% of subjects with type 2 RCD within five years and is also possible, although much less likely, in type 1 RCD (Al-Toma et al. 2007; Ilus et al. 2014b; Malamut et al. 2009). Risk factors for RCD are male sex, history of recurrent dietary lapses, older age, seronegativity, and severe clinical presentation at celiac disease diagnosis (Ilus et al. 2014b).

Prednisone and azathioprine are used for the treatment of type 1 RCD, leading in most cases to some clinical response, although less often to histological recovery (Goerres et al. 2003; Malamut et al. 2009). Different immunosuppressive and chemotherapies have been used for type 2 RCD, but none of these prevent the development of lymphoma (Malamut et al. 2009). The course of type 1 RCD is usually benign with a 5-year survival of up 93-96%, versus that of only 44-58% in type 2 (Al-Toma et al. 2007; Malamut et al. 2009). Progression to lymphoma, malnutrition, sepsis, and thrombotic events are common causes of mortality in the latter (Ilus et al. 2014b; Malamut et al. 2009).

8.3 Complications

According to most studies, the overall risk for malignancies is not increased either among diagnosed and treated celiac disease patients or undetected and untreated subjects compared to general population (Ilus et al. 2014a; Tio et al. 2012). The risk for some malignancies such as breast cancers is in fact decreased (Ilus et al. 2014a). However, there is a well-established increased risk especially for EATL (Catassi et al. 2002; Silano et al. 2008; Tio et al. 2012) as well as other non-Hodgkin's lymphomas (NHL) (Ilus et al. 2014a) at least among clinically diagnosed celiac disease patients. In recent studies the lymphoma risk has been lower than previously reported, possibly due to the inclusion of a higher proportion of patients with mild clinical presentation and/or good dietary adherence (Catassi et al. 2002; Ilus et al. 2014a).

As regards other malignancies, a 3-4-fold increased risk has been reported for small-intestinal adenocarcinoma among diagnosed celiac disease patients, the absolute risk being lower than that of NHL (Emilsson et al. 2020; Ilus et al. 2014a). Additionally, the risk for colon carcinoma may be increased (Ilus et al. 2014a),

although some earlier studies have not shown this (Grainge et al. 2012; Viljamaa et al. 2006).

There is evidence that strict GFD protects against the development of lymphoma (Holmes et al. 1989; Viljamaa et al. 2006). This is supported by the finding that the risk for lymphoproliferative diseases is highest in the first years after celiac disease diagnosis, as well as by the association between incomplete mucosal recovery and increased malignancy risk (Ilus et al. 2014a; Lebwohl et al. 2013).

Initiation of GFD increases BMD, but if diagnosis is made after the achievement of peak bone mass, osteoporosis/osteopenia may persist in 9-62% of subjects even after several years on GFD (Larussa et al. 2017). Normal BMD is associated with mucosal healing, whereas impaired mucosal healing, older age at diagnosis, and nutritional deficiency related to gluten-free food are suspected factors behind the persistently low BMD (Larussa et al. 2017; Pekki et al. 2015) (Chapter 7.1). Low BMD may lead to increased risk for fractures, but although high persentages of persistent osteoporosis have been presented, the actual fracture risk among treated patients compared to general population is unclear (Larussa et al. 2012). According to a meta-analysis, however, any fracture at some point in life is almost twice as common among celiac disease patients than among those without the disease (Heikkilä et al. 2015).

Untreated celiac disease appears to be associated with adverse pregnancy outcomes, such as intrauterine growth restriction and preterm delivery (Tersigni et al. 2014). Likewise, unrecognized celiac disease is reported to be more common among women with unexplained infertility than among general population (Singh et al. 2016), although at present the results are somewhat contradicting (Celdir et al. 2021; Grode et al. 2018b). Adverse pregnancy outcomes do not seem to be more frequent among treated celiac disease patients than among non-celiac disease controls (Grode et al. 2018a) and GFD may have a protective effect at least in some disease-related reproductive disorders (Tersigni et al. 2014).

Whether the risk for other complications than those described above is similarly present among screen-detected patients with often mild or asymptomatic clinical presentation as in the clinically-detected cases remains debatable. There is some evidence that the risk for lymphoproliferative diseases and esophageal carcinoma is also elevated in unrecognised celiac disease (Lohi et al. 2009), but there are opposite findings as well (Godfrey et al. 2010). Interestingly, reduced BMD may be even more common among screen-detected and asymptomatic patients than among those found due to clinical symptoms (Mustalahti et al. 1999).

8.4 Mortality

Clinically-detected celiac disease patients have somewhat increased mortality from lymphoproliferative diseases even on GFD (Koskinen et al. 2020; Sultan et al. 2015), but it is unclear whether this also applies to screen-detected subjects (Corrao et al. 2001; Godfrey et al. 2010; Lohi et al. 2009). Some authors also report an increased overall mortality among celiac disease patients with hazard ratios (HR) of approximately 1.2-1.4 (Lebwohl et al. 2020; West et al. 2004), whereas no such association has been found in other studies (Koskinen et al. 2020; Sultan et al. 2015). As much as 4-fold higher overall mortality has been reported among undetected subjects compared to non-celiac disease controls (Rubio-Tapia et al. 2009), but, again, no increased risk has been found in several other studies (Canavan et al. 2011; Choung et al. 2017; Godfrey et al. 2010; Lohi et al. 2009).

The risk for both overall mortality (West et al. 2004) and mortality from lymphoproliferative diseases (Koskinen et al. 2020) has been greatest during the first one to two years after the diagnosis. Some, but, again, not all studies have also reported an association between cardiovascular deaths and celiac disease (Lebwohl et al. 2020; Sultan et al. 2015).

9 SCREENING FOR CELIAC DISEASE

The risk of celiac disease is considered high enough to justify screening among FDRs of patients and certain other known risk groups (Chapter 9.3), whereas untargeted population-based screening is not recommended in any of the current guidelines (Table 6). Yet several open questions remain regarding even family screening, such as optimal starting age, screening of SDRs and more distant relatives and frequency and need for repeated screening after a one-time negative screening result. This issue is further complicated by the wide variation in the reported celiac disease risk among relatives and poorly defined impact of additional risk factors such as HLA haplotype and gender at individual level (Chapter 6.1).

9.1 WHO screening criteria

The World Health Organization has provided specific requirements that should be fulfilled before launching a screening program for any disease (Table 5). Although celiac disease fulfills most of the criteria, particularly the natural course of untreated disease – especially among asymptomatic subjects – and the cost-effectiveness of screening are poorly known (Chapter 8.3 and Chapter 9.6). For example, the possibly increased risk for lymphoproliferative malignancies among undetected patients is not considered high enough to justify mass screening (Catassi et al. 2002). Furthermore, while there is some evidence that population-based screening could be cost-effective, this applies only if a number of assumptions are met, including sufficiently increased mortality in untreated compared to treated patients and presence of a long diagnostic delay without screening (Hershcovici et al. 2010). Given that increased overall mortality among celiac disease is debatable (Chapter 8.4), the risk of complications and quality of life among screened subjects should be evaluated and studies on cost-effectiveness conducted based on these aspects.

Table 5. WHO criteria for mass screening and their validity in celiac disease.

Criteria	Validity in celiac disease
1. The disease is common and well-defined.	Fulfills the criteria with an estimated prevalence of ~1% in Western countries.
2. Accurate and simple screening tests are available.	Positive predictive value with positive TGA combined with EMA 94 % [1]
3. The screening test is culturally acceptable.	Blood testing is culturally acceptable in majority of countries.
4. Treatment is available.	GFD relieves symptoms and mucosal damage, but its benefits in asymptomatic subjects and those with potential celiac disease are unclear.
5. Clinical detection is difficult.	The disease is underdiagnosed and clinical picture is variable.
6. The disease will lead to severe complications if untreated.	GFD reduces risk for complications, such as lymphoma and growth failure, but the natural course of untreated and particularly asymptomatic celiac disease remains unclear.
7. Testing and treatment is cost-effective.	Unclear.

EMA, endomysial antibodies; GFD, gluten-free diet; TGA, transglutaminase 2 antibodies; WHO, World Health Organization. [1] Katz et al. 2011. Adapted from Ludvigsson et al. 2015.

9.2 Screening of family members

Most international guidelines recommend screening of the FDRs of celiac disease patients (Table 6). However, the National Institutes of Health and the US Preventive Service Task Force state that routine screening is not recommended since the benefits and harms of screening of asymptomatic relatives remain unclear (NIH 2004; Bibbins-Domingo et al. 2017). Additionally, the British Society of Gastroenterology recommends active case-finding and testing only for symptomatic FDRs (Ludvigsson et al. 2014). Here, it is important to realize that many "asymptomatic" subjects may actually have mild clinical findings and experience a beneficial response to GFD (Chapter 9.6).

Table 6. Screening recommendations for celiac disease in family members.

Reference	Organization	Recommendation
NIH 2004	NIH	Routine screening cannot be recommended.
Hill et al. 2005	NASPGHAN, children	FDR
Husby et al. 2012	ESPGHAN, children	FDR
Murch et al. 2013	BSPGHAN and Coeliac UK, children	FDR
Rubio-Tapia et al. 2013	ACG, children and adults	FDR, SDR who have >1 relative already diagnosed with celiac disease.
Ludvigsson et al. 2014	British Society of Gastroenterology, adults	Symptomatic FDR
Downey et al. 2015	NICE, children and adults	FDR
Bibbins-Domingo et al. 2017	USPSTF, children and adults	Not stated
Al-Toma et al. 2019	ESsCD, children and adults	FDR, SDR who have >1 relative already diagnosed with celiac disease.

ACG, American College of Gastroenterology; BSPGHAN, British Society for Paediatric Gastroenterology; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; ESsCD, European Society for study of Coeliac Disease; FDR, first-degree relative; NASPGHAN, North American Society for Pediatric Gastroenterology, Hepatology and Nutrition; NICE, National Institute for Health and Care Excellence; NIH, National Institutes of Health; SDR, second-degree relative; USPSTF, US Preventive Services Task Force.

Some guidelines recommend that the screening should be extended to SDRs if there is already more than one affected family member (Al-Toma et al. 2019; Rubio-Tapia et al. 2013); however, the evidence supporting such an approach is limited (Chapter 6.1). Altogether, the use of the current screening recommendations is variable, and a study from the USA revealed that almost 30% of even symptomatic FDRs were not tested for celiac disease (Faye et al. 2018).

9.3 Screening in other risk groups

Besides family members, screening of certain other risk groups for celiac disease has been variably recommended. Most pediatric and adult guidelines recommend screening patients with type 1 diabetes and autoimmune thyroid disease (Al-Toma et al. 2019; Downey et al. 2015; Hill et al. 2005; Husby et al. 2012; Murch et al. 2013), although some prefer testing only those with symptoms (Rubio-Tapia et al. 2013).

Other risk groups often recommended to be screened are individuals with Down's, Williams', and Turner's syndrome and patients with autoimmune liver disease, IBS, and IgA deficiency (Al-Toma et al. 2019; Downey et al. 2015; Hill et al. 2005; Husby et al. 2012; Ludvigsson et al. 2014; Murch et al. 2013).

9.4 Repeated screening

The need for repeated screening for celiac disease after one-time negative testing remains unclear. The studies conducted hitherto assessing the incidence of novel seroconversion among family members have usually been small and follow-up times short (Table 7). Moreover, the re-screening may not have been performed systemically (Bonamico et al. 2006; Goldberg et al. 2007; Wessels et al. 2018) and the follow-up times in person-years and IRs are rarely reported (Biagi et al. 2008). These limitations hamper the estimation of IRs for seroconversion (Table 7).

Table 7. Incidence rate (IR) of celiac disease seroconversion in follow-up studies comprising one-time screening-negative family members.

Reference*	Study period and country	Relatives	Definition of seroconversion	Follow-up time, years	Seroconverted cases, n	Cumulative Incidence, %	IR[1]
Niveloni et al. 2000	1989−1997, Argentina	44 FDR, adolescents and adults	Positive EMA	7.2, median	2	4.5	631/100,000
Pittschieler et al. 2003	1990−2001, Italy	86 FDR, children	Positive EMA	2-12 [2]	5	5.8	484/100,000
Högberg et al. 2003	1975−2000, Sweden	90 FDR, all ages	Positive EMA or TGA or positive AGA in children	20-25	2 [3]	2.2	89/100,000
Bonamico et al. 2006	ND, Italy	193 HLA DQ2/8 positive FDR, all ages	Positive EMA and/or TGA	13, 20 and 25	3	1.6	NA[4]
Goldberg et al. 2007	1996−2005, USA[5]	151 FDR, 20 SDR, all ages	Positive EMA	1.5 non-seroconverted and 1.7 seroconverted, median	6	3.5	NA
Biagi et al. 2008	1996–2006, Italy	64 FDR, adults	Positive EMA	4.3, median	1 [6]	1.6	437/100,000 [7]

Uenishi et al. 2014	2000–2012, Brazil	205 FDR, all ages	Positive TGA and/or EMA with HLA DQ2/8	4.3, median	8	3.9	916/100,000
Wessels et al. 2018	1994−2016, Netherlands[5, 8]	341 FDR, all ages[9]	Positive EMA and/or TGA with HLA DQ2/8	NA	20	5.9	NA

AGA, anti-gliadin antibodies; EMA, endomysial antibodies; FDR, first-degree relatives; HLA, human leukocyte antigen; IR, incidence rate; NA, not available; SDR, second-degree relatives; TGA, transglutaminase 2 antibodies. *Only studies published from 2000 onwards are included. [1] Person-years were estimated using the median or maximum follow-up time, except for Biagi et al., who reported the annual incidence; [2] Every year for a 12 year-period or until seroconversion; [3] The two seroconverted subjects already had histological changes at first evaluation; [4] Follow-up times given only for the three seroconverted subjects; [5] Retrospective study; [6] One person received celiac disease diagnosis in clinical practice before the second screening; [7] Reported by authors; [8] Re-screening was systematically offered only to HLA-DQ2 and/or DQ8 positive children; [9] HLA DQ2/8 unknown in 90 and positive in 251 relatives

Besides the need for it, the optimal frequency of re-screening among once seronegative at-risk individuals remains to be determined. Pittschieler et al. (Pittschieler et al. 2003) tested annual screening for a 12-year period among 86 FDR children, but the study consisted of only five new cases after two to five years of follow-up (Table 7). The re-screening interval among children with type 1 diabetes has been more studied, and, based on a meta-analysis, screening for celiac disease at diabetes diagnosis and after two and five years was recommended (Pham-Short et al. 2015). It is unclear how to continue the screening of the type 1 diabetes patients in adulthood, but regular screening is recommended at least by European Society for Study of Coeliac Disease (ESsCD) (Al-Toma et al. 2019).

Determination of the presence of at-risk genetics might theoretically help targeting repeated screening. Accordingly, Wessels et al. suggested an algorithm with annual screening for children under ten years of age carrying HLA DQ2/DQ8 and having an affected FDR (Wessels et al. 2018). The British Society for Paediatric Gastroenterology and ESPGHAN provide expert opinion-based recommendations for repeated screening in childhood between every two to three years in an initially seronegative asymptomatic child belonging to a celiac disease risk group and carrying HLA DQ2/8 (Husby et al. 2012; Murch et al. 2013). In adult guidelines, determination of HLA DQ2/8 is recommended to rule out celiac disease and avoid subsequent testing among at-risk groups, while the subsequent screening of the HLA-positive seronegative subjects has not been specified (Al-Toma et al. 2019; Ludvigsson et al. 2014).

9.5 Adherence to gluten-free diet in screen-detected patients

Self-rated dietary adherence in Finland has been comparable among clinically-detected (strict adherence in 82-88%) and screen-detected (strict adherence in 71-91%) adults and children with celiac disease (Kinos et al. 2012; Kivelä et al. 2017; Paavola et al. 2012; Ukkola et al. 2011, Viljamaa et al. 2005b), and similar findings have also been reported from the USA (Mahadev et al. 2016). There is nevertheless some evidence of poorer adherence among screen-detected than clinically-detected subjects in small studies (Fabiani et al. 2000) and increased risk of dietary lapses among asymptomatic screen-detected adults (Ukkola et al. 2011). However, more recent studies have reported comparable adherence among initially symptomatic and asymptomatic children and adults (Kinos et al. 2012; Mahadev et al. 2016; Webb et

al. 2015). In one study, the screen-detected children actually had even better adherence than those found on a clinical basis (Kivelä et al. 2017). There is evidence of good long-term dietary adherence among adults detected by screening in childhood, regardless of the presence or lack of symptoms at diagnosis (Kivelä et al. 2018).

9.6 Symptoms and quality of life among screen-detected patients

The impact of early diagnosis of celiac disease by active screening on the symptoms and quality of life remain scarcely studied, particularly among subjects who consider themselves asymptomatic at diagnosis. According to previous evidence, 52-65% of screen-detected children and 45-84% of adults have some symptoms at the time of the screening (Kinos et al. 2012; Kivelä et al. 2017; Mahadev et al. 2016; Ukkola et al. 2011; Viljamaa et al. 2005b). These symptoms tend to be milder than those in clinically-detected subjects but, on the other hand, anemia has been reported in 5-22% and poor growth in 17-31% among otherwise asymptomatic, clinically undetected children (Kivelä et al. 2017; Korponay-Szabó et al. 2007). Regarding quality of life at the time of screening, it has been reported to be impaired among symptomatic adults compared to non-celiac disease controls, but this was not seen among initially asymptomatic adults (Ukkola et al. 2011). Among screen-detected adolescents quality of life seems to be similar to non-celiac disease controls as well as to clinically-detected patients (Myléus et al. 2014; Nordyke et al. 2013).

Studies conducted in Finland in particular have found the symptoms to abate comparably in screen-detected and clinically-detected children and adults on GFD (Kinos et al. 2012; Kivelä et al. 2017; Paavola et al. 2012). There are also opposite findings in two studies from the USA, reporting no change in gastrointestinal symptoms among screened family members after one year on GFD (Rubio-Tapia et al. 2008) and that symptom improvement was more unlikely among screened than clinically-detected subjects (Mahadev et al. 2016). This issue is complicated by the fact that up to 30-50% of patients apparently asymptomatic at diagnosis report a beneficial clinical response during GFD (Kinos et al. 2012; Ukkola et al. 2011). In general, self-perceived health and quality of life also seem to improve similarly among screened and clinically-detected subjects during long-term GFD – again at least in Finland (Kivelä et al. 2018; Paavola et al. 2012; Viljamaa et al. 2005b). There are also data from other countries reporting similar quality of life among screened

subjects compared to general population and non-celiac disease controls after one year (Nordyke et al. 2013) and ten years (Van Koppen et al. 2009) on GFD. However, a subgroup of asymptomatic screen-detected individuals may have increased risk for anxiety and impaired health on GFD (Kivelä et al. 2018; Ukkola et al. 2011). Furthermore, a US study reported screen-detected celiac disease patients to be less likely to be satisfied with their diagnosis than those found in clinical routine (Mahadev et al. 2016). The role of screening in the prevention of long-term complications, such as fractures, growth disturbances, and malignancies, remains little studied, particularly among initially asymptomatic subjects (Chapter 8.3).

THE PRESENT STUDY

10 AIMS

The main aims of the present dissertation project were to evaluate the clinical presentation, prevalence, and incidence of celiac disease among at-risk relatives of patients. In addition, possible individual risk factors for first-time and later screening positivity for celiac disease were investigated.

The more specific aims of the three individual studies were:

1. To investigate whether the clinical features of celiac disease differ between affected siblings in multiple case families (**I**).

2. To evaluate the overall prevalence of celiac disease, particularly in screen-detected form, among relatives with different degrees of kinship with the affected index celiac disease patient, and further to determine possible relative- and index patient-related risk factors for screening positivity (**II**).

3. To assess the incidence of and individual factors affecting *de novo* celiac disease or seropositivity for celiac disease autoantibodies among at-risk relatives approximately ten years after the initial negative screening (**III**).

11 MATERIALS AND METHODS

11.1 Participants and study design

11.1.1 Study design

The study cohorts for Studies **I**-**II** and the background population for Study **III** were participants of a large family screening project carried out in 2006-2010 at the Celiac Disease Research Center, Tampere University and Tampere University Hospital. First, children and adults with previously diagnosed celiac disease were invited to participate at occasions organized by the Finnish Celiac Society and via announcements in newspapers and Pirkanmaa child health clinics. Next, the enrolled subjects or their caregivers were asked to invite close relatives of the patients for a screening study, with one of the main goals to evaluate the prevalence of celiac disease among at-risk relatives. The aim was specifically to recruit FDRs, but more distant relatives and spouses could also participate. Altogether 4,155 subjects with self-reported celiac disease and their relatives from 732 families participated from different parts of the country (Figure 3).

Blood samples for celiac disease serology and determination of HLA genotype were drawn from all participants during the study visit or in a local laboratory facility and utilized for the analyses in Studies **I**-**II**. Subjects with self-reported celiac disease or novel seropositivity for EMA were interviewed by a study nurse or study doctor at study visit or, in case of a long distance, by telephone. Family trees were collected from all families as meticulously as possible. Altogether 148 screened subjects with new EMA-positivity were referred to healthcare for diagnostic endoscopy. The result of histology was further collected and recorded by a study nurse or a study physician from individual medical records from subjects with self-reported previous celiac disease and from subjects with new EMA-positivity.

For Study **III**, relatives who were EMA-negative at the initial screening in 2006-2010 were invited to join a follow-up study conducted in 2017-2021 at the Celiac Disease Research Center. During the study visit, all voluntary participants underwent comprehensive interviews and blood samples were drawn for serology. For

participants with long distance, the interviews were conducted by telephone and blood samples were drawn at local laboratories. Possible celiac disease diagnosis made in clinical practice between the two study periods or *de novo* positive seroconversion in the follow-up study were recorded. Relatives with a new positive seroconversion were directed to an appropriate healthcare unit for official celiac disease diagnosis.

11.1.2 Subjects in Study I

The study population was formed by confirming the diagnoses of 1,035 patients with self-reported celiac disease and the 148 relatives found to be EMA-positive in the first screening study in 2006-2010. Of these, altogether 920 had biopsy-proven celiac disease. The final cohort consisted of 200 siblings (100 sibling pairs from separate families) with celiac disease (Figure 3). The first sibling to be diagnosed was defined as the index patient.

11.1.3 Subjects in Study II

For Study **II**, participants of the family screening project whose self-reported celiac disease diagnoses could not be verified and patients without previous non-celiac disease relatives were excluded, as were also relatives who were not related (e.g. spouses) or had no confirmed index patient (Figure 1 in original publication **II**). An index patient denoted the first diagnosed celiac disease case if there was more than one previously diagnosed patient within the same family. The final study group consisted of altogether 2,714 screened non-celiac relatives and 229 non-index patients with previously confirmed celiac disease from 624 families (Figure 3).

11.1.4 Subjects in Study III

Altogether 2,541 out of the 2,967 relatives with negative EMA result in the first screening study in 2006-2010 could be contacted (Figure 1 in original publication **III**). Of these, 640 participated in the follow-up study in 2017-2021. After exclusion of relatives without a confirmed index patient and subjects who were not related to the index patient, the final study cohort included 599 relatives from 339 families (Figure 3). The kinship with the index patient was verified from family trees for each

re-screened relative, and defined as the closest, i.e., FDR if possible. If there was more than one previously affected FDR, the consanguinity (sibling/parent/offspring) was based on the relative who was the reason for the celiac disease suspicion/study participation.

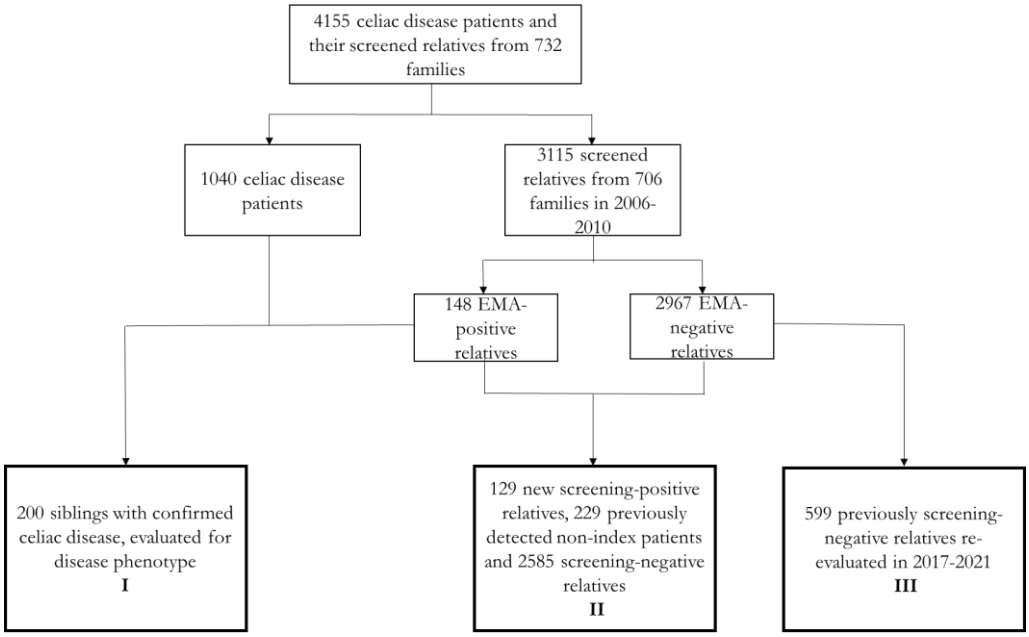

Figure 3. Flowchart of patient collection in Studies **I-III**. EMA, endomysial antibodies

11.2 Data collection

11.2.1 Clinical data

Demographic data were collected from all participants of the first screening study in 2006-2010. More detailed interviews were conducted with the previously diagnosed celiac disease index and non-index patients and new EMA-positive relatives (**I**, **II**). The interview comprised questions about the time of celiac disease diagnosis, clinical presentation before diagnosis among previously diagnosed patients or before the

study visit among new EMA-positive relatives, and presence of possible co-morbidities.

In Study **III**, the initially EMA-negative relatives were asked about demographic data and presence of recurrent or chronic gastrointestinal and extraintestinal symptoms before the study visit, chronic conditions other than celiac disease and self-initiated GFD. In addition, we inquired whether they had received a celiac disease diagnosis after the first screening and before the follow-up study in 2017-2021, as well as their age at and symptoms before the diagnosis.

In Study **I**, the symptoms were further categorized as 1) gastrointestinal symptoms, 2) malabsorption and anemia, 3) extraintestinal symptoms, and 4) asymptomatic. Malabsorption was defined as weight loss and/or laboratory signs of malabsorption such as folate deficiency or hypoalbuminemia. Gastrointestinal symptoms included abdominal pain, constipation, diarrhea, dysphagia, and heartburn, and extraintestinal manifestations dermatitis herpetiformis, failure to thrive in children, dental enamel defects, recurrent aphtous stomatitis, ataxia, arthritis, and elevated liver enzymes. Unspecific symptoms such as back pain and fatigue were disregarded.

In Study **III**, particular attention was paid to possibly celiac disease-related self-reported gastrointestinal and extraintestinal symptoms, such as abdominal pain, constipation, growth failure in childhood, and DH.

In both Studies **I** and **III**, the duration of symptoms before screening or celiac disease diagnosis was defined as no symptoms, ≤5 years and >5 years. The longest symptom duration was considered if the subject had several different symptoms.

The co-morbidities elicited in all studies (**I**-**III**) included selective IgA deficiency and autoimmune diseases such as Sjögrens' syndrome, autoimmune thyroidal disease and type 1 diabetes. In addition, histories of fractures and malignancies, presence of osteoporosis/osteopenia and chronic gastrointestinal and cardiovascular diseases were assessed.

The kinship of the relatives with the index patient was categorized as FDRs (siblings, parents, offspring), SDRs (grandparents, grandchildren, aunts, uncles, nephews, nieces, and half-siblings) and more distant (first- and second-degree cousins, great-grandchildren, great-grandparents, greataunts, and greatuncles) (**II**, **III**). If there was more than one previously diagnosed celiac disease patient (FDR or SDR) in the same family, the screened relative was defined to belong to a multiple case family (**II**, **III**).

11.2.2 Serology

Serum EMAs were measured by indirect immunofluorescence (in-house) using human umbilical cord as an antigen and considering titers 1: ≥ 5 positive (**I**-**III**). Serum TGAs were measured by enzyme-linked immunosorbent assays at Celiac Disease Research Center laboratory: Inova QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego CA, USA (**I**, **II**), cut-off for positivity either >30 (**I**) or >20 U/L (**II**) and EliA Celikey test, Phadia, Freiburg, Germany cut-off for positivity >7 U/L (**III**) according to manufacturer's instructions. The corresponding IgG class serological tests were used if selective IgA deficiency was suspected based on abnormal EMA staining pattern and low TGA value (**I**-**III**). Information on possible previously determined celiac disease autoantibodies (EMA, TGA, and/or ARA) was collected from the patient records of the self-reported celiac disease patients (**I**, **II**) and re-screened relatives diagnosed between the two studies (EMA and/or TGA) (**III**).

From the previously diagnosed celiac disease patients, results of histopathology of the small bowel biopsies and/or possible skin biopsies were verified from earlier patient records (**I**-**III**). The degree of villous atrophy was classifierd as partial (PVA), subtotal (SVA), and total (TVA) based on the original pathology reports, these corresponding approximately to Marsh-Oberhuber grades IIIa-c respectively (Dickson et al. 2006) (**I**, **II**). The diagnosis of DH was based on demonstration of IgA deposits in the papillary dermis adjacent to the lesion by indirect immunofluorescence (Collin et al. 2017) (**I**, **II**).

The diagnosis of celiac disease index patients in the first study (2006-2010) was based on the demonstration of small-bowel mucosal villous atrophy in duodenal biopsy or characteristic findings in skin biopsy (Collin et al. 2017) in case of DH (**I**-**III**). In Study **III**, the diagnosis of celiac disease received after the initial screening and before the follow-up study had to be established in healthcare according to the most recent Finnish celiac disease guidelines, which also permit serology-based diagnostic criteria in selected sircumstances (Celiac disease, Current Care Guidelines 2018).

11.2.3 Genetic analyses

Genotyping for the celiac disease-associated HLA alleles was performed using the SSPTM DQB1 low-resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden) (**I**-**III**), DELFIA® Coeliac Disease Hybridization Assay Kit (PerkinElmer Life and

Analytical Sciences, Wallac Oy, Turku, Finland) (**II**) or tagging SNP approach (Monsuur et al. 2008) (**I**-**III**).

In Study **I**, the celiac disease associated haplogenotypes were further categorized into HLA DQ2.5 positives (DQ2.5/DQX, DQ2.5/2.5, DQ2.5/8, DQ2.5/2.2, 2.5/7 or DQ2.2/DQ7), to HLA DQ8 positives (DQ8/DQX, DQ8/8, DQ8/2.2 or DQ8/7), and to both DQ2 and DQ8 negatives.

In Studies **II** and **III**, the haplogenotype was further categorized into high, intermediate, and low risk groups. The high risk included DQ2.5/DQ2.2 (A1*05-B1*0201/A1*02-B1*0202) or homozygosity for DQ2.5 (A1*05-B1*0201/A1*05-B1*0201 [DQ2.5/2.5]). The intermediate risk included DQB1*02 heterozygosity and/or DQ8 positivity (A1*05-B1*0201/X [DQ2.5/X], A1*05-B1*0201/A1*03-B1*0302 [DQ2.5/8], A1*02-B1*0202 [DQ2.2/2.2 and DQ2.2/X], A1*02-B1*0202/A1*03-B1*0302 [DQ2.2/8] and A1*03-B1*0302 [DQ8/8 and DQ8/X]). Low risk was categorized as negativity for both DQ2 and DQ8 (Romanos et al. 2009).

11.3 Definition for screening positivity and negativity

Screening positivity was defined as positivity for both EMA and TGA irrespective of their specific numerical value, together with a presence of the celiac disease-associated HLA DQ2 and/or DQ8 (**II**, **III**) (Pietzak et al. 2009; Rubio-Tapia et al. 2008). In Study **III** relatives with negative EMA in the first study (2006-2010) were considered as having had a negative screening result at the initial screening regardless of the TGA result.

11.4 Statistics

In all three studies, the results are given as medians with quartiles and with number of cases and/or percentages (**I**-**III**). In addition, odds ratios (OR) with 95% confidence intervals (CI) are given in Study **II** and IR and incidence rate ratios (IRR) with 95% CI in Study **III**. Categorical variables were studied either by McNemar test (**I**), chi-square test (**I**-**III**) or by Fisher's Exact test (**III**) and continuous variables by Wilcoxon signed rank test (**I**) or Mann-Whitney test (**II**, **III**). A p-value <0.05 was considered significant (**I**-**III**).

In Study **I**, the index case and his/her sibling in the same sibling pair were compared according to clinical phenotype and frequency of having the same HLA haplotype was assessed among pairs with and without concordant clinical phenotype.

In Study **II**, the ORs for new seropositivity were evaluated with binary logistic regression. In logistic regression, the characteristics of the index patient were set as variables for each screened relative and the group with the greatest number of subjects was used as a reference category. The variable for HLA risk was high vs. intermediate risk group. FDRs were analyzed separately as siblings, parents, and offspring, but SDRs and more distant relatives as whole groups. Index patient and relative-related factors affecting screening positivity were assessed. To determine the independent risk factors, the statistically significant characteristics in univariate analysis were further assessed with multivariable binary logistic regression. Three models were used as follows: Model 1 notified the significant characteristics of the screened relatives except for HLA risk, Model 2 the significant characteristics of the index patient and screened relative except for HLA risk and Model 3 the significant characteristics of the index patient and screened relative, including HLA risk.

In Study **III**, person-time at risk was follow-up time from first screening either to celiac disease diagnosis outside the study protocol or to re-screening. The IRs were calculated for the whole study group and separately for subjects carrying HLA DQ2/8, having high and intermediate risk HLA, and being <30 years and ≥30 years at initial screening and for women and men. IRRs were estimated in univariate analysis using age <30 years and ≥30 years at initial screening, sex and HLA high and intermediate risk groups as covariates. Multivariable Poisson regression analysis was further performed for statistically significant IRs.

SPSS Statistics for Windows version 27 (IBM Corp Armonk, NY, USA) and STATA Statistical Software (StataCorp. LLC, Lakeway Drive, TX, USA) were used for the analyses in Studies **I** and **III** and SPSS and Confidence Interval Analysis Program (Altman et al. 2000) in Study **II**.

11.5 Ethics

The study designs and recruitment of participants were approved by the Ethics Committee of Pirkanmaa Hospital District. The Declaration of Helsinki was followed and the participants were given written and oral information on the purpose of the study and the significance of the positive screening result. All participants and/or their caregivers gave written informed consent.

12 RESULTS

12.1 Demographic data

12.1.1 Celiac disease patients and screening-positive relatives

Altogether 72% of the first and later diagnosed siblings (**I**), 56% of the screening-positive relatives and 75% of the index and non-index patients (**II**), and 60% of the screening-positive relatives/celiac disease patients (**III**) were females.

Median age at diagnosis in Study **I** was 40 years, in Study **II** 40 years among screening-positive relatives, 39 years among index patients and 36 years among non-index patients, and in Study **III** 34 years at celiac disease diagnosis/re-screening positivity. Altogether 19% of the first and later diagnosed siblings (**I**), 23% of the screening-positive relatives and 18% of the index and non-index patients (**II**), and 13% of the screening-positive relatives/celiac disease patients (**III**) were <18 years of age at diagnosis or at the time of screening.

Both the celiac disease patients and the screening-positive subjects in Study **II** belonged most often to age category 41-50 years, whereas the least common age group was 61-70 years (Figure 4). When evaluating only the proportion of screening positivity among the screened n=2,714 relatives, the figures remained similar, with the highest proportion found in the age group 41-50 years (Figure 5).

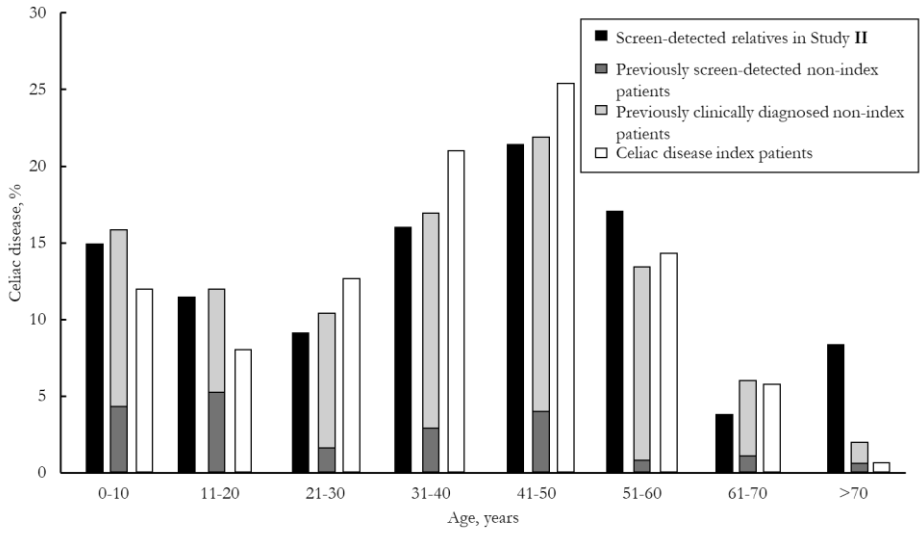

Figure 4. Distribution of age at diagnosis/screening of the previously detected celiac disease index patients (n=624), non-index patients (n=229) and those with new screening positivity (n=129) in Study **II**.

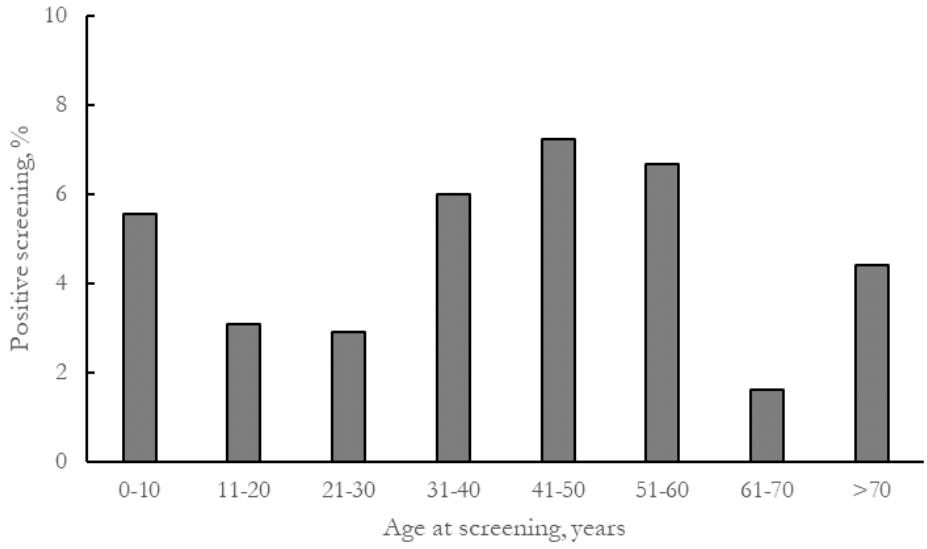

Figure 5. Proportion of screening positivity among 2,714 screened relatives in different age groups in Study **II**.

In Study **III**, new celiac disease diagnosis/screening positivity was found most frequently in the age group 0-20 years (Figure 6), although the age of new cases ranged 5-79 years.

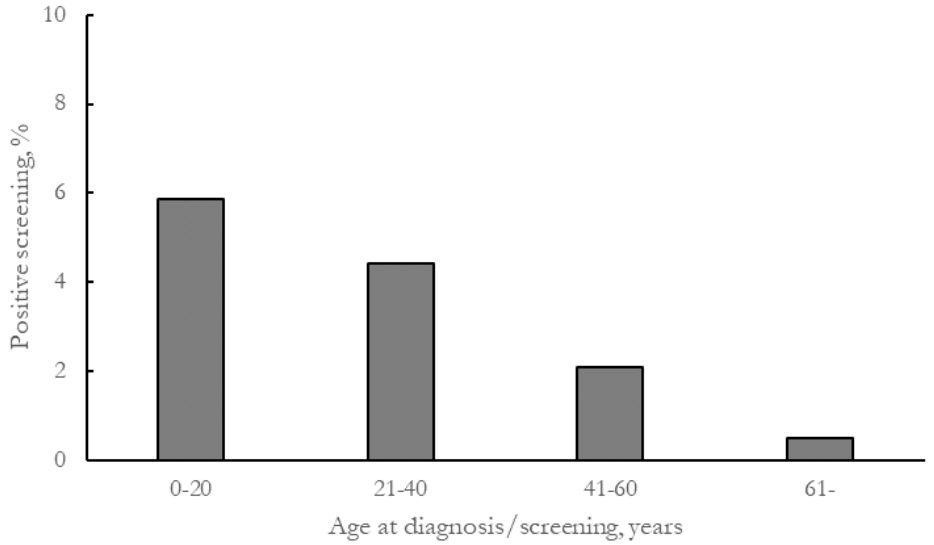

Figure 6. Proportion of celiac disease diagnosis/screening positivity among 599 re-screened relatives in different age groups in Study **III**.

12.1.2 Screened and re-screened relatives

Altogether 56% of the 2,714 screened relatives in Study **II** and 66% of the 599 re-screened relatives in Study **III** were females. The median ages at the time of the study visit were 36 years (**II**) and 52 years (**III**). Seventy-six percent of the relatives in Study **II** and 93% in Study **III** were FDRs (Figure 7).

In Study **III** the groups of participants (n=640) and non-participants (n=2,327) did not differ in age at initial testing, initial TGA value or HLA distribution (Supplementary table 1 in original publication **III**).

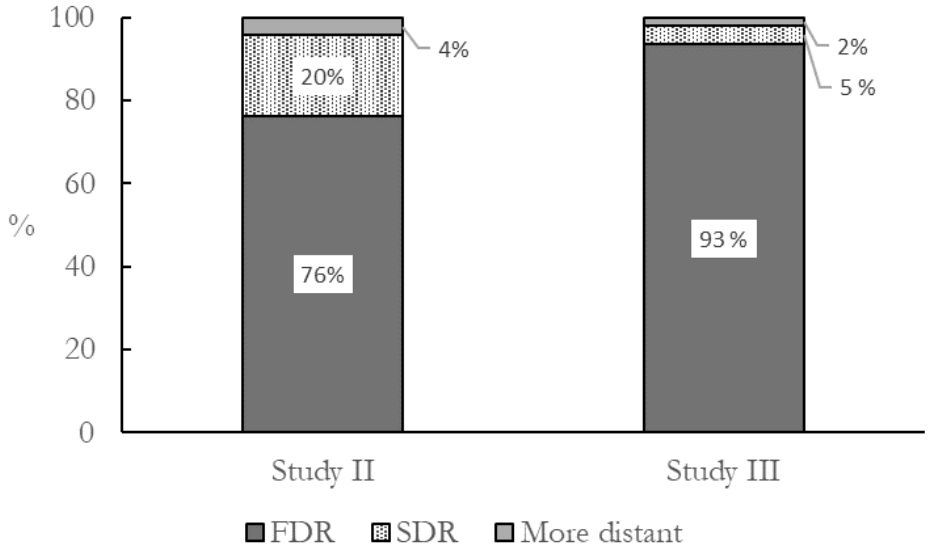

Figure 7. Distribution of screened relatives (n=2,714) in Study **II** and re-screened relatives (n=599) in Study **III** according to kinship with the index patient. Index patient was defined either as the first diagnosed relative (**II**) or the closest relative (FDR) (**III**) in families with more than one previously detected celiac disease patient. FDR, first-degree relative; SDR, second-degree relative.

12.2 Clinical characteristics of first and later diagnosed siblings

12.2.1 Distribution of clinical phenotypes in 100 sibling pairs

Among the 100 sibling pairs, gastrointestinal presentation was the most common phenotype, being present in 78% of the index patients and siblings. Of the gastrointestinal symptoms, diarrhea was the most common separate symptom (43%).

Anemia and/or malabsorption was reported by 33% and extraintestinal symptoms by 23% of the index patients and siblings. The most common extraintestinal symptom was DH (17%). None had ataxia, arthritis or elevated liver enzymes. Altogether 10% (n=20, all siblings) were asymptomatic at diagnosis and 24% (n=5 first diagnosed and n=42 later diagnosed siblings) were detected in screening. Of the 42 later diagnosed screened siblings, 24 were detected in the Study **I**.

12.2.2 Differences between index patients and later diagnosed siblings

The index patients were younger, had less often partial villous atrophy and were more often negative for one or more antibodies tested (TGA, EMA, ARA) at diagnosis and more often female than their later diagnosed siblings (Table 8). Siblings did not differ significantly in prevalence of fractures, malignancies, or associated conditions (Table 1 in original publication **I**).

Table 8. Demographic data and clinical characteristics in 100 sibling pairs with celiac disease (**I**)*.

	Index patients, n=100	Siblings, n=100	p value
Age at diagnosis, median (Q_1, Q_3), years	37 (22, 47)	43 (25,52)	**<0.001**
Female, %	81	63	**0.008**
Clinical presentation at diagnosis[1], %			
Malabsorption or anemia	45	20	**<0.001**
Extra-intestinal	33	12	**<0.001**
Asymptomatic	0	20	**<0.001**
Degree of villous atrophy at diagnosis, %			**0.047**
Total	29	27	
Subtotal	49	31	
Partial	20	41	
Positive celiac antibodies at diagnosis[2], %	89	100	**0.030**

Q1 and Q3, first and third quartiles. *Only statistically significant values are presented. [1] Symptomatic patients may have had several overlapping presentations; [2] Transglutaminase 2, endomysial or anti-reticulin antibodies. Antibody data were missing from 33 index patients and 23 siblings and the comparisons were made between 54 pairs. Numbers in bold face indicate significant values.

The index patients also presented more often with malabsorption/anemia and extraintestinal symptoms at diagnosis (Table 8), and with DH (25% vs. 9% (p=0.002). There was no significant difference in the duration of symptoms or presence of gastrointestinal symptoms between the first and later diagnosed siblings (Table 1 in original publication **I**), or in presence of diarrhea, abdominal pain and constipation from gastrointestinal symptom subgroups or in oral symptoms or failure to thrive from extraintestinal symptoms.

Twenty-one of the 100 sibling pairs had concordant clinical phenotype, including 14 with gastrointestinal presentation (Table 9). Of the 79 pairs with discordant phenotype, 28 index patients had gastrointestinal symptoms combined with malabsorption/anemia and/or extraintestinal symptoms, while the later diagnosed sibling had only gastrointestinal symptoms. In nine pairs the later diagnosed sibling had other clinical manifestations in addition to gastrointestinal symptoms when their index patients had only gastrointestinal symptoms.

Table 9. Phenotype concordance in 100 sibling pairs in Study **I**. From the 21 pairs with concordant phenotype, the HLA haplotype was concordant in eight pairs (shown in parentheses).*

		Index patients, n=100						
		GI	MA	EI	GI + EI	MA + EI	GI + MA	GI + MA + EI
Siblings, n=100	GI	**14** [2.5/X and 2.5/2.5]	3	3	8	1	13	7
	MA	3	**1** [2.5/8]	0	1	0	0	0
	EI	0	0	**1**	0	0	1	0
	GI + EI	3	0	2	**1** [2.5/X]	0	3	0
	MA + EI	0	0	0	0	**0**	0	1
	GI + MA	6	1	1	1	1	**4**	0
	GI+ MA + EI	0	0	0	0	0	0	**0**
	Asymptomatic	6	4	2	3	0	5	0

HLA, human leukocyte antigen; GI, gastrointestinal; MA, malabsorption or anemia; EI, extra-intestinal. *Data on HLA haplotype was available in 17 pairs with concordant phenotype. Pairs with concordant phenotype appear in bold face and the results are given as number of sibling pairs.

12.3 HLA distribution among celiac disease patients and screening-positive relatives

The most frequent HLA haplotype in Study **I** was DQ2.5/X, carried by 51% of the celiac disease patients (48% of the index patients and 53% of the siblings). Seventy-four percent of the screening-positive relatives in Study **II** and 64% of the screening-positive relatives/new celiac disease patients in Study **III** had intermediate HLA risk, while high-risk HLA was present in 29% (**I**), 27% (**II**) and 36% (**III**) of the celiac disease patients/screening-positive relatives.

In Study **I**, the frequency of having the same HLA haplotype was assessed among sibling pairs with and without concordant clinical phenotype. In both siblings the data on HLA haplotype was available in 66 pairs. The clinical phenotype was concordant in 17 pairs and eight of these (47%) had concordant HLA haplotype. Among 49 pairs with discordant clinical phenotype, concordant HLA haplotype was present more often than among 17 pairs with concordant clinical phenotype (78% vs. 47%, p=0.018 respectively).

12.4 Prevalence of celiac disease among relatives

Altogether 129 (4.8%) out of the 2,714 screened non-celiac disease relatives in Study **II** were screening-positive (Figure 3). The prevalence was 5.1% (n=106) in FDRs, 3.6% (n=19) in SDRs, and 3.5% (n=4) in more distant relatives. In more detailed analysis among FDRs, the prevalence was 6.5% in siblings, 4.7% in parents, and 4.0% in offspring. Among relatives whose index patient was SDR or more distant and who did not have an affected FDR in the family, the prevalence of screen-detected celiac disease was 3.4% (n=12) among SDRs and 4.2% (n=3) among more distant relatives. The combined prevalence of celiac disease in the new screen-detected and previously diagnosed non-index patients was 12.2% in all relatives and in separate analysis 12.5% in FDRs, 10.9% in SDRs, and 12.8% in more distant relatives (Figure 8). The overall prevalence decreased among FDRs from siblings to parents and offspring as did the screen-detected celiac disease (Figure 3 in original publication **II**).

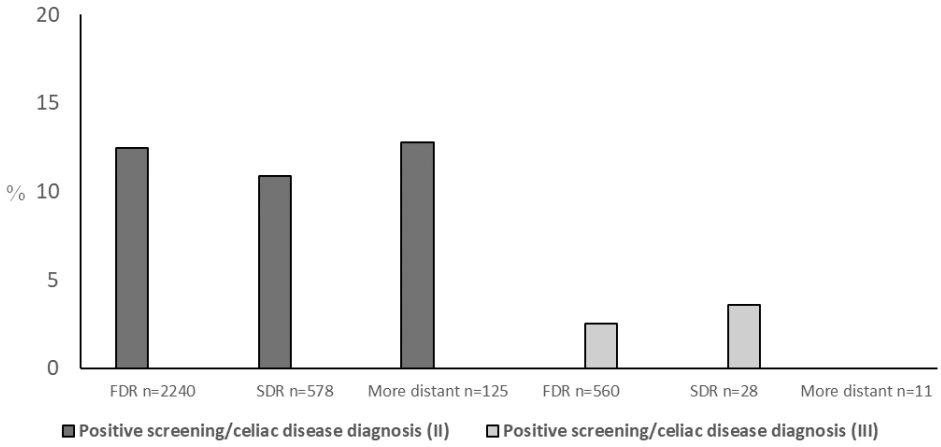

Figure 8. Proportion of combined celiac disease prevalence in Study **II** or new celiac disease diagnosis/screening positivity in Study **III** according to kinship with the index patient. Index patient is defined as the first diagnosed relative (**II**) or the closest relative (FDR) (**III**) in families with more than one previously detected celiac disease patient. FDR, first-degree relative; SDR, second-degree relative.

12.5 Incidence of celiac disease/screening positivity among once screening-negative relatives

Of the 599 initially EMA-negative relatives who participated to the second study, 15 (2.5%) were either diagnosed with celiac disease between initial and new screening (n=8) or were screening-positive in the new study (n=7). One of them was SDR and the other 14 FDRs (Figure 8). Median time from initial screening to the follow-up study was 11.4 years. The follow-up time was 6,785.9 person-years from first screening to diagnosis or re-screening, giving an IR of 221/100,000 person-years for celiac disease/screening positivity. The corresponding figure was 336/100,000 person-years among the relatives carrying HLA DQ2/8 and zero among those without the risk haplotypes.

12.6 Comparison of screening-positive and screening-negative relatives

The screening-positive relatives more often carried high-risk HLA than those who were screening-negative in both Studies **II** and **III** (Table 10). In addition, the re-

screened relatives with celiac disease diagnosis/screening positivity in Study **III** were younger than the screening-negative subjects. There was no significant difference in number of relatives <18 years of age at screening either in Study **II (**23% vs. 26%, p=0.404) or in Study **III** (13% vs. 6%, p=0.245). Furthermore, both groups showed comparable sex distribution, number of members in multiple case families and distribution of degree of kinship with the index patient (Table 10).

Table 10. Characteristics of the 2,714 screened relatives in Study **II** and 599 re-screened relatives in Study **III** with and without screening positivity[1].

	Screening-positive, n=129 (**II**)		Screening-negative, n=2,585 (**II**)			Screening-positive, n=15 (**III**)		Screening-negative, n=583 (**III**)		
	n	%	n	%	p value	n	%	n	%	p value
Age at study visit, median (Q1, Q3), years	40 (20, 53)		36 (17, 55)		0.556	34 (24, 52)		52 (34, 66)		**0.027**
Females, %	72	55.8	1435	55.5	0.946	9	60.0	385	65.9	0.633
Member of multiple case family[2]	35	27.1	631	24.4	0.483	7	46.7	449	76.9	0.058
Kinship with index patient					0.260					0.640
First-degree relative	106	82.2	1,961	75.9		14	93.3	546	93.5	
Second-degree relative	19	14.7	515	19.9		1	6.7	27	4.6	
More distant relative	4	3.1	109	4.2		0	0	11	2.1	
HLA risk group[3]					**<0.001**					**<0.001**
High risk[4]	27	26.5	156	7.0		5	35.7	37	7.4	
Intermediate risk[5]	75	73.5	1,394	62.3		9	64.3	316	63.3	
Low risk[6]	0	0	686	30.7		0	0	146	29.3	

FDR, first-degree relative; HLA, human leukocyte antigen; SDR, second-degree relative; Q1 and Q3, first and third quartile. [1] Positivity for endomysial antibodies and transglutaminase 2 antibodies and presence of HLA DQ2/DQ8 (II, III) or previous celiac disease diagnosis after the initial screening and before the re-screening study (III); [2] Relative has ≥2 previously affected FDR or SDR; [3] Data missing from 376 (II) and 86 (III) relatives; [4] DQ2.5 homozygotes and DQ2.5/2.2; [5] DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive; [6] DQ2/8 negative. Numbers in bold face indicate significant values.

In Study **III**, the new celiac disease patients/screening-positive relatives were younger at the time of the initial screening study in 2006-2010 than those who were screening-negative (23 vs. 41 years, p=0.028), whereas there was no significant difference in the proportion of those <18 years of age (Table 1 in original publication **III**). The former also had higher median TGA value at initial screening (10 U/mL vs. 8 U/mL, p=0.033), even though all relatives had been EMA negative at the time of the first study. The groups did not differ in duration of symptoms (57% with celiac disease diagnosis/positive screening result and 72% with negative result reported symptom duration >5 years before the study visit or celiac disease diagnosis, p=0.333) or in the presence of co-morbidities (Table 1 in original publication **III**).

Altogether 86% of the new celiac disease patients/screening-positive relatives and 71% of the seven screen-detected cases reported some symptoms at diagnosis/screening (Table 2 in original publication **III**). These two subgroups did not differ significantly in any of the study parameters (Table 2 in original publication **III**).

In a separate analysis among screening-positive FDRs and SDRs in Study **II**, SDRs were younger and more often members of multiple case families, whereas the groups did not differ in sex or HLA risk group (Table 11).

Table 11. Characteristics of the 125 screening-positive[1] first-degree (FDR) and second-degree (SDR) relatives in Study **II**.

	FDR, n=106		SDR, n=19		
	n	%	n	%	p value
Age at screening, median (Q_1, Q_3), years	42 (26, 56)		29 (5, 42)		**0.007**
Age <18 years at screening	19	17.9	8	44.4	**0.026**
Women	58	54.7	12	63.2	0.495
Multiple case family[2]	22	20.8	9	47.4	**0.020**
HLA risk group[3]					0.224
High[4]	25	30.1	2	13.3	
Intermediate[5]	58	69.9	13	86.7	

HLA, human leukocyte antigen; Q1 and Q3, first and third quartile. [1] Positivity for endomysial antibodies and transglutaminase 2 antibodies and presence of HLA DQ2/DQ8; [2] Screened relative has at least two relatives previously diagnosed with celiac disease; [3] Data missing from 27 relatives; [4] DQ2 homozygous (DQ2.5/2.5 or 2.2/2.5); [5] DQ2 or DQ8 (DQ2.5, 2.2 or 8). Numbers in bold face indicate significant values.

12.7 Factors affecting screening positivity/celiac disease diagnosis

In Study **II**, the age of index <18 years at diagnosis was risk factor for screening positivity in univariate logistic regression analysis (Table 3 in original publication **II**). From the characteristics of the screened relative, being a sibling compared to other degrees of kinship with the index and 41-60 years of age at screening compared to other age groups and having the high-risk HLA compared to intermediate risk HLA were risk factors for screening positivity. However, only high-risk HLA remained a statistically significant independent risk factor in multivariable analysis (OR 2.94 [1.80-4.78]). Autoimmune comorbidities, sex, degree of villous atrophy, DH and high vs. intermediate risk HLA as characteristics of index patient were not significant risk factors in univariate analysis. Of the characteristics of the screened relative, age <18 years at screening, sex or being a member of a multiple case family were not risk factors in univariate analysis (Table 3 in original publication **II**).

In Study **III,** the IR for celiac disease diagnosis/screening positivity in the new screening study was higher among subjects <30 years than among those ≥30 years

of age at initial screening and among carriers of high-risk HLA than among those with intermediate risk, whereas sex had no effect. Only high-risk HLA remained significant in multivariable analysis (Table 12).

Table 12. Incidence rates (IR) and IR ratios (IRR) for celiac disease/screening positivity in Study **III** using age at initial screening, gender, and human leukocyte antigen (HLA) group as covariates. Significant covariates were further adjusted in multivariable analysis.

	Univariate		**Multivariable**
	IR	IRR (95% CI)	IRR (95% CI)
Age at initial screening			
<30 years	406/100,000	**3.51 (1.09-13.1)**	2.83 (0.95-8.46)
≥30 years	116/100,000		
Sex			
Women	202/100,000	1.27 (0.37-4.02)	
Men	258/100,000		
HLA group			
High[1]	1073/100,000	**4.41 (1.16-14.7)**	**4.62 (1.55-13.8)**
Intermediate[2]	243/100,000		

CI, confidence interval. [1] DQ2.5/2.5 and DQ2.5/2.2; [2] DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive. Numbers in bold face indicate significant values.

13 DISCUSSION

13.1 Clinical picture among screen-detected patients

Celiac disease-related symptoms at the time of screening were reported by 57% of the later diagnosed siblings in Study **I** and 71% of the screening-positive relatives in Study **III**. As a potential explanation for the differing percentage, symptom classification was more specific in Study **I** than in Study **III**. Of note, analysis made long time after the diagnosis may result in misleadingly lower percentage of symptoms due to recall bias (Ukkola et al. 2011; Viljamaa et al. 2005b). The time from diagnosis was not reported in Study **I**, but since only approximately half of the later diagnosed siblings were detected during the study and the rest earlier, it is likely that time from diagnosis to study visit was longer than that among the screen-detected relatives in Study **III**. Another confounding factor is that the initially "asymptomatic" screen-detected subjects may realize the actual presence of symptoms only after initiation of GFD (Kinos et al. 2012; Ukkola et al. 2011). It should also be noted that in both studies (**I** and **III**) the number of screen-detected cases was relatively low.

In any case, the prevelance of symptoms in Studies **I** and **III** is mostly in line with that reported previously (52-69%) (Kinos et al. 2012; Kivelä et al. 2017; Mahadev et al. 2016) and demonstrates that a major part of screen-detected patients may suffer from unrecognized clinical symptoms not recognized in healthcare (Kivelä et al. 2017; Mahadev et al. 2016; Ukkola et al. 2011). This lends further support to systematic screening of at-risk relatives, particularly as there is evidence that not even those who are symptomatic are currently adequately tested (Faye et al. 2018). Somewhat contradictorily, however, the screening-positive and negative relatives in Study **III** reported symptoms equally often. This reflects the wide extent of various unspecific symptoms in general population (Katz et al. 2011) and serves as a reminder that not every symptom before screening is necessarily celiac disease-related. On the other hand, the reported clinical response for up to 96% of the screen-detected children (Kivelä et al. 2017) and 74% of the adults (Paavola et al. 2012) indicates that most of the patients consider the treatment beneficial.

13.2 Comparison of first and later diagnosed siblings

The index siblings in Study **I** were younger and had more severe clinical picture at diagnosis than those diagnosed later, which is in line with the studies by Gudjónsdóttir et al. and Mustalahti et al. (Table 13). It seems logical that symptomatic subjects consult healthcare earlier. Accordingly, Kivelä et al. found symptomatic children with celiac disease to be younger at diagnosis than asymptomatic patients (Kivelä et al. 2017). Patients diagnosed earlier have also been reported to be more often females than males (Schosler et al. 2015), possibly due, at least in part, to more active healthcare use among the former (Pinkhasov et al. 2010). There was also a tendency for shorter duration of symptoms among the later diagnosed siblings, which may reflect the awareness of the increased celiac disease risk in families with one already affected patient; however, more studies on this issue are needed. Conversely, age at celiac disease testing may be rather incidental in asymptomatic or mildly symptomatic patients, making comparison of age at diagnosis between siblings more complicated. As regards type of symptoms, this was similar in only 21% of the sibling pairs. More specifically, gastrointestinal symptoms were equally reported, but otherwise the different manifestations of the disease appeared to be somewhat randomly distributed, even when vague symptoms such as fatigue and joint pain were disregarded.

Remarkably, the HLA genotype was even more often similar among pairs whose clinical phenotype was discordant. This concurs with earlier results (Table 13) and speaks against a major role of HLA in differences in the nature or severity of symptoms among the sibling pairs. Similarly, HLA genotype does not seem to explain the difference in age at diagnosis between the siblings (Gudjonsdóttir et al. 2009; Mustalahti et al 2002). Besides HLA, non-HLA genes may also contribute to the phenotype (Gudjonsdóttir et al. 2009, Chapter 3.5), but the fact that the clinical presentation may differ even among monozygous twins (Hervonen et al. 2000) supports the role of additional environmental factors such as gut microbiome (Wacklin et al. 2010).

As a special case, the index patients suffered more often from DH than the later diagnosed siblings, possibly because the condition often manifests with visible bullous rash and marked itching (Collin et al. 2017) and thus leads to prompt healthcare contact. Additionally, it has been proposed that DH develops as a complication of long-term untreated celiac disease (Salmi et al. 2015). In line with this, concomitantly with increasing incidence of celiac disease, incidence of DH has decreased (Salmi et al. 2011). In that sense it is interesting that – although not

statistically significant – there was a trend for longer diagnostic delay in the index patients compared to their siblings.

Table 13. Effect of genetics on clinical features of celiac disease among affected family members.

Reference	Patients	Compared clinical phenotypes	Compared genotypes	Effect of genotype to the phenotype	Effect of genpotype to the age at diagnosis
Mustalahti et al. 2002	28 sibling pairs of all ages	Symptomatic vs. silent (screen-detected)	HLA-DQ2/DQ8 haplotypes and HLA-DQB1*0201 homozygosity vs. heterozygosity	No difference in DQ2/8 distribution or in number of DQB1*0201 homozygous patients.	No correlation between DQB1*0201 homozygosity and age. Symptomatic siblings were younger at diagnosis.
Karell et al. 2002	110 sibling pairs	Classic celiac disease vs. DH	HLA-DQ2/DQ8 haplotypes	No difference in DQ2/8 distribution.	
Karinen et al. 2006b	144 FDRs in families with ≥2 affected siblings, adults	Different clinical features at diagnosis	Number of HLA-DQB1*0201 alleles (two vs. one vs. zero)	More severe villous atrophy, higher frequency of abdominal pain and diarrhea and lower hemoglobin* among DQB1*0201 homozygous.	HLA DQB1*0201 homozygous patients were younger than those with one or zero alleles.
Gudjonsdottir et al. 2009	107 families with ≥2 affected siblings, children	Classic symptoms, milder symptoms, clinically silent	DQ2 homozygotes (2.5/2.5 and 2.5/2.2) vs. DQ2.5 heterogygotes vs. other HLA and CTLA 4 +49 A/G genotypes	Significant association between clinical phenotype and CTLA4 +49A/G polymorphism, but no association according to other genotypes.	No correlation between genotypes and age. Siblings with more severe symptoms were younger at diagnosis.

CTLA 4, cytotoxic T-lymphocyte-associated protein 4; DH, dermatitis herpetiformis, FDRs, first-degree relatives; HLA, human leukocyte antigen. *After adjusting for gender and degree of villous atrophy.

13.3 Definition of screening positivity

In Studies **II** and **III**, the definition of screening positivity was based on previous findings that positivity for EMA and TGA and presence of HLA DQ2/8 result in a PPV of up to 96-100% for biopsy-proven celiac disease (Fasano et al. 2003; Rubio-Tapia et al. 2008). Hence, only one out of the 130 EMA-positive subjects in Study **II** had positive EMA and HLA compatible with celiac disease but borderline negative TGA (19 U/l, cut-off >20). Moreover, all EMA-positive screen-detected relatives in Study **III** were also TGA-positive. Further supporting the use of the chosen approach instead of histology-based outcome is the fact that up to 36-47% of the seropositive subjects has refused endoscopy in earlier studies (Fasano et al. 2003; Katz et al. 2011).

In Study **III**, a negative result in the initial screening was defined as negative EMA regardless of the TGA level. This was based on our long experience with the in-house test used (Ladinser et al. 1994) and the overall high specificity of EMA for celiac disease (Valdimarsson et al. 1996, Ylönen et al. 2020). Moreover, there are significant differences in diagnostic performance between TGA assays (Husby et al. 2012; Husby et al. 2020; Werkstetter et al. 2017), and some studies have reported particularly low PPVs for TGA-positivity in EMA negative at-risk relatives (Bonamico et al. 2006; Rubio-Tapia et al. 2008). Therefore, the chosen approach was believed to provide sufficient sensitivity with low risk for false positivity. It is nevertheless possible that some of the EMA-negative relatives in Study **III** actually had celiac disease. Moreover, the fact that the initial TGA value was higher among relatives with new celiac disease/screening positivity than those who were screening-negative indicates that some relatives may already have had an early stage of the disease at initial testing (Mariné et al. 2009). Altogether, the significance of low/borderline positive TGA among EMA negative relatives is an important subject for future studies.

13.4 Prevalence of celiac disease among at-risk relatives

The prevalence of celiac disease in Study **II** was 4.8% among all screened at-risk relatives and, after combining the screen-detected and previously diagnosed patients, 12.2%. The corresponding figures among FDRs were 5.1% and 12.5%. Most earlier

studies have been conducted among FDRs, in whom the prevalence has varied, with some exceptions, from 2.6% to 6.8% (Martins et al. 2010; Oliveira et al. 2012) (Table 4). Markedly higher figures have been reported by Wessels et al. (2016), who found the prevalence of screen-detected celiac disease to be 15.0%, and by Rubio-Tapia et al. (2008) and Bonamico et al. (2006) who reported prevalences of 11.3% and 9.5%, resepectively.

Comparison of these percentages is hampered by the divergent diagnostic criteria used, i.e., biopsy-proven vs. serology-based. In addition, the prevalence of previously detected celiac disease is usually not reported. As an exception, two earlier studies have also reported combined prevalences, namely 4.3% among parents and 9.8% among siblings (Bourgey et al. 2007) of the index patients, and 16.4% among all FDRs (Rubio-Tapia et al. 2008). Of note, this high percentage reported by Wessels et al. could be explained by the fact that they offered repeated screening for once seronegative children (Wessels et al. 2016).

13.5 Age at screening positivity

One novel finding of this dissertation was that celiac disease can be found in all ages after gluten initiation in both initial testing and during later re-screening (Figures 5 and 6).

As regards the first-time screening, the highest proportion of screen-detected celiac disease in Study **II** was found among those aged 41-50 years. Moreover, the age between 41-60 years was a risk factor for positive screening results in univariate analysis, although both kinship with the index patient and presence of high-risk HLA overrode the age effect. The proportion of screen-detected patients also increased from childhood to middle age, which accords with earlier reports that celiac disease is rarer among children and adolescents than in adult population (Mäki et al. 2003; Vilppula et al. 2009). In turn, the smaller screening positivity among elderly patients could be due to the increased frequency of EMA-negativity, up to 12.7%, in this age group (Salmi et al. 2006). Interpretation of these findings is hampered by the variable proportion of previously detected relatives in each age category, which may be affected by temporal changes in celiac disease incidence (Kivelä et al. 2015; Virta et al. 2009) and active childhood testing. Furthermore, the individual healthcare behaviour and diagnostic approach in healthcare may have an affect, if, for example, celiac disease has not been suspected in elderly population.

In separate analyses, the screening-positive SDRs were younger and more often <18 years of age at screening than the screening-positive FDRs in Study **II**. The lack of studies on this issue and the relatively small number of SDRs make it impossible to draw any firm conclusions about this finding. In addition, the classification of the index patient may have made a difference here, since kinship was determined solely on the basis of the first diagnosed patient in the family, and with different determination the SDRs might actually have been included among the FDRs.

As regards re-screening in Study **III**, there was no difference in the number of celiac disease patients/screening-positive relatives and screening-negative relatives who were <18 years of age. However, the IR in re-screening was higher among subjects who were <30 of age at initial screening than among those who were ≥30 years, although HLA risk overrode this effect. A peak in celiac disease incidence has previously been reported among children <10 years of age with genetic risk for celiac disease in the multicenter TEDDY study (Hagopian et al. 2017), whereas in a US population-based screening the prevalence of celiac disease did not differ between pediatric age groups (Fasano et al. 2003). In any case, it could be assumed that new cases develop more often among children than among adults and re-screening children <10 years of age only has been recommended by Wessels et al. (2018). However, it is impossible to determine any specific age limit for re-screening given the present knowledge, and the results of Study **III** provide further evidence that new cases develop in adulthood and even among the elderly (Vilppula et al. 2009).

The optimal frequency of screening is another question to be resolved, although more active re-screening in childhood could be justified in light of the possibility of permanent complications such as poor growth and delayed puberty (Jericho et al. 2017; Nurminen et al. 2019), even in a relatively short period of time. Of note, systematic re-screening among once seronegative children has only been tested by Pittschieler et al. (2003), who reported five new cases and an IR of 484/100,000 person-years in annual testing during a follow-up period of up to 12 years.

Due to such limited evidence, only expert opinions are available about the re-screening interval in children. For example, the British Society of Gastroenterology recommend triennial testing of asymptomatic children who carry HLA DQ2/8, but subsequent surveillance of symptomatic seronegative children is not specified (Murch et al. 2013). Altogether, it is likely that large multicenter studies with systematic screening at certain pre-defined timepoints are needed to ascertain the optimal screening frequency for celiac disease.

13.6 Kinship with the index patient in screening

In FDRs, the prevalence of screen-detected celiac disease decreased to some extent from siblings (6.5%) to parents (4.7%), and offspring (4.0%), while the corresponding figures in an earlier meta-analysis were 8.9%, 3.0%. and 7.9% (Singh et al. 2015). Of note, these numbers may be affected by age at screening. For example, the youngest siblings and offspring may not have been tested earlier or they had not yet developed celiac disease, whereas their middle-aged parents have likely been more often already detected in healthcare. Here the overall prevalence, also including the previously detected patients was also highest among siblings. However, kinship was not a significant risk factor in multivariable analysis, implying that being a sibling is not as major a risk factor as previously thought (Singh 2015).

Although the prevalence of screening positivity was highest among FDRs, it was also surprisingly high among SDRs and more distant relatives, although the latter groups were quite small. Only very limited previous data are available on the risk of celiac disease in this subgroup, the pooled prevalence of the few studies being 2.3% among SDRs (Singh et al. 2015). Here the screening-positive SDRs belonged more often to multiple case families than the screening-positive FDRs, which is in line with earlier observations (Book et al. 2003; Fraser et al. 2006). In fact, figures as high as 17% for seroposivity have been reported in first-degree cousins with two affected siblings in the family (Book et al. 2003). These results suggest that screening of at least SDRs in multiple case families should be considered, although further studies on this issue and particularly on the screening of more distant relatives are called for. As regards re-secreening of other relatives than FDRs, there was one new case among the SDRs in Study **III**, the number of subjects being too low to permit any firm conclusions.

13.7 Role of HLA risk determination in family screening

Celiac disease can be practically excluded from subjects without HLA-DQ2 or HLA-DQ8 (Chapter 2.2), indicating that determination of these haplotypes could help to target screening. It is good to realize that of the 2,714 relatives screened in Study **II**, 29% did not have the HLA risk, which is somewhat less than that previously reported in FDRs and those with autoimmune comorbidities (Bourgey et al. 2007; Choung et al. 2020; Pietzak et al. 2009; Vriezinga et al. 2014). The corresponding figure in general population is 50-60% (Kårhus et al. 2018; Liu et al. 2017). HLA

determination could be used either in targeting the first screening or in selecting the once seronegative relatives needing further testing and surveillance. In fact, some of the current guidelines recommend both starting screening with HLA and using it for targeting in follow-up (Al-Toma et al. 2019; Husby et al. 2012) while some prefer to use it only to guide the re-screening (Ludvigsson et al. 2014; Murch et al. 2013). However, this approach has also been challenged due to the high percentage of HLA DQ2/8 among the at-risk relatives (Rubio-Tapia et al. 2013). Here the IR was markedly higher among relatives carrying the risk haplotypes (336 vs. 221/100,000 person-years among all relatives) in Study **III**, indicating that subsequent screening could be omitted from a significant proportion of the relatives without the risk alleles. This lends further support to the recommendations to use HLA determination to guide the surveillance of once-seronegative relatives (Al-Toma et al. 2019; Husby et al. 2012; Ludvigsson et al. 2014; Murch et al. 2013).

In more detailed analysis, the high-risk HLA (DQ2.5/2.5 or 2.5/2.2) was the most important risk factor for screening positivity/celiac disease in both first testing and re-screening, overriding the other individual risk factors. This information could thus help to further target screening, although it must be born in mind that the majority of patients carry the intermediate risk haplotype (Chapter 12.3). Although such a similar approach has not been studied before, a two-step screening strategy determining HLA-DQ2.5 allele has been proposed for subjects with Down's syndrome (Csizmadia et al. 2000). In addition, determining HLA-DQ1*02 allele as a first step for screening children has been advocated, since up to 97% of pediatric celiac disease patients carry this risk allele either alone or with HLA-DQA1*05 allele and/or DQ8 (Poddighe et al. 2019). It is open to speculation whether the use of high-risk HLA is feasible in determining screening intervals. Assessment of the more detailed HLA risk might also have prognostic significance, as both the higher number (2 vs. 0-1) of DQ2.5, DQ2.2 and DQ8 haplotypes as well as the homozygosity for DQ2.5 could increase mortality due to lymhoproliferative diseases in celiac disease (Schneider et al. 2021).

One factor affecting the recommendations to use either HLA DQ2/8 or more specific high-risk HLA determination is cost-effectiveness. This includes the price and availability of genetic tests, as well as other factors such as the risk of complications among undetected patients. Moreover, the opinions of at-risk subjects and/or their families about HLA testing should be elicited (Husby et al. 2012), particularly as knowledge of disease susceptibility could cause anxiety. In the future, combining non-HLA genes with HLA may offer an opportunity for even more precise risk calculation (Romanos et al. 2014).

13.8 Advantages and downsides of family screening

The present dissertation provided further data on the efficiency of screening of at-risk relatives to improve the diagnostic yield. Particularly, despite the good knowledge of celiac disease in Finland (Vilppula et al. 2008), up to 36% of the affected relatives in Study **II** were undetected before the first testing. Moreover, the re-screening in Study **III** revealed that IR remained markedly higher among once EMA-negative family members compared to the previously reported celiac disease incidence in general Finnish population. This indicates that not only first screening but also re-screening of at-risk relatives results in a significant number of new cases. Study **I** showed that the clinical presentation of celiac disease varies substantially even between siblings, demonstrating the difficulty of clinical case-finding among the family members, let alone in the population at large (Katz et al. 2011).

It must be realized that screening does not only improve the diagnostic yield but may also prevent complications related to celiac disease. In Study **I**, the later diagnosed siblings had milder disease and shorter duration of symptoms than the index patients, indicating that family screening could reduce the diagnostic delay and thereby also the risk of persistent symptoms (Paarlahti et al. 2013; Sansotta et al. 2018). In fact, most of the screen-detected subjects in Studies **I** and **III** had actually reported symptoms before screening, although these either had not led to a healthcare visit or were not found to be caused by celiac disease. Interestingly, these symptoms may subside on GFD as in clinically-detected subjects (Kinos et al. 2012; Kivelä et al. 2017, Paavola et al. 2012). Besides preventing persistent symptoms, early screening and initiation of GFD could at least in theory also prevent other complications, such as poor growth and reduced bone accrual in childhood (Kivelä & Kurppa 2018) and infertility (Singh et al. 2016), NHL (Lohi et al. 2009) and osteoporosis/osteopenia (Mustalahti et al. 1999) in adulthood (Figure 9). Of note, although there was no signifant difference in the number of low-energy fractures or miscarriages between screening-positive and -negative relatives in Study **III**, the number of cases was fairly low, and the study was not specifically designed to investigate this issue. Overall, more studies are needed on the complication risk among screened subjects.

Further supporting the rationality of screening – at least in some countries – is the finding that clinically- and screen-detected celiac disease patients adhere to GFD equally well in long-term follow-up (Iorfida et al. 2021; Kurppa et al. 2012b). Similar long-term dietary adherence between these groups has been reported regardless of whether the subjects had symptoms before diagnosis (Kivelä et al. 2018), although

more dietary lapses among asymptomatic screened subjects have also been reported (Ukkola et al. 2011). However, there are also less promising results e.g. from Italy, reporting poorer adherence among mass-screened than among clinically-detected subjects in 20-year follow-up (Cozzi et al. 2022) and adherence of only 23% among screened adolescents compared to 68% in clinically-identified patients in five-year follow-up (Fabiani et al. 2000). Altogether it seems that at least in countries with generally good GFD adherence, neither the initial clinical picture nor being screen-detected appears to affect long-term compliance. Other factors, such as concomitant type 1 diabetes and intention to prevent symptoms may in turn have some effect (Kivelä et al. 2022).

As a downside of screening, celiac disease may also increase anxiety and burden on individual level. GFD products are more expensive than their regular counterparts and following a strict GFD restricts social life (See et al. 2015), both of which can lead to poorer long-term dietary adherence (Kivelä et al. 2022). In addition, poorer symptom improvement on GFD among screen-detected compared to clinically-detected subjects has been reported (Mahadev et al. 2016; Rubio-Tapia et al. 2008). Special attention should be paid to apparently asymptomatic subjects, who may be at greatest risk for anxiety (Kivelä et al. 2018) and impaired quality of life on GFD (Ukkola et al. 2011). All the above-mentioned issues should be considered before launching any wide-scale screening procols (Figure 9), and the effect of the diagnostic yield and opportunities to maintain GFD in different countries should also be evaluated.

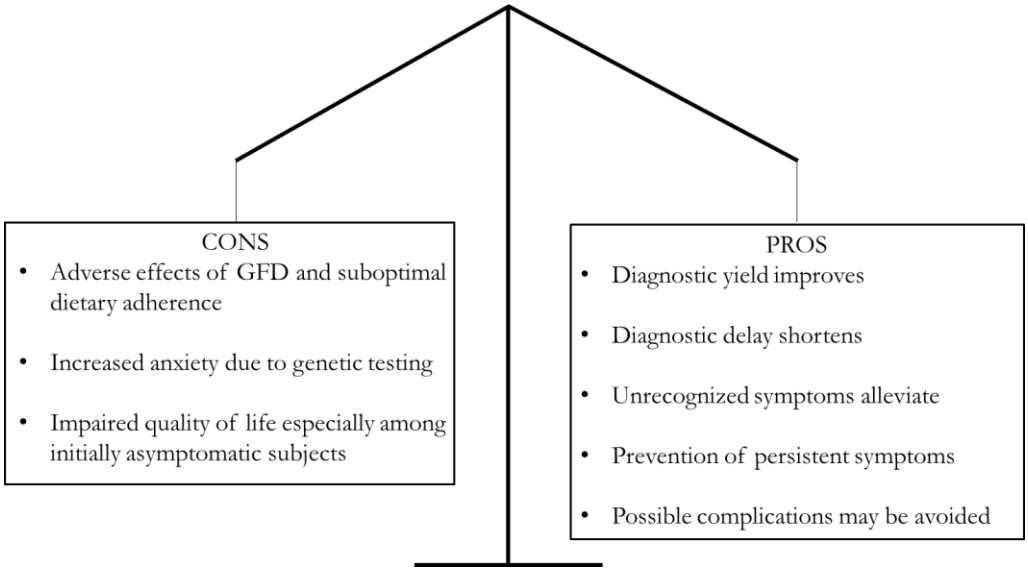

Figure 9. Possible aspects that should be considered before implementation of family screening for celiac disease. GFD, gluten-free diet.

13.9 Strengths and limitations

The main strength of the present dissertation was the opportunity to study large and well-defined cohorts of patients with previously detected celiac disease and their relatives of all ages and from different parts of Finland. Furthermore, the possible earlier diagnoses were meticulously verified from systematically maintained patient records, and accurate, well-validated serological tests were used at all stages of screening. The large number of new screen-detected relatives in Study **II** also made it possible to evaluate various index- and relative-related characteristics as possible risk factors for screening positivity, although HLA data was not available on all participants. Additionally, structured interviews were conducted at the time of serological testing, thereby reducing the risk of possible bias in reporting of symptoms among the screen-detected cases (**I**, **III**).

As a further strength, all initially EMA-negative relatives were systematically invited to the follow-up Study **III** to obtain reliable estimates on the incidence of celiac disease/screening positivity in this group. Although only some of them eventually participated, the sample size and follow-up time were large and long enough to detect a moderate number of new celiac disease cases/screening-positive relatives. In addition, the groups of participants and non-participants were

comparable in most factors that might influence the screening result. The systemic approach also enabled us to calculate the exact follow-up time for each participant and subsequently to determine IR.

There is a possibility that some cases with seronegative celiac disease were missed in Studies **II** and **III,** when only serological testing was used, although this relatively rare condition affects less than 2% of adult celiac disease patients (Volta et al. 2016a), with increasing prevalence by age (Salmi et al. 2006). A possible bias may have been caused by reporting of symptoms, since the patients previously detected in healthcare were interviewed retrospectively and experiencing symptoms is always somewhat subjective. The reported signs and symptoms at previous diagnosis may also have been affected by different diagnostic approaches among the healthcare physicians. In addition, symptoms among screened relatives in Study **II** were not assessed.

The dissertation included ethnically homogenous population, so these results may not be generalized to other countries and ethnicities with different prevalences of celiac disease (Singh et al. 2018). The homogeneity of the population and selection bias may have also led to overestimation of celiac disease risk among SDRs and more distant relatives. Despite the relatively large sample size, we were not able to confirm what proportion of relatives eventually participated to initial screening study from each family, and it is possible that for example only more symptomatic relatives among SDRs participated. In addition, the effect of high-risk HLA may not be generalized to countries where HLA-DQ8 is a more significant risk genotype than HLA-DQ2 for celiac disease (Sollid 2017). It must also be noted that knowledge of celiac disease in Finland is good, likewise the availability of GFD products, which may not be the case in all countries.

13.10 Summary and future directions

To conclude, the present study demonstrated that celiac disease may present with heterogenous clinical phenotype even within the same family, and that HLA distribution does not explain these differences. Furthermore, the findings from the family screening confirmed the conclusion that this approach can improve the currently suboptimal diagnostic yield of celiac disease. In light of the present and previous findings on the celiac disease risk among FDRs, targeting of screening could be recommended for all FDRs after the index patient is diagnosed, regardless of the relatives' age or exact kinship with the index patient. Additionally, follow-up screening could be omitted from approximately 30% of relatives without HLA

DQ2/8 and more specific high-risk HLA was the most important risk factor for celiac disease/screening positivity. Therefore, determination of at least "crude" HLA risk or even the presence of high-risk HLA could be useful in targeting re-screenings among once-seronegative at-risk relatives. This dissertation also provided insights into the significant risk of celiac disease among SDRs and more distant relatives, although further studies on this group are needed.

One important topic for future research is to assess the utility and cost-effectiveness of family screening in general. This requires information on the complication risk in unrecognized celiac disease as well as quality of life and long-term dietary adherence among screen-detected relatives, with special attention to initially asymptomatic subjects. As regards optimal screening frequency among once-seronegative relatives, large multicenter studies are probably needed. There is also a clear need for studies evaluating the cost-effectiveness of using HLA either in first-time testing or in re-screening. In addition, it should be ascertained whether more detailed risk analysis could be provided by combining high-risk HLA and non-HLA genetics. Finally, it is essential to evaluate families' opinions on genetic testing before launching any wide-scale screening protocols exploiting HLA determination. Based on the present findings, some updates could also be made to the next revision of the Finnish Current Care Guidelines. These include screening of the SDRs in multiple case families and re-screening frequency of approximately ten years in adults and more actively in childhood.

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APPENDIX

English translation of the original Finnish interview for celiac disease patients/EMA-positive subjects in Study I and II

1. Time of diagnosis/screening?

_____

2. Age at diagnosis/screening?

_____

3. Site of diagnosis?

_____

4. Serology done

Yes ( ) No ( )

5. Result of serological test available

Yes ( ) No ( )

6. Endomysial antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

7. Tissue transglutaminase antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

8. Anti-gliadin antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

9. Anti-reticulin antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

10. Comments about serology (exact test results, IgA deficiency/IgG class antibodies etc.)

_____

11. Duodenal histology

Total villous atrophy/M3c ( )

Subtotal villous atrophy/M3b ( )

Partial villous atrophy/M3a ( )

Other/M1-M2 ( )

Normal ( )

Unknown ( )

12. Comments about histology (reason for refusal, possible gluten challenge, more detailed histology etc.)

_____

13. Skin biopsy in case of dermatitis herpetiformis

Positive IgA deposits ( )

Other ( )

Unknown ( )

14. Symptoms before diagnosis?

_____

15. Duration of symptoms before diagnosis? (By longest duration among different symptoms)

No symptoms ( )

Under 1 year ( )

1-5 years ( )

5-10 years ( )

Over 10 years ( )

16. Previous celiac disease diagnosis found by screening?

Yes ( ) No ( )

17. Chronic comorbidities

Type 1 diabetes

Yes ( ) No ( )

Autoimmune thyroidal disease

Yes ( ) No ( )

Sjögren's syndrome

Yes ( ) No ( )

Osteoporosis/osteopenia

Yes ( ) No ( )

Fractures

Yes ( ) No ( )

What caused: _____

Gastrointestinal disease

Yes ( ) No ( )

Cardiovascular disease

Yes ( ) No ( )

Enamel damage

Yes ( ) No ( )

Malignancies

Yes ( ) No ( )

What malignancy: _____

IgA deficiency

Yes ( ) No ( )

Other comorbidities

_____

English translation of the original Finnish interview for participants in Study III

1. Earlier diagnosis after the initial screening and before the current study?

Yes ( ) No ( )

Questions related to earlier celiac disease diagnosis after the initial screening

2. Age at diagnosis?

_____

3. Site of diagnosis?

_____

4. Serology done

Yes ( ) No ( )

5. Result of serological test available

Yes ( ) No ( )

6. Endomysial antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

7. Tissue transglutaminase antibodies

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

8. Other serology

Positive ( )

Negative ( )

Not taken ( )

Unknown ( )

9. Hemoglobin at diagnosis _____

10. Other abnormal laboratory values

_____

11. Duodenal histology

Total villous atrophy/M3c ( )

Subtotal villous atrophy/M3b ( )

Partial villous atrophy/M3a ( )

Other/M1-M2 ( )

Normal ( )

Unknown ( )

12. Comments about histology (reason for refusal, possible gluten challenge, more detailed histology etc.)

_____

13. Skin biopsy in case of dermatitis herpetiformis

Positive IgA deposits ( )

Other ( )

Unknown ( )

14. Reason for celiac disease suspicion?

_____

Questions for all participants

15. Symptoms before diagnosis or the study visit

Diarrhea/loose stools

Yes ( ) No ( )

Vomiting

Yes ( ) No ( )

Abdominal pain

Yes ( ) No ( )

Constipation

Yes ( ) No ( )

Flatulation

Yes ( ) No ( )

Joint pain/arthritis

Yes ( ) No ( )

Dental enamel defects

Yes ( ) No ( )

Anemia

Yes ( ) No ( )

Poor growth in childhood

Yes ( ) No ( )

Delayed puberty

Yes ( ) No ( )

Fatigue

Yes ( ) No ( )

Mood disorders

Yes ( ) No ( )

Skin symptoms

Yes ( ) No ( )

16. Duration of symptoms before diagnosis or the study visit? (By longest duration among different symptoms)

No symptoms ( )

Under 1 year ( )

1-5 years ( )

5-10 years ( )

Over 10 years ( )

17. Other symptoms before diagnosis or the study visit, and their duration?

_____

18. Chronic comorbidities

Type 1 diabetes

Yes ( ) No ( )

Autoimmune thyroidal disease

Yes ( ) No ( )

Rheumatoid disease

Yes ( ) No ( )

Osteoporosis/osteopenia

Yes ( ) No ( )

Fractures

Yes ( ) No ( )

What caused: _____

Gastrointestinal disease

Yes ( ) No ( )

Cardiovascular disease

Yes ( ) No ( )

Miscarriages

Yes ( ) No ( )

Malignancies

Yes ( ) No ( )

What malignancy: _____

Other comorbidities

_____

Questions for re-screened relatives

19. Self-initiated gluten-free diet without celiac disease diagnosis?

Yes ( ) No ( )

20. Reason for study participation (who is the index relative)?

_____

PUBLICATIONS

PUBLICATION I

The phenotype of celiac disease has low concordance between siblings, despite a similar distribution of HLA haplotypes

Saana Kauma, Katri Kaukinen, Heini Huhtala, Laura Kivelä, Henna Pekki, Teea Salmi, Päivi Saavalainen, Katri Lindfors, Kalle Kurppa

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nutrients

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Article

The Phenotype of Celiac Disease Has Low Concordance between Siblings, Despite a Similar Distribution of HLA Haplotypes

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Abstract: The factors determining the presentation of celiac disease are unclear. We investigated the phenotypic concordance and the distribution of human leukocyte antigen (HLA) risk haplotypes in affected siblings. One hundred sibling pairs were included. Clinical and histological parameters and HLA haplotypes were compared between the first diagnosed indexes and their siblings. The phenotype was categorized into gastrointestinal, extra-intestinal, malabsorption/anemia, and asymptomatic. The phenotype was fully concordant in 21 pairs. The most common concordant phenotype was gastrointestinal (14 pairs). Indexes had more anemia/malabsorption and extra-intestinal symptoms than siblings (45% vs. 20%, $p < 0.001$ and 33% vs. 12%, $p < 0.001$, respectively). Twenty siblings and none of the indexes were asymptomatic. The indexes were more often women (81% vs. 63%, $p = 0.008$). They were also more often seronegative (11% vs. 0%, $p = 0.03$) and younger (37 vs. 43 year, $p < 0.001$), and had more severe histopathology (total/subtotal atrophy 79% vs. 58%, $p = 0.047$) at diagnosis. The indexes and siblings were comparable in other disease features. Pairs with discordant presentation had similar HLA haplotypes more often than the concordant pairs. The phenotype was observed to vary markedly between siblings, with the indexes generally having a more severe presentation. HLA did not explain the differences, suggesting that non-HLA genes and environmental factors play significant roles.

Keywords: celiac disease; sibling; phenotype; gluten-free diet; environmental factors; genotype

1. Introduction

Celiac disease is an immune-mediated condition with an estimated prevalence of 1–2% in Western countries [1–3]. The first-degree relatives of patients have approximately 2–10 times the average risk for the disease, whereas in identical twins the concordance rate can be as high as 80% [4–6]. Human leukocyte antigen (HLA) DQ2 and DQ8 haplotypes have been identified as the main genetic risk factors, without which celiac disease is very unlikely [7]. At population level, approximately 40% of individuals have these risk haplotypes, but only a fraction of them will eventually develop

the disease [2,7]. This might be partly explained by the effect of non-HLA genes, but considerable differences in the prevalence between genetically similar populations and the rising true incidence support the additional role of environmental factors [3,8,9].

In recent decades, we have also come to appreciate that the phenotype of celiac disease is very heterogeneous. The classical presentation with malabsorption and failure to thrive in early childhood has become rare. Nowadays, patients often have different gastrointestinal or extra-intestinal symptoms that may appear at any age, or they can even be completely asymptomatic [10–12]. The reason for this phenotypic diversity remains obscure [13–17], but the observed variability even between identical twins suggests that it is not solely determined by genetics [5,18]. Overall, the concordance of the clinical picture between affected relatives has been scarcely studied. This information could improve our understanding of the complex interactions between genetic and environmental factors in celiac disease, and possibly increase the diagnostic yield of this markedly under-recognized condition [3].

In this study, we aimed to evaluate the concordance of the clinical and histological presentation and the HLA risk haplotypes of untreated celiac disease in close relatives who both have the disease. Specifically, the comparisons were made between the first affected index patients and their siblings, who usually have/had a shared environment in childhood and are genetically markedly similar [6].

2. Materials and Methods

2.1. Patients and Study Design

The study was carried out in Finland at the University of Tampere and Tampere University Hospital. Previously diagnosed celiac disease patients and their relatives were invited to participate through advertisements in newspapers and via local celiac societies. All participants were interviewed by a study nurse or physician with expertise in celiac disease, and blood samples were drawn for further serological and genetic analyses between 2006–2010. Relatives with new celiac autoantibody positivity were referred to the local hospital for diagnostic endoscopy. In addition to the interview, the medical records of the patients were surveyed to confirm the diagnosis and to supplement the clinical, histological, and serological data at diagnosis. Diagnosis had to be based on the demonstration of villous atrophy in duodenal biopsy in both children and adults. The exclusion criteria were study refusal and unclear celiac disease diagnosis.

Altogether, 1035 patients and 3031 of their relatives from 732 families were enrolled (Figure 1). Among the 3031 relatives, 148 new cases of celiac disease were detected by screening. Thus, 1183 subjects had either previously diagnosed or newly diagnosed celiac disease. Of this number, 263 were excluded due to insufficient data for the study analyses. Of the remaining 920 patients, only families with at least two affected subjects (n = 492) entered the next stage. In order to simplify the statistical evaluation, only two first-affected siblings from families with multiple cases were enrolled. The final study group comprised 200 subjects (100 sibling pairs) who underwent comparison for all study variables as described below (Figure 1). The first diagnosed subject is defined as the index and the later diagnosed subject is defined as the sibling. The 200 patients included in the final analyses were diagnosed between the years 1972–2009.

2.2. Clinical Characteristics

The clinical information collected included demographic data and the family history of celiac disease, the main disease presentation/reason for disease suspicion, and the possible presence of co-existing autoimmune conditions, fractures, and malignancies.

For the purposes of the study, the clinical presentation at diagnosis was categorized as follows: malabsorption or anemia, gastrointestinal symptoms, extra-intestinal symptoms, or asymptomatic. Malabsorption was defined as weight loss and/or characteristic laboratory abnormalities, such as low folate or hypoalbuminemia. Gastrointestinal symptoms included abdominal pain, diarrhea, constipation, heartburn, and dysphagia. Extra-intestinal manifestations included dermatitis

herpetiformis, recurrent aphthous stomatitis, enamel damage as confirmed by a dentist, failure to thrive (pediatric diagnosis), ataxia, unspecific arthritis, and elevated liver enzymes that were normalized by a gluten-free diet [19]. Non-specific and/or vague symptoms such as fatigue, infertility, back pain, and headache were disregarded. A patient could have had several symptoms simultaneously at diagnosis and thus be included in several symptom groups.

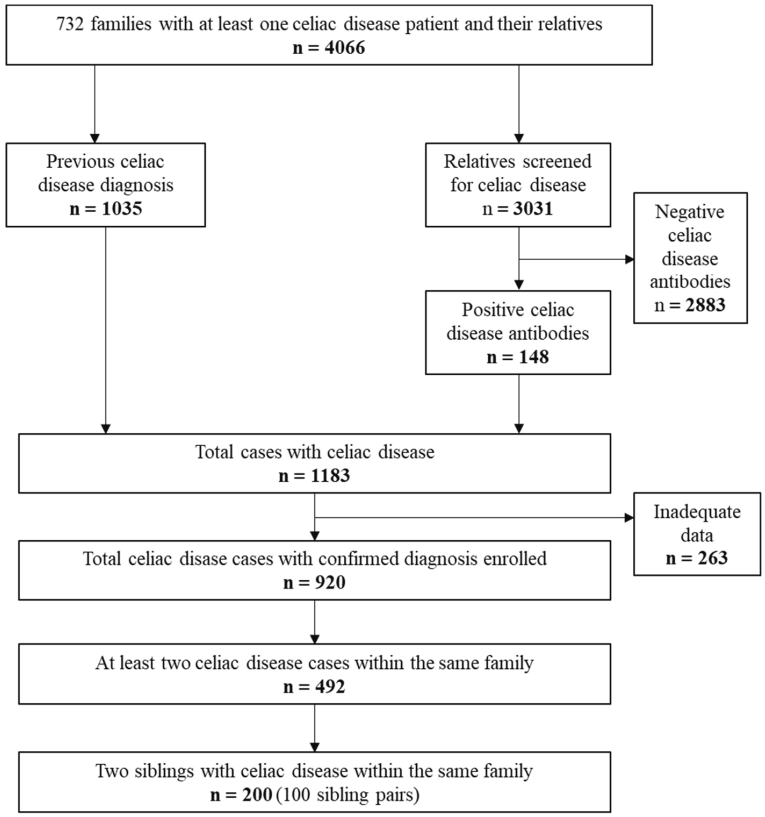

Figure 1. Flowchart of the study.

The diagnostic delay, defined as the duration of possible symptoms before the diagnosis, was also recorded and further divided into ≤5 years and >5 years.

2.3. Histology

The results of the histopathologic evaluation of the small-bowel mucosal biopsies were collected from patient records. According to our national guidelines, at least four representative biopsies are routinely taken from the duodenum in cases of suspected celiac disease [20]. Only correctly orientated cuttings are accepted for precise morphometric evaluation [21]. The diagnosis of celiac disease is based on the demonstration of either total, subtotal, or partial villous atrophy, comparable to the Marsh–Oberhuber classifications IIIa, IIIb, and IIIc, respectively [22]. In cases of dermatitis herpetiformis, the diagnosis is based on demonstration of granular IgA deposits in the papillary dermis by direct immunofluorescence examination in a skin biopsy [20].

2.4. Serology and Genetics

Information on possible earlier determined celiac disease autoantibodies was obtained from patient records. In addition, serum endomysial (EmA) and tissue transglutaminase antibodies (tTGab) were measured in all participants from the blood samples taken at the study visit. EmA was measured by the indirect immunofluorescence method as previously described [20] and titers 1:≥5 were considered positive. A commercial ELISA test (QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA, USA) was used to test tTGab, with a cut-off of >30.0 U/l for seropositivity according to the manufacturer's instructions. In cases of IgA deficiency, the autoantibodies were determined by IgG class.

Genotyping for celiac disease-associated HLA alleles was performed with the SSP™ DQB1 low-resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden) and/or tagging SNP approach [23]. Haplotypes were categorized into HLA DQ2 positives (DQ2.5/DQX or DQ2.2/DQ7), HLA DQ8 positives (DQ8/DQX), and both DQ2 and DQ8 negatives.

2.5. Statistical Analyses

Statistical analyses were performed using SPSS Statistics for Windows (IBM Corp. Armonk, NY, USA) and STATA Statistical Software (StataCorp. LP, Lakeway Drive, TX, USA). Categorical variables were studied by McNemar and Chi-Squared tests and continuous variables were studied by the Wilcoxon signed rank test. A p-value < 0.05 was considered statistically significant.

2.6. Ethics

The study design and patient enrolment was accepted by the Ethics Committee of Pirkanmaa Hospital District. The study protocol follows the ethical guidelines of the Declaration of Helsinki. All participants gave written informed consent.

3. Results

3.1. Clinical Data

Twenty-four (12%) of the later diagnosed siblings were diagnosed in the present study. Twenty-six (13%) of the 200 patients were <18 years of age (range 5–17 years) at the time of the study and 37 (19%) of the 200 patients were <18 years of age (range 2–17 years) at diagnosis. Of the latter 37 subjects, 21 were first diagnosed and 16 later diagnosed siblings. Among the sibling pairs, there was only one dizygotic twin pair and no identical twins. The index patients were significantly younger at diagnosis and more often females compared to the later diagnosed siblings (Table 1). Gastrointestinal symptoms were the most common and equally distributed presentation was observed in both groups, but the indexes had malabsorption/anemia and extra-intestinal symptoms significantly more often (Table 1). Five index patients and 42 siblings were detected by screening; twenty were asymptomatic, all of them siblings. Seven of the asymptomatic patients were <18 years of age at diagnosis. The index subjects had more severe histological damage (less partial and more subtotal villous atrophy) and were more often seronegative at diagnosis, whereas the groups did not differ in terms of current age, length of diagnostic delay, or the presence of fractures, malignancies, and autoimmune comorbidities (Table 1). When looking at the whole study cohort, patients suffering from malabsorption or anemia had more severe villous atrophy compared to the asymptomatic subjects (total 33% vs. 28%, subtotal 50% vs. 22% and partial 17% vs. 50%, $p = 0.012$, respectively).

Among all subjects, the most common gastrointestinal symptom was diarrhea (43%) and the most common extra-intestinal symptom was dermatitis herpetiformis (17%). When comparing the symptom subgroups between the siblings, diarrhea (42% vs. 44%, $p = 0.878$), abdominal pain (45% vs. 34%, $p = 0.117$), and constipation (6% vs. 5%, $p = 1.000$) were equally presented among the indexes and siblings, as were oral symptoms (4% vs. 1%, $p = 0.375$) and failure to thrive (7% vs. 3%, $p = 0.289$).

Dermatitis herpetiformis was more common among the index patients than among the siblings (25% vs. 9%, $p = 0.002$). None of the study subjects had ataxia, arthritis, or elevated liver enzymes.

Table 1. Diagnostic characteristics and presence of complications and comorbidities in 100 sibling pairs with celiac disease.

	Index Patients, *n* = 100	Siblings, *n* = 100	*p* Value
Age at diagnosis, median (Q_1, Q_3), year	37 (22, 47)	43 (25, 52)	<0.001
Age at study visit, median (Q_1, Q_3), year	52 (38, 59)	51 (36, 58)	0.704
Female, %	81	63	0.008
Diagnostic delay [1], %			0.073
>5 years	44	27	
≤5 years	56	74	
Clinical presentation at diagnosis, % [2]			
Gastrointestinal	80	75	0.215
Malabsorption or anemia	45	20	<0.001
Extra-intestinal	33	12	<0.001
Asymptomatic	0	20	<0.001
Degree of villous atrophy at diagnosis, %			0.047
Total	29	27	
Subtotal	49	31	
Partial	20	41	
Positive celiac antibodies at diagnosis [3], %	89	100	0.030
Fractures, %	24	21	0.736
Malignancy [4], %	4	5	1.000
Associated diseases, %			
Thyroidal disease	20	9	0.052
Type 1 diabetes	1	4	0.375
Sjögren's syndrome	2	1	1.000
IgA deficiency	1	0	1.000

[1] Duration of symptoms before the diagnosis. Asymptomatic patients excluded. [2] Symptomatic patients could have had several overlapping presentations. [3] Tissue transglutaminase, endomysium, or reticulin antibodies. Data missing from 33 indexes and 23 siblings. Comparison made between 54 pairs. [4] For example, breast and thyroidal cancer.

Altogether, 21 pairs were concordant and 79 pairs were discordant for the celiac disease phenotype, as defined in the present study (Table 2). Gastrointestinal symptoms represented the most common concordant phenotype, and it was observed in 14 of the 21 pairs with concordant disease manifestation. Regarding partial concordance, in 28 pairs the index subject suffered simultaneous gastrointestinal symptoms and malabsorption/anemia and/or extra-intestinal symptoms, while the sibling had only gastrointestinal symptoms. Conversely, in nine pairs the sibling had both gastrointestinal and other symptoms, while the index suffered only gastrointestinal symptoms.

Table 2. Phenotype concordances at celiac disease diagnosis in 100 sibling pairs. Results are presented as numbers of sibling pairs.

Title		Index Patients, *n* = 100						
		GI	**MA**	**EI**	**GI + EI**	**MA + EI**	**GI + MA**	**GI + MA + EI**
Siblings, *n* = 100	GI	14	3	3	8	1	13	7
	MA	3	1	0	1	0	0	0
	EI	0	0	1	0	0	1	0
	GI + EI	3	0	2	1	0	3	0
	MA + EI	0	0	0	0	0	0	1
	GI + MA	6	1	1	1	1	4	0
	GI+ MA + EI	0	0	0	0	0	0	0
	Asymptomatic	6	4	2	3	0	5	0

GI, gastrointestinal; MA, malabsorption or anemia; EI, extra-intestinal. Pairs with concordant phenotype bolded.

3.2. Genetics

Celiac disease-related HLA haplotype data was available for 66 pairs (Table 3). The remaining 34 pairs had one or more allele missing from the HLA-typing and were excluded from the haplotype comparisons. The most common haplotype among both the indexes and siblings was DQ2.5/DQX, followed by DQ2.5 homozygosity, and DQ2.5/DQ8. The other celiac disease-associated HLA haplotypes were present only in a small number of individuals. The HLA haplotype was equal in 46 (70%) out of the 66 pairs (Table 4). Of them, there were 17 pairs with a concordant clinical presentation, of whom eight pairs (47%) had the same haplotype (DQ2.5/DQX in six pairs). Of the remaining 49 pairs with a discordant clinical presentation, 38 (78%) had equal haplotype (DQ2.5/DQX in 19 pairs). This difference in the prevalence of the same haplotypes between pairs with and without concordant clinical presentation (47% vs. 78%) was statistically significant ($p = 0.018$).

Table 3. Overall distribution of human leukocyte antigen (HLA) haplotypes in index cases and siblings with celiac disease. DQX = other than DQ2.5, DQ2.2, DQ7, or DQ8.

HLA	Index Patients, *n* = 66 %	Siblings, *n* = 66 %
DQ2 positive		
DQ2.5/DQX	48	53
DQ2.5/DQ2.5	29	21
DQ2.5/DQ8	11	12
DQ2.5/DQ2.2	2	5
DQ2.5/DQ7	3	2
DQ2.2/DQ7	0	2
DQ2 negative, DQ8 positive		
DQ8/DQX	2	2
DQ8/DQ8	0	0
DQ8/DQ2.2	2	0
DQ8/DQ7	3	3
DQ2 negative, DQ8 negative	2	3

Table 4. Concordance of human leukocyte antigen (HLA) haplotypes in 66 sibling pairs. Results are presented as numbers of sibling pairs. Pairs with similar genotype bolded. DQX = other than DQ2.5, DQ2.2, DQ7, or DQ8.

		Index Patients, *n* = 66										
	HLA	**DQ 2.5/X**	**DQ 2.5/2.5**	**DQ 2.5/8**	**DQ 2.5/2.2**	**DQ 2.5/7**	**DQ 2.2/7**	**DQ 8/X**	**DQ 8/8**	**DQ 8/2.2**	**DQ 8/7**	**DQ 2/8 Neg.**
Siblings, *n* = 66	DQ 2.5/X	**25**	5	1	0	1	0	0	0	0	2	0
	DQ 2.5/2.5	2	**12**	0	0	0	0	0	0	0	0	0
	DQ 2.5/8	1	1	**6**	0	0	0	0	0	0	0	0
	DQ 2.5/2.2	2	0	0	**1**	0	0	0	0	0	0	0
	DQ 2.5/7	1	0	0	0	**0**	0	0	0	0	0	0
	DQ 2.2/7	0	0	0	0	1	**0**	0	0	0	0	0
	DQ 8/X	0	0	0	0	0	0	**1**	0	0	0	0
	DQ 8/8	0	0	0	0	0	0	0	**0**	0	0	0
	DQ 8/2.2	0	0	0	0	0	0	0	0	**0**	0	0
	DQ 8/7	0	1	0	0	0	0	0	0	1	**0**	0
	DQ2/8 Neg.	1	0	0	0	0	0	0	0	0	0	**1**

4. Discussion

We observed substantial phenotypic variation between the first diagnosed indexes and the later diagnosed siblings with celiac disease. Gastrointestinal symptoms were frequently seen in both

siblings, but they often co-existed with additional randomly distributed extra-intestinal manifestations, and within a significant portion of the pairs the clinical presentation was completely different. Familial phenotype concordance has not been previously studied using a similar approach, but some studies have shown that the intestinal form of celiac disease and dermatitis herpetiformis can occur within the same family [24,25]. Interestingly, variation in the clinical phenotype is not restricted to celiac disease, as similar heterogeneity has been reported, for example, in inflammatory bowel disease [26] and systemic lupus erythematosus [27].

The haplotypes were even more likely to be similar if the siblings were discordant for the clinical presentation, suggesting that HLA genotype does not predict the clinical outcome. Previously, Karell et al. investigated the distribution of HLA haplotypes in 110 sibling pairs with dermal and intestinal celiac disease and, in accord with us, found no significant association with clinical outcome [25]. Mustalahti et al. studied 28 asymptomatic and symptomatic sibling pairs [13], while Greco et al. studied 145 patients categorized into 16 separate phenotypes [14], and no phenotype–HLA haplotype associations were observed in either study. In contrast, there have been studies reporting the association of non-HLA variants with distinct celiac disease phenotypes [28–30]. For instance, certain genotypes of haptoglobin and CTLA4 have been associated with clinically mild or silent disease [29,30], whereas a particular interleukin-10 genotype seems to predispose to early-onset and histologically severe disease [28]. In any case, the role of both HLA and non-HLA risk variants seems to be at most modest, as supported by the few studies conducted using monozygous twins. Hervonen et al. investigated the co-occurrence of intestinal disease and dermatitis herpetiformis in six monozygous twin pairs: three pairs had the concordant phenotype and two pairs had the discordant phenotype [5]. Bardella et al. reported variance in both the clinical presentation and even the overall risk of developing the disease in five monozygous twin pairs [18]. In comparison, the age of onset and the disease risk can also vary between monozygous twins in children with type 1 diabetes [31]. Thus, the limited role of genetics and the additional effect of environmental factors as modifiers of the disease risk and phenotype seem to be common features in autoimmune diseases.

The environmental factors involved in modulating the celiac disease phenotype and its development remain undetermined, as does how their effect is mediated. Hitherto, studies have focused on searching for possible modifiers of general celiac disease risk. For example, high amounts of gluten and gastrointestinal infections in infancy may increase the risk [32,33], whereas cesarean section, the age of gluten introduction, and breastfeeding are unlikely to play a role [34–36]. It remains unclear if these same factors affect the phenotype. One topic of interest relevant here is gut microbiota. We previously studied the duodenal microbiota of 32 celiac disease patients and observed that the bacterial profile, as well as the overall richness and diversity of the microbiota, varies depending on the phenotype [17]. The causality of these findings is difficult to evaluate, particularly since many of the previously mentioned environmental and genetic factors may affect both the structure and function of the microbiota [37,38]. In any case, a better understanding of the environmental factors could, besides further elucidating the pathogenesis, enable the development of interventions to reduce the disease risk and/or prevent the most severe outcomes. Large multicenter studies including patients with well-defined phenotypes are likely needed to fully decipher the complex association between phenotype, genotype, and environmental factors in celiac disease.

The first diagnosed siblings generally had a more severe disease presentation, demonstrated, for example, by their higher frequency of anemia and advanced villous atrophy. Similarly, Gudjónsdóttir et al. investigated the severity of symptoms in 105 sibling pairs and reported more severe symptoms among the indexes [29]. One explanation for the more advanced presentation in the first diagnosed siblings in the present study could be the longer disease history, even though the difference was not statistically significant [39]. Celiac disease is likely suspected with a lower threshold or screened even without apparent symptoms in the non-index siblings with a known family history for the condition, who can thus have a less severe phenotype despite being diagnosed at a later age. Another influencing factor could be the higher frequency of females among the indexes, as women

have been shown to use more health care services compared to men [40]. In any case, the task of deciphering the factors behind differences in disease severity between siblings is complex, as various individual aspects may contribute to the timing of the diagnosis and the phenotype. In a broad sense, we found it important that the later diagnosed siblings had milder disease at diagnosis, as this indicates that family screening of celiac disease could simultaneously improve the diagnostic yield and prevent long-term complications due to earlier initiation of dietary treatment.

The main strength of the present study is the large and well-defined study cohort. The limitations are the subjective nature and challenging definition of the symptoms, plus possible recall bias. In order to categorize the phenotype as reliably as possible, the most non-specific symptoms were excluded, and one author made the classification and analyses systemically. However, the original diagnoses were made by several physicians, who could have had different approaches, for example, to clinical evaluation and laboratory testing. Altogether, complex interactions between the many confounders, such as the individual experience of symptoms, the implementation of screening, and differences between the groups in the ages at diagnosis and the number of subjects diagnosed in childhood, could influence the ultimate phenotype and were impossible to fully control for statistically. Genetic analysis was also limited to the assessment of the frequency of known celiac disease HLA risk haplotypes, and thus no deeper insight into the role of non-HLA genes and gene-to-gene interactions could be attained.

5. Conclusions

In conclusion, we found the clinical presentation of celiac disease to have a wide variation between the affected siblings, with the indexes generally having a more severe presentation at diagnosis. It is therefore important for physicians to remember possible atypical presentations, and to suspect the disease with a low threshold among the patient's close relatives. Furthermore, HLA did not explain the differences, suggesting that non-HLA genes and environmental factors play significant roles. The ultimate reasons for the substantial phenotype variation in celiac disease remain to be determined in future studies.

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PUBLICATION II

Presence of high-risk HLA genotype is the most important individual risk factor for coeliac disease among at-risk relatives

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Presence of high-risk HLA genotype is the most important individual risk factor for coeliac disease among at-risk relatives

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Summary

Background: Family screening has been advocated as a means to reduce the major underdiagnosis of coeliac disease. However, the precise risk of the disease in relatives and the impact of patient- and relative-related individual factors remain obscure.

Aims: To investigate the individual risk of coeliac disease among patients' relatives.

Methods: Altogether 2943 relatives of 624 index patients were assessed for the presence of previous coeliac disease diagnosis, or were screened for the disease. Coeliac disease-associated human leucocyte antigen (HLA) genotype was determined from all participants. The association between individual factors and new screening positivity was assessed by logistic regression.

Results: There were 229 previously diagnosed non-index relatives with coeliac disease and 2714 non-affected (2067 first-degree, 647 more distant) relatives. Of these 2714 relatives, 129 (4.8%) were screening-positive (first-degree 5.1%, second-degree 3.6%, more distant 3.5%). The combined prevalence of the previously diagnosed and now detected cases in relatives was 12.2% (6.3% clinically detected, 5.9% screen-detected). In univariate analysis, age <18 years at diagnosis (odds ratio 1.60, 95% CI 1.04-2.45) in index, and age 41-60 years (1.73, 1.10-2.73), being a sibling (1.65, 1.06-2.59) and having the high-risk genotype (3.22, 2.01-5.15 DQ2.5/2.5 or DQ2.5/2.2 vs other risk alleles) in relatives were associated with screening positivity. Only high-risk HLA remained significant (2.94, 1.80-4.78) in multivariable analysis.

Conclusions: Unrecognised coeliac disease was common among at-risk relatives even in a country with an active case-finding policy, and also in relatives more distant than first-degree. The presence of a high-risk genotype was the most important predictor for screening positivity. ClinicalTrials.gov identifier NCT03136731.

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1 | INTRODUCTION

Coeliac disease is a gluten-driven chronic gastrointestinal condition affecting individuals with a predisposing human leucocyte antigen HLA-DQ2 and/or HLA-DQ8 haplogenotype.[1] Estimated prevalence of the disease is up to 1%-3% in general population, but currently, most of the affected patients remain unrecognised.[2-5] This substantial underdiagnosis could be improved by active testing of either specific at-risk groups or even the whole population with serum coeliac autoantibodies. At present, most authorities do not recommend untargeted screening mainly because of inconsistent data on the prognosis of unrecognised coeliac disease at the population level.[6-9] However, particularly, the first-degree (FDR) and, sometimes, also second-degree (SDR) relatives of patients are often considered to have a sufficiently high disease risk to justify screening.[9-13]

Selecting an optimal screening strategy is complicated by a wide variation in the reported family risk[14-16] possibly due to different poorly defined patient- and relative-related individual factors, such as age at screening, gender, HLA haplogenotype and degree of consanquinity.[1,16-23] Limited data on these factors make optimal timing of screening, testing of other than FDRs, and the benefits of genetic risk stratification debatable.[13,15,16,23] The heterogenous and often small study cohorts and different diagnostic outcomes in earlier studies further hamper the interpretation of the results and emphasise the need for additional evidence.[14] Besides optimised implementation of the screenings, better understanding of the individual risk factors could provide novel insights into pathogenesis. In fact, precise risk stratification in coeliac disease is becoming increasingly important as we may be entering the era of primary preventions.[24,25]

Here we aimed to study the impact of various index patient- and relative-related factors on the risk of coeliac disease. This was established by serological and genetic testing of a large and well-defined cohort of relatives of previously diagnosed coeliac disease patients.

2 | METHODS

2.1 | Patients and study design

The study was conducted in Tampere University and Tampere University Hospital. The participants were enrolled by inviting children and adults with previously diagnosed coeliac disease and their close relatives to a voluntary family screening via newspaper announcements and local coeliac societies. The aim was to recruit particularly FDRs and SDRs of the index patients, although more distant relatives could also participate. All subjects reporting coeliac disease, or in the case of children, their parents/caregivers were interviewed systemically by a study nurse or a physician (File S1). Patient records were obtained with participants' permission in order to confirm the original diagnosis and other relevant medical data. Patients with lacking medical records or unclear diagnoses were excluded. Blood samples were collected from all study participants for the determination of coeliac disease serology and HLA type.

The first family member diagnosed was defined as the index if there was more than one coeliac disease patient in the same family. The degree of consanguinity between index patients and relatives was documented based on self-report by the participant or caregivers. The non-index family members were further divided into FDRs (siblings, parents and offspring), SDRs (grandparents, grandchildren, aunts, uncles, nieces, nephews and half-siblings) and more distant (first- and second-degree cousins, great-grandchildren, great-grandparents, great-uncles and great-aunts).[26] The screened relatives were considered to belong to a multiple-case family if they had ≥1 FDR or SDR in addition to the index previously diagnosed with coeliac disease. Families with neither confirmed index patient nor any non-coeliac relatives to screen were excluded, as were individuals found to be unrelated to or having an unclear relation to the index patient.

2.2 | Ethics

The study design and recruitment of the participants were approved by the Ethics Committee of Pirkanmaa Hospital District. The Declaration of Helsinki was followed. The participants were informed in advance of the purpose of the study and the significance of the screening results. All participants/caregivers provided written informed consent. The study is registered in ClinicalTrials.gov, identifier number NCT03136731.

2.3 | Clinical data

Demographic information was collected from all participants. In addition, age at diagnosis, severity of small-bowel mucosal damage[27] (partial-, subtotal- and total villous atrophy) as reported by the pathologists and the presence of dermatitis herpetiformis or possible autoimmune co-morbidity (eg type 1 diabetes, Sjögren's syndrome, Addison's disease) were recorded from the previously diagnosed coeliac disease patients. Possible symptoms preceding the diagnosis or screening were assessed from the previously diagnosed coeliac disease patients and new screening-positive relatives.

2.4 | Serological testing, genetics and diagnostic outcome

Serum endomysial (EmA) and tissue transglutaminase antibodies (TGA) were tested from all relatives without previous coeliac disease diagnoses. EmAs were measured by indirect immunofluorescence using the human umbilical cord as an antigen and considering titres 1: ≥5 positive.[28] An enzyme-linked immunosorbent assay (QUANTA Lite h-tTG IgA; INOVA Diagnostics) was used to test TGA, applying a cut-off >20 U/L for seropositivity.[29] IgG-class EmA and TGA were used only if IgA deficiency was suspected based on abnormal EmA staining pattern and low TGA.[30]

Genotyping for the coeliac disease-associated HLA alleles was performed using the SSPTM DQB1 low-resolution kit (Olerup SSP AB), DELFIA® Coeliac Disease Hybridization Assay Kit (PerkinElmer Life and Analytical Sciences, Wallac Oy) or tagging SNP approach.[31] The genotypes were categorised based on predisposing alleles for coeliac disease to high risk (A1*05-B1*0201/A1*05-B1*0201 [DQ2.5/2.5] or A1*05-B1*0201/A1*02-B1*0202 [DQ2.5/2.2]), intermediate risk (A1*05-B1*0201/X [DQ2.5/X], A1*05-B1*0201/A1*03-B1*0302 [DQ2.5/8], A1*02-B1*0202 [DQ2.2/2.2 and DQ2.2/X], A1*02-B1*0202/A1*03-B1*0302 [DQ2.2/8] and A1*03-B1*0302 [DQ8/8 and DQ8/X]) and low risk (DQ2/DQ8 negative).[32]

The main diagnostic outcome—considered to signify coeliac disease in the present study—was positivity for both TGA and EmA and the presence of the disease-associated HLA DQ2 and/or DQ8 haplotype.[23] The possible new seropositive family members were either recruited for prospective studies or received a referral to health care for possible additional investigations or follow-up outside the study protocol.

2.5 | Statistics

The results are given either as number of cases, percentages, medians with lower and upper quartiles or as odds ratios (OR) with 95% CIs. The FDRs were analysed both as a whole group and also separately (offspring, siblings, parents) and SDRs and more distant relatives as whole groups. Statistical significance of categorical variables was assessed by chi-square test and that of continuous variables by Mann-Whitney test, considering P values <0.05 significant. The association between index-related factors and positive screening outcomes was studied by setting the properties of the index as variables for each screened relative. The ORs for new seropositivity were then evaluated by binary logistic regression first in univariate analysis. The reference category was defined as the group with the greatest number of subjects. Next, statistically significant independent risk predictors were determined by multivariable binary logistic regression for characteristics significant in the univariate analysis. Three different models were applied for the multivariable analysis as follows: *Model 1* notifies the significant characteristics of the screened relative excluding HLA, *Model 2* the significant characteristics of both relative and index excluding HLA and *Model 3* the characteristics of *Model 2* and high- vs intermediate-risk HLA of the screened relative. Statistical analyses were performed using either SPSS Statistics for Windows (IBM Corp.) or Confidence Interval Analysis Program[33] as appropriate.

3 | RESULTS

Altogether 4155 subjects were enrolled (Figure 1). White North European origin was the only ethnic background reported. After applying the exclusion criteria, 624 index patients with coeliac disease and 2943 of their relatives were included. Among the relatives, there were altogether 229 previously diagnosed non-index coeliac disease patients in 152 multiple-case families (Figure 1), including 45 who were screen-detected. The characteristics of the index and non-index coeliac disease patients are shown in Table S1. The median age of the 2714 relatives without previous coeliac disease diagnosis was 36 (range 1-91) years and 55.5% were females. Altogether 2067 (76.2%) were FDRs (30.1% offspring, 28.9% siblings, 17.2% parents), 534 (19.7%) SDRs and 113 (4.2%) more distant relatives. The distribution of HLA risk alleles did not differ significantly either between men and women or between FDRs, SDRs and more distant relatives (data not shown).

Altogether 129 (4.8%) of the 2714 screened relatives without previously diagnosed coeliac disease were screening-positive (Figure 1), the prevalence being 5.1% in FDRs (siblings 6.5%, parents 4.7%, offspring 4.0%), 3.6% in SDRs and 3.5% in other relatives. The combined prevalence of the newly detected relatives and previous coeliac disease in non-index relatives was 12.2% (6.3% clinically detected, 5.9% screen-detected) as a whole and 12.5% in FDRs, 10.9% in SDRs and 12.8% in more distant relatives (Figure 1). Of the 2714 screened relatives, three had IgA deficiency and one EmA positive subject had negative (19 U/L) TGA. High-risk HLA haplogenotypes were more frequent in the newly detected screening-positive relatives than in screening-negative relatives, whereas the groups were comparable in demographic data, prevalence of multiple-case families and relation with the index (Table 1). The presence of screening positivity was not affected by sex (FDR: sisters 6.2% vs brothers 6.9%, $P = 0.670$; mothers 5.1% vs fathers 4.1%, $P = 0.591$; daughters 4.1% vs sons 4.3%, $P = 0.766$; SDR: women 4.1% vs men 2.9%, $P = 0.430$; more distant relatives: women 3.0% vs men 4.3%, $P = 1.000$). Clinical data were available from 87 of the new screening-positive relatives, of whom 62.1% reported experiencing possible coeliac disease-related symptoms before the study.

When the newly detected screening-positive FDRs (n = 106) and SDRs (n = 19) were compared, the former were older (median 42 vs 29 years, $P = 0.007$) and less often below 18 years of age (17.9% vs 44.4%, $P = 0.026$) and members of multiple-case families (20.8% vs 47.4%, $P = 0.020$), whereas there were no significant differences between the FDRs and SDRs in sex or distribution of HLA risk alleles (data not shown).

The highest frequencies of both previously diagnosed coeliac disease and screening positivity detected in the present study were seen in subjects aged between 41 and 50 years of age and the lowest frequencies in those older than 60 years (Figure 2). The overall prevalence among relatives was highest in siblings, and there was a trend for decreasing frequency of new screening positivity from siblings to more distant relatives, while the prevalence was distributed more equally among the previously diagnosed patients (Figure 3).

Age below 18 years at diagnosis in index patients and age between 41 and 60 years at screening, being a sibling, and carrying high-risk HLA alleles in relatives were significantly associated with

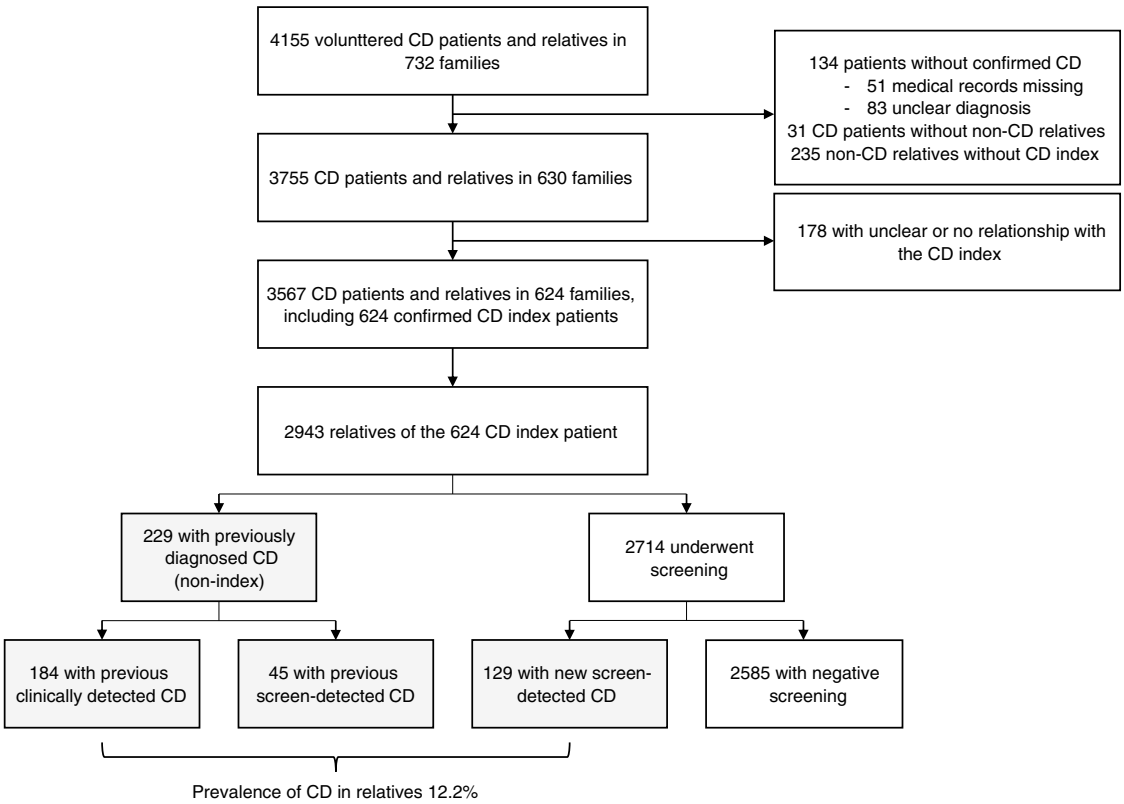

FIGURE 1 Flowchart of the study. CD, coeliac disease

screening positivity in univariate regression analysis (Table 2). In multivariable analysis, a significant association was observed with being a sibling in *Model 1* (notifying consanguinity and age at screening) and with the presence of high-risk HLA group in *Model 3* (notifying all characteristics significant in univariate analysis) (Table 2).

4 | DISCUSSION

We found unrecognised coeliac disease to be common (4.8%) among the index patients' relatives despite a high rate of previously diagnosed family members. The combined prevalence of all relatives detected either by clinical suspicion (6.3%) or by present and earlier screening (5.9%) was 12.2%, which is approximately five times our population-based estimate.[4] Most earlier studies have concentrated on FDRs, in whom prevalences have ranged from 1.3% to 44.1%.[14,34] This heterogeneity may be due to differences in the overall incidence of coeliac disease[1] and demographic features of the relatives. In fact, most earlier studies have been small and included only a few hundred screened FDRs.[14] Another salient factor may be the definition of screening positivity adopted. The seropositivity definition used here, providing that validated tests are used, may yield less biased results than duodenal biopsy, which is frequently declined, particularly by asymptomatic screening-positive subjects.[35,36] Serology is actually gaining a more important role in screening studies and even diagnosis, in spite of not yet being a universally accepted diagnostic criterion.[37-39] In the few relatively large studies with at least partially similar design, 4.2%-5.6% of FDRs have been screening-positive.[36,40,41] Closer to our findings, Rubio-Tapia et al[22] reported a prevalence of 16.4% in FDRs in which they—exceptionally—also counted the proportion (5.1%) of previous diagnoses in non-index family members.

In detailed analysis, siblings had the highest frequency of seropositivity among the FDRs. Despite concurring with previous reports,[14] the differences between consanguinities were smaller here and not significant in multivariable analysis, possibly because confounding factors have not been similarly considered in earlier studies.[16,20] Likewise, although the SDRs and more distant relatives had less often newly detected seropositivity than the FDRs, the groups did not differ in either the combined prevalences or multivariable analysis. This might be due to similar HLA distribution within the groups and indicates that coeliac disease risk in other relatives than FDRs is higher than previously thought.[15,39,40] It must be noted, however, that seropositive SDRs belonged more often to multiple-case families than the FDRs, although this was not a significant risk

TABLE 1 Clinical characteristics and HLA distribution in 2714 at-risk relatives with positive[a] or negative screening outcome

	Positive screening, n = 129		Negative screening, n = 2585		
	n	%	n	%	*P* value
Age at screening, median (Q_1, Q_3), y	40 (20, 53)		36 (17, 55)		0.556
Age <18 y at screening	29	22.7	667	26.0	0.404
Women	72	55.8	1435	55.5	0.946
Member of multiple-case family[b]	35	27.1	631	24.4	0.483
Degree of consanguinity with index					
First-degree relatives	106	82.2	1961	75.9	0.260[c]
Sibling	51	48.1	732	37.3	0.073[d]
Offspring	33	31.1	783	39.9	
Parent	22	20.8	446	22.7	
Second-degree relatives[e]	19	14.7	515	19.9	
More distant relatives	4	3.1	109	4.2	
HLA risk group[f]					<0.001
High[g]	27	26.5	156	7.0	
Intermediate[h]	75	73.5	1394	62.3	
Low[i]	0	0	686	30.7	

[a]Positive endomysium and transglutaminase antibodies and presence of human leucocyte antigen (HLA) DQ2 and/or DQ8.
[b]At least two first- or second-degree relatives previously diagnosed with coeliac disease.
[c]First-degree vs second-degree vs more distant.
[d]Among first-degree relatives.
[e]Grandparent, grandchild, aunt, uncle, niece, nephew, half-sibling.
[f]Data missing from 376 screened relatives.
[g]DQ2.5 homozygotes and DQ2.5/2.2.
[h]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.
[i]DQ2 and DQ8 negative.

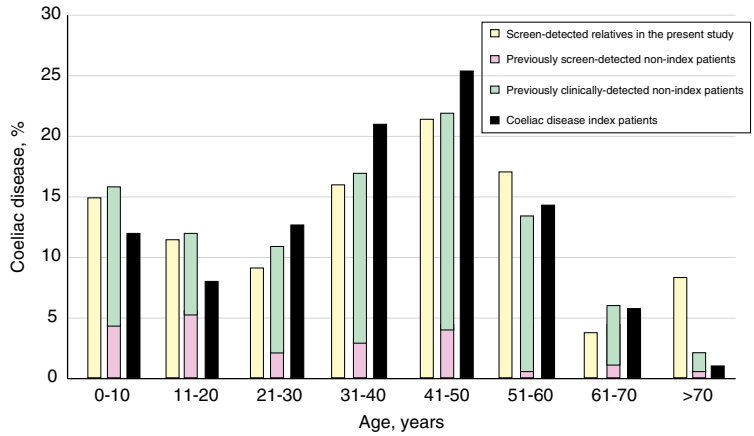

FIGURE 2 Age distribution at coeliac disease diagnosis in previously diagnosed index patients and non-index relatives with coeliac disease and current age of new screening-positive relatives

factor as such. However, the somewhat arbitrary classification of index in the multiple-case families according to the order of the coeliac disease diagnosis and the general homogeneity of Finnish population may have affected the analysis.[40] Of note, although evidence has been scant,[15] the American College of Gastroenterology recommends screening more distant relatives than FDRs,[13] and our findings give further support for this approach. However, additional studies on this issue are warranted.

Age at screening was not a significant factor associated with screening positivity in multivariable analysis, but the prevalence of affected cases increased from childhood to middle age, after which it decreased. The former is in line with the similar increase in the

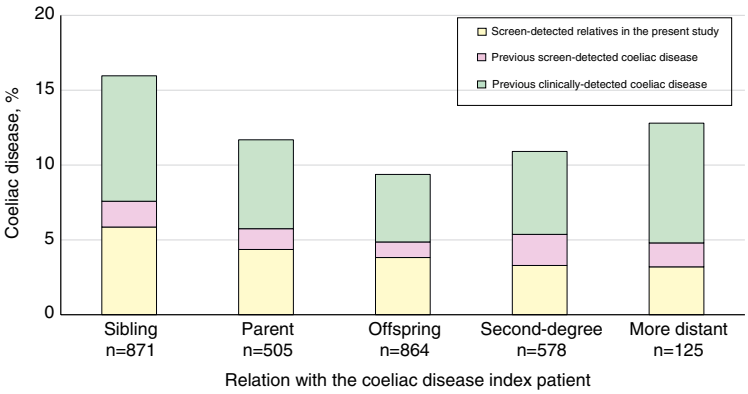

FIGURE 3 Percentage of coeliac disease in the relatives of index patients, divided according to degree of consanguinity and pathway leading to the diagnosis

TABLE 2 Logistic regression analysis of different index- and relative-related characteristics for positive screening outcome[a] in 2714 at-risk relatives

		Multivariable		
	Univariate	Model 1[b]	Model 2[c]	Model 3[d]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Index characteristics				
Age <18 y at diagnosis	1.60 (1.04-2.45)		1.34 (0.80-2.23)	1.41 (0.80-2.51)
Women	0.73 (0.49-1.07)			
High vs intermediate risk HLA	1.37 (0.89-2.12)			
Dermatitis herpetiformis	0.91 (0.55-1.52)			
Autoimmune co-morbidity	1.39 (0.86-2.23)			
TVA vs PVA/SVA	1.02 (0.68-1.62)			
Relative characteristics				
Age <18 y at screening	0.84 (0.55-1.28)			
Women	1.01 (0.71-1.45)			
High[e] vs intermediate[f] risk HLA	3.22 (2.01-5.15)			2.94 (1.80-4.78)
Consanguinity with index				
Offspring	1	1	1	1
Sibling	1.65 (1.06-2.59)	1.67 (1.00-2.79)	1.51 (0.88-2.58)	1.30 (0.71-2.35)
Parent	1.17 (0.67-2.03)	1.58 (0.82-3.05)	1.29 (0.61-2.72)	1.05 (0.45-2.44)
Second-degree relative	0.88 (0.49-1.57)	0.86 (0.48-1.55)	0.84 (0.46-1.52)	0.90 (0.46-1.76)
More distant relative	0.87 (0.30-2.51)	0.91 (0.31-2.62)	0.84 (0.29-1.27)	1.24 (0.41-3.76)
Multiple-case family	1.15 (0.77-1.72)			
Age at screening, years				
0-20	1	1	1	1
21-40	1.06 (0.65-1.74)	0.97 (0.58-1.61)	1.01 (0.60-1.69)	1.06 (0.59-1.91)
41-60	1.73 (1.10-2.73)	1.21 (0.71-2.07)	1.30 (0.75-2.26)	1.62 (0.88-2.97)
61-	0.70 (0.37-1.33)	0.48 (0.23-1.01)	0.57 (0.26-1.27)	0.51 (0.19-1.34)

Note: Characteristics notified in multivariable analysis were [b]consanguinity with the index and relative's age at screening; [c]model 1 and age of index <18 y at diagnosis; [d]model 2 and high vs intermediate risk HLA of relatives.

Abbreviations: OR, odds ratio; PVA, partial villous atrophy; SVA, subtotal villous atrophy; TVA, total villous atrophy.

[a]Positive endomysium and transglutaminase antibodies and presence of human leucocyte antigen (HLA) DQ2 and/or DQ8.

[e]DQ2.5 homozygotes and DQ2.5/2.2.

[f]DQ2.5 heterozygotes and DQ2.2 and/or DQ8 positive.

overall prevalence of coeliac disease by age,[4,17,42] whereas the later decrease could be caused by a higher frequency of seronegative disease among the elderly.[43] Another explanation could be increased mortality in unrecognised diseases, but this is debatable.[44-47] Although interpretation is complicated by temporal changes in the recognition and true prevalence of coeliac disease,[4,48] our results suggest that age should not affect the implementation of family screening. A more challenging issue is the follow-up of the seronegative relatives.[16,41] Further evidence is needed, but a previously reported peak in the seroconversion rate in early life and possible risk of permanent complications in growing children could justify more frequent re-testing in childhood.[18,49,50] Of note, somewhat contrary to some earlier reports,[4,14] sex had no significant effect here. This finding should be interpreted with some caution due to the high proportion of women among the index and non-index patients and possible gender differences in healthcare-seeking behavior.[51] On the other hand, there are also previous reports consistent with our findings[36] and it is possible that stronger HLA risk in family members independent of sex factors might contribute. Unfortunately, we were not able to study the role of symptoms but based on previous evidence they are a poor predictor of coeliac disease in subjects undergoing screening.[52-54]

The presence of a high-risk haplotype was the only factor significantly affecting (OR ~3) the risk of newly identified screening positivity in multivariable analysis and clearly overrode the other hypothesised factors. Similar findings have been reported in a few smaller studies.[16,20] In light of these findings, and that the high-risk haplotype may even increase the risk for complications,[55] determination of the HLA genotype could be beneficial, as it would make it possible to target screening most actively at those with high genetic risk and even to omit testing of low-risk relatives. It must, however, be realised that most of the affected relatives still carry the much more frequent intermediate-risk haplotypes and the majority of cases would thus be missed if only high-risk subjects were included. Although more studies are needed, the role of HLA risk group determination may be more useful in re-screening of initially seronegative relatives, since making a distinction between the high- and intermediate-risk HLA might help to target the possible re-testing, as also suggested by Wessels et al.[16] In the future, improved genetic score considering the additional contribution of non-HLA coeliac disease risk variants to disease risk of, as well as more precise stratification of the HLA-DQ alleles, might enable more precise screening protocols.[56-58] In any case, before a more specific genetic risk score is validated, serological testing of at least all FDRs after an index patient is diagnosed likely remains the simplest and most effective approach for the initial screening.

4.1 | Strengths and limitations

The main strengths of our study were the exceptionally large and well-defined cohort of index patients and their relatives, the meticulously collected family trees and the knowledge of various individual factors. Furthermore, the screening was conducted with well-validated serological tests, although only IgA class assays were systematically used. As a limitation, we had no exact data on those refusing to participate in the study or the individual symptoms or gluten consumption of the relatives. Furthermore, although largely as a whole, the study may still have been too small to reveal all significant associations in the regression analysis, and the high proportion of previous diagnoses—a sign of active case finding and at-risk group screening—further complicates interpretation of the results. It must also be noted that our participants were ethnically very homogeneous which, although beneficial in association analyses, for example, may overestimate the coeliac disease risk of more distant relatives than FDRs and limit the generalisability of the study.

4.2 | Conclusions

The prevalence of unrecognised coeliac disease was high in all ages and also in more distant than FDRs despite a high rate of previously diagnosed non-index relatives. Moreover, further supporting more active screening, the diagnostic yield was suboptimal even in a country with high coeliac disease awareness and broad healthcare coverage. The presence of the high-risk genotype is the most important predictor for coeliac disease and HLA determination could thus be useful to target serological screening of at-risk relatives.

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AUTHORSHIP

Guarantor of the article: KKu.

Author contributions: SP, KL, LK, HH, KKa and KKu designed the study. SP and HH were responsible for statistical analyses. PS, RT, KKa and KKu collected the data. SP and KKu drafted the manuscript and SP wrote the final version. KL, JC, LK, HH, PS, RT, KKa and KKu reviewed the paper for important intellectual content. All authors interpreted the results and approved the final draft submitted.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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Supplementary Table 1. Clinico-demographic characteristics and HLA distribution of index patients[†] and their non-index relatives with previously diagnosed coeliac disease

	Index patients, n=624		Non-index patients, n=229	
	n	%	n	%
Age at diagnosis, median (Q_1, Q_3), years	39 (25, 49)		36 (19, 49)	
Age <18 years at diagnosis	100	16.1	54	24.0
Women	482	77.2	157	68.6
Autoimmune co-morbidity[‡]	148	25.7	39	18.2
Degree of villous atrophy at diagnosis[§]				
Total	138	25.7	50	25.1
Subtotal	221	42.4	77	38.7
Partial	179	33.3	72	36.2
HLA risk group[¶]				
High[††]	104	23.8	39	22.8
Intermediate[‡‡]	323	74.0	131	76.6
Low[§§]	10	2.3	1	0.6

[†]First-affected coeliac disease patient in family; [‡]E.g. type 1 diabetes, autoimmune thyroidal diseases, Addison´s disease; [§,¶]Data missing from [§]116 and [¶]245 patients; [††]DQ2.5 homozygotes and DQ2.5/2.2; [‡‡]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive; [§§]DQ2 and DQ8 negative

PUBLICATION III

Coeliac disease re-screening among once seronegative at-risk relatives: A long-term follow-up study

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Coeliac disease re-screening among once seronegative at-risk relatives: A long-term follow-up study

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Abstract

Background: Serological screening of the relatives of coeliac disease patients is widely endorsed. However, the need for and the optimal timing of possible re-testing of once seronegative at-risk individuals for coeliac disease remain unclear.

Objective: We investigated this issue by inviting a large cohort of previously screening-negative relatives of patients with coeliac disease to participate in a follow-up study.

Methods: Altogether 599 relatives of coeliac disease index patients not diagnosed with coeliac disease in a screening study carried out in 2006–2010 were asked about possible later diagnosis or re-tested with coeliac disease autoantibodies in 2017–2021. Besides incidence, the possible impact of various patient-related clinical factors and HLA haplotype on the later diagnosis or screening positivity was examined.

Results: Fifteen (2.5%) relatives were either diagnosed with a coeliac disease ($n = 8$) during the follow-up period or were found to be screening-positive in the re-testing ($n = 7$), giving a combined annual incidence of 221/100,000 person-years in all relatives and 336/100,000 among those carrying coeliac disease-associated HLA DQ2/DQ8. The new cases more often carried the high-risk (DQ2.5/2.5 or DQ2.5/2.2; 35.7% vs. 7.4%, respectively, $p < 0.001$) HLA and were younger at initial screening (23.3 vs. 40.5 years, $p = 0.028$) and – in spite of a negative screening outcome – had higher median transglutaminase antibody level in the first study than those not affected. There were no significant differences between the affected and non-affected relatives in other demographic data, degree of kinship with the index, current symptoms or frequency of chronic co-morbidities.

Conclusion: The incidence rate for later coeliac disease diagnosis or new seropositivity in relatives who had been tested once was 221/100,000 person-years in all and 336/100,000 among those carrying at-risk HLA genetics after ~10 years of follow-up. HLA-typing could help to target a subgroup of relatives who would benefit most from re-testing.

KEYWORDS
coeliac disease, genetics, gluten, kinship, serology, HLA, testing

INTRODUCTION

Despite the availability of sensitive and non-invasive serological tests, the majority of coeliac disease patients remain unrecognised and at increased risk for ill-health and severe long-term complications, often even without apparent symptoms.[1,2] Consequently, most of the current guidelines recommend improving the diagnostic yield for this treatable disorder by screening specific at-risk groups, particularly first-degree relatives (FDR) of previously identified coeliac disease patients.[3,4] There nevertheless remain many open questions regarding the actual implementation of the screening, including optimal starting age, testing of other than FDRs and the possible use of genetic risk stratification.[5–7]

The need for and timing of possible re-testing after a negative result in the first serological testing is even more unclear, as a single seronegative result does not exclude coeliac disease for life.[8] Interestingly, although the incidence of coeliac autoimmunity may peak already in early childhood, according to population-based studies the prevalence continues to increase with age, new cases appearing even among the elderly.[8–10] However, there is a paucity of studies systematically investigating the frequency of de novo seropositivity/coeliac disease diagnosis after childhood in previously screening-negative individuals.[11–13] Moreover, the possible patient-related factors affecting the likelihood of a positive screening result, such as age at re-testing, sex, comorbidities and individual profile for the coeliac disease-associated HLA genetics remain unidentified.

We aimed to further elucidate the above-mentioned unresolved issues by re-screening a large cohort of at-risk relatives with coeliac disease who some 10 years earlier had been excluded in a previous screening study.

Key summary

Established knowledge on this subject:

- Screening of at-risk relatives could be used to improve diagnostic yield of coeliac disease.
- The need for and the optimal timing of possible re-testing of once seronegative individuals remain obscure.

New findings of this study:

- The incidence rate for later coeliac disease diagnosis or new seropositivity in the once tested relatives was 221/100,000 person-years after 10 years of follow-up.
- The figure increased to 336/100,000 among those carrying at-risk HLA genetics.
- More detailed HLA-typing could help to target a subgroup of relatives who would benefit most from re-screening.

METHODS

Patients and study design

The study was conducted in Tampere University and Tampere University Hospital in the period 2017–2021. It continued an earlier family screening study carried out in the same centre in the period 2006–2010.[6] The first study comprised altogether 3115 non-coeliac relatives of previously diagnosed coeliac disease index patients from 706 families (Figure 1). The main intention was to invite FDRs, but more distant relatives were also approved. All participants were tested for serum IgA-class endomysium (EmA) and tissue transglutaminase antibodies (TGA) and coeliac disease-associated HLA. Corresponding IgG-class antibody tests were used in cases of selective IgA deficiency. Altogether 148 of the screened relatives had positive EmA – the main screening outcome and definition for seropositivity – and were referred to local healthcare facilities for further diagnostic investigations (Figure 1). The remaining 2967 relatives were informed that one-time negative testing does not exclude coeliac disease for a lifetime and that they should contact their local healthcare facilities in case of symptoms or signs suggestive of the disease.

The present follow-up study aimed to recruit a representative sample of the aforesaid 2967 non-coeliac relatives for re-testing (Figure 1). All previously screening-negative relatives with contact information available were invited to participate in the present study. Exclusion criteria were refusal and difficulties in communication. In addition, families with an inconclusive coeliac disease diagnosis of the index patient and subjects not related to the index patient were excluded after updating the original family tree data (Figure 1). During the study visit, the participants were interviewed by a physician or study nurse with expertise in coeliac disease and blood samples were taken for serology. For participants unable to travel for a face-to-face visit, the interviews were conducted by telephone and blood was drawn at local laboratory facilities from which it was sent to the research centre for analysis. Relatives with a new coeliac disease suspicion were referred to an appropriate healthcare unit for further diagnostic investigations.

Clinical data and diagnostic findings

The clinical data collected included age at present, sex and possible coeliac disease diagnosis in clinical routines between the first screening and the present study and the presence of chronic or

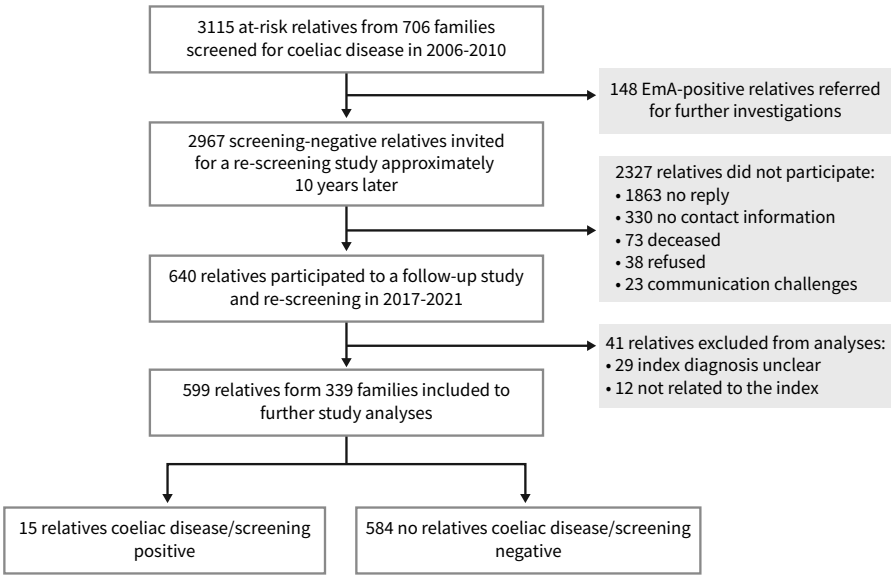

FIGURE 1 Flowchart of the study

recurrent symptoms and co-morbidities. Particular attention was paid to coeliac disease-related gastrointestinal and extraintestinal manifestations, such as diarrhoea, vomiting, abdominal pain, weight loss, constipation, arthralgia, fractures, dermatological or neurological symptoms, poor growth and anaemia.[14] Possible self-initiated gluten reduction was also elicited. Duration of symptoms was further categorised as no symptoms, symptoms ≤5 years and symptoms >5 years. Low-energy fracture was defined as a fracture resulting from trauma that would not normally result in fracture in a healthy individual.[15]

Age at diagnosis and symptoms preceding the diagnosis were elicited from subjects diagnosed with coeliac disease in clinical routine after the first screening and before the present study. Diagnoses in clinical routine were verified from patient records and made according to current guidelines.[16]

The degree of consanguinity between the relative and coeliac disease index patient was classified to FDR (siblings, parents and offspring), second-degree relative (SDR; grandparents, grandchildren, aunts, uncles, nephews, nieces and half-siblings) and more distant (first- and second-degree cousins, great-grandchildren, great-grandparents, greataunts and greatuncles). The relationship was defined as the closest for example, FDR when possible and verified from the familial data. Among the FDRs, consanguinity was based on the relative who was the reason for study participation/coeliac disease suspicion. A subject was defined to belong to a multiple case family if there was already more than one affected FDR and/or SDR.

Serological testing and genetics

EmA were measured by indirect immunofluorescence using human umbilical cord as an antigen and considering titres 1: ≥5 positive. A commercial EliA test (Celikey, Phadia) was used to test TGA, applying a cut-off of >7 U/L for seropositivity. At the initial screening in 2006–2010, TGA was tested with commercial ELISA test (QUANTA Lite h-tTG IgA, INOVA Diagnostics), applying a cut-off of >20 U/L for seropositivity. The corresponding IgG class serological tests were used if selective IgA deficiency was suspected based on abnormal EmA staining pattern and low TGA value. For the purposes of the study, a positive screening result was defined as positivity for both antibodies (EmA, TGA) and presence of coeliac disease-related HLA.[17]

The presence and subtype of the coeliac disease-associated HLA alleles were determined from each participant in connection with the first screening study using the SSPTM DQB1 low-resolution kit (Olerup SSP AB) or tagging SNP approach.[18] Individual HLA-type was further categorised based on estimated predisposition to coeliac disease to high risk (A1*05-B1*0201/A1*05-B1*0201 [DQ2.5/2.5] or A1*05-B1*0201/A1*02-B1*0202 [DQ2.5/2.2]), intermediate risk (A1*05-B1*0201/X [DQ2.5/X], A1*05-B1*0201/A1*03-B1*0302 [DQ2.5/8], A1*02-B1*0202 [DQ2.2/2.2 and DQ2.2/X], A1*02-B1*0202/A1*03-B1*0302 [DQ2.2/8] and A1*03-B1*0302 [DQ8/8 and DQ8/X]) and low risk (DQ2/DQ8 negative).[19]

Ethics

All study participants/caregivers gave written consent after receiving comprehensive information on the purpose of the study and the significance of the screening results. The study design and recruitment of the participants were approved by the Ethics Committee of Pirkanmaa Hospital District and the Declaration of Helsinki was adhered to in all stages.

Statistics

The results are given as medians with quartiles, number of cases and percentages, or as incidence rates (IR) and incidence rate ratios (IRR) with 95% confidence intervals. Chi-square test or Fisher's Exact test were used to analyse the statistical significance of categorical variables and Mann-Whitney test for continuous variables as appropriate. p value <0.05 was considered significant. IR was calculated by applying person-time at risk either according to the time elapsing from the first screening to the date of the coeliac disease diagnosis outside the study protocol or to the positive result in the present study. In univariate analysis IRRs were estimated using Poisson regression using age groups <30 years and ≥30 years at first study, sex and HLA high- and intermediate-risk groups as covariates. Statistically significant covariates were further analysed in multivariable Poisson regression. Statistical analyses were performed using SPSS Statistics for Windows, version 27 (IBM Corp.) and STATA Statistical Software (StataCorp. LP, Lakeway Drive).

RESULTS

Altogether 640 relatives participated in the present study and 599 of them were included in the final analyses (Figure 1). Of these, 560 (93.5%) were FDRs (252 siblings, 208 offspring, 100 parents), 28 (4.7%) SDRs and 11 (1.8%) more distant relatives. The median age was 40.2 (range 1.5–76.7) years at first screening and 51.8 (range 10.6–90.8) years at present and 65.6% were females. The participants were more often females and less often under 18 years of age at first study round than the non-participants, whereas there were no significant differences in the median TGA values at first screening, current age, being a member of a multiple-case family or distribution of the HLA haplotypes (Table S1).

Median time from the first screening to the present study was 11.4 years (7.8–14.5 years). Altogether 15 (14 FDRs, 1 SDR) relatives had either received a coeliac disease diagnosis during the later follow-up after the first screening and before the present study (n = 8) or were found to be screening-positive in the present study (n = 7, Figure 1), giving a cumulative incidence of 2.5%. The follow-up time was 6785.9 person-years, giving an IR of 221/100,000 person-years for coeliac disease/screening positivity. These 15 cases were more often carriers of the high-risk HLA haplotypes and were younger and – despite having had a negative screening outcome – had a higher median TGA value at first testing than the 584 seronegative relatives, whereas the groups were comparable in sex, being a member of a multiple-case family, presence and duration of symptoms before the diagnosis or new screening positivity and frequency of co-morbidities (Table 1). Two subjects had suspected IgA deficiency and both of them were IgG-class antibody testing-negative.

The clinically diagnosed (n = 8) and screening-positive (n = 7) relatives did not differ significantly on any study parameters (Table 2). Three of the screening-positive subjects were from the same family and the remaining 12 from separate families. Coeliac disease was confirmed endoscopically in all clinically diagnosed patients except one elderly subject who was diagnosed based on TGA level >10x upper limit of normal. The biopsy has also been taken from four of the now detected screening-positive subjects and three of them are still considering undergoing endoscopy. Three of the four subjects had subtotal villous atrophy consistent for coeliac disease, whereas one (EmA 1:100, TGA Celikey® 26.0 IU/L) subject was reported to have normal mucosal morphology. One half of the HLA haplotype was uncertain in one clinically diagnosed subject, although she was found to be carrying HLA DQ2.5.

IR was 336/100,000 person-years among subjects carrying the coeliac disease-related HLA. The rate was higher for subjects aged <30 years than for those ≥30 years at the time of the first study and those with high-risk HLA compared to intermediate risk in univariate analysis, whereas there was no significant difference between women and men (Table 3). In multivariable analysis, only HLA remained significant (Table 3).

Twenty-seven relatives reported to maintain self-initiated gluten-free diet before the current serological screening. None of these subjects had a new screening positivity. Strictness of the diet was not assessed. They were more often female (85.2% vs. 65.8% (respectively), p = 0.036) and had longer symptom duration, >5 years at present (92.6% vs. 70.7%, p = 0.045), than those screening-negative subjects on a gluten-containing diet. Subjects maintaining and not maintaining the diet did not differ in TGA value either at first (INOVA 8.0 U/L vs. 8.0 U/L, (respectively) p = 0.555) or present screening (Celikey 0.5 IU/L vs. 0.5 IU/L, p = 0.629) or in present age (52.1 vs. 52.0 years, p = 0.619). Of those maintaining a self-initiated gluten-free diet, 11 (52.4%) carried and 10 did not carry HLA risk for coeliac disease; data were missing from six subjects.

DISCUSSION

We found a cumulative incidence of 2.5% and IR of 221/100,000 person-years for new coeliac disease diagnosis or screening positivity in once-seronegative relatives. The IR is high compared to the figures of 30–45/100,000 observed in clinically diagnosed Finnish patients during the past decade,[20,21] and also compared to the estimated seroconversion rates of 16–90/100,000 seen after one-time negative testing in general adult population.[8,11] Previous reports among re-tested relatives are scarce, likely since re-screening has often not been performed systemically,[22–24] follow-up times have been short[12,25] and studies have comprised <100 relatives.[12,13,26] As an exception, Biagi et al. recently reported an IR of 437/100,000, but this was based on only one new case.[12] Furthermore, based on the median follow-up times, IRs ranging from 89/100,000 to 916/100,000 can again be indirectly estimated from the earlier publications.[12,13,22,25,26] Additionally, two retrospective studies reported cumulative incidences of 5.9%[23] and 3.5%[24] without giving an explicit follow-up time. Different diagnostic definitions hamper the comparisons although there usually has been a good correlation between the

TABLE 1 Characteristics of the relatives who had coeliac disease excluded in the first screening but either had a later coeliac disease diagnosis or new screening positivity[a] or remained seronegative

	Diagnosis/positive screening, $n = 15$		Negative screening, $n = 584$		
	Median	Quartiles	Median	Quartiles	p value
Age at first screening	23.3	12.5, 40.6	40.5	22.3, 53.5	**0.028**
Current age, years	33.6	24.3, 51.9	52.0	33.9, 65.5	**0.027**
Follow-up time[b], years	10.9	5.0, 11.9	11.4	10.7, 12.0	**0.021**
Initial TGA value, U/ml	10	8, 29	8	7, 10	**0.033**
	n	%	n	%	
Females	9	60.0	385	65.9	0.633
Age <18 years at first screening	6	40.0	119	20.4	0.099
HLA risk group[c]					**<0.001**
High[d]	5	35.7	37	7.4	
Intermediate[e]	9	64.3	316	63.3	
Low[f]	0	0	146	29.3	
Member of a multiple case family[g]	7	46.7	449	76.9	0.058
Relation with the index					0.640
First-degree relative	14	93.3	546	93.5	0.455[h]
Sibling	6	42.9	246	42.1	
Offspring	7	50.0	201	34.4	
Parent	1	7.1	99	17.0	
Second-degree relative	1	6.7	27	4.6	
More distant relative	0	0	11	2.1	
Presence of symptoms[i]					0.333
No symptoms	2	14.3	46	8.1	
≤5 years	4	28.6	114	20.1	
>5 years	8	57.1	406	71.7	
Co-morbidity					
Autoimmune thyroidal disease	4	26.7	82	14.0	0.250
Rheumatoid disease	0	0	31	5.3	1.000
Type 1 diabetes	0	0	8	1.4	1.000
Osteoporosis or osteopenia	0	0	22	3.8	1.000
Any fractures	3	20.0	186	31.8	0.410
Low-energy fractures	1	7.1	83	14.2	0.707
Gastrointestinal disease	1	6.7	99	17.0	0.486
Cardiovascular disease	2	13.3	125	21.4	0.749
Miscarriages	1	11.1	91	23.6	0.691

[a]Positive endomysial and transglutaminase antibodies and HLA DQ2/8.

[b]Time from the first screening to the present study or new coeliac disease diagnosis.

[c]Data missing from 86 subjects.

[d]DQ2.5/2.5 and DQ2.5/2.2.

[e]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

[f]DQ2/8 negative.

[g]Subject has ≥2 previously diagnosed first-/second-degree relatives.

[h]Comparison between first-degree relatives.

[i]Gastrointestinal and extraintestinal manifestations; HLA, human leucocyte antigen; TGA, tissue transglutaminase antibody (Inova®, cut-off > 20 U/L). Bolded numbers indicate significant values.

TABLE 2 Characteristics of the 15 initially seronegative at-risk relatives who either received a later coeliac disease diagnosis or had new screening positivity[a] in the present study

	Coeliac disease $n = 8$		Positive screening $n = 7$		
	Median	**Quartiles**	**Median**	**Quartiles**	***p* value**
Age at initial, years	24.4	5.2, 54.1	23.3	12.5, 40.3	0.728
Age at diagnosis or current screening	33.0	13.0, 55.0	33.6	24.3, 51.6	0.908
Follow-up time[b], years	6.5	2.3, 10.5	11.7	10.9, 11.9	N/A
Initial TGA value, U/ml	16	8, 29	8	7, 29	0.599
	n	**%**	**n**	**%**	
Females	6	75.0	3	42.9	0.315
Age <18 years at first testing	3	37.5	3	42.9	1.000
HLA risk group[c]					0.266
High[d]	4	57.1	1	14.3	
Intermediate[e]	3	42.9	6	85.7	
Low[f]	0	0	0	0	
Member of a multiple case family[g]	4	50.0	3	42.9	1.000
Relation with the index					1.000
First-degree relative	7	87.5	7	100.0	1.000[h]
Sibling	3	42.9	3	42.9	
Offspring	3	42.9	4	57.1	
Parent	1	14.3	0	0	
Second-degree relative	1	12.5	0	0	
More distant relative	0	0	0	0	
Presence of symptoms[i]					0.298
No symptoms	0	0	2	28.6	
≤5 years	3	42.9	1	14.3	
>5 years	4	57.1	4	57.1	
Co-morbidity					
Autoimmune thyroidal disease	1	12.5	3	42.9	0.569
Rheumatoid disease	0	0	0	0	-
Type 1 diabetes	0	0	0	0	-
Osteoporosis or osteopenia	0	0	0	0	-
Any fractures	2	25.0	1	14.3	1.000
Low-energy fractures	1	12.5	0	0	1.000
Gastrointestinal disease	1	12.5	0	0	1.000
Cardiovascular disease	1	12.5	1	14.3	1.000
Miscarriages	0	0	1	33.3	0.333

[a]Positive endomysial and transglutaminase antibodies and HLA DQ2/8.

[b]Time from the first screening to the present study or new coeliac disease diagnosis.

[c]Data missing from 86 subjects.

[d]DQ2.5/2.5 and DQ2.5/2.2.

[e]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

[f]DQ2/8 negative.

[g]Subject has ≥2 previously diagnosed first-/second-degree relatives.

[h]Comparison between first-degree relatives.

[i]Gastrointestinal and extraintestinal manifestations before diagnosis or before present screening; HLA, human leucocyte antigen; TGA, tissue transglutaminase antibody (Inova®, cut-off > 20 U/L).

TABLE 3 Incidence rates (IR) and incidence rate ratios (IRR) for coeliac disease/positive screening using age at initial screening, sex and HLA group as covariates

	Univariate		Multivariable
	IR	IRR (95% CI)	IRR (95% CI)
Age at initial screening			
<30 years	406/100,000	**3.51 (1.09–13.1)**	2.83 (0.95–8.46)
≥30 years	116/100,000		
Sex			
Women	202/100, 000	1.27 (0.37–4.02)	
Men	258/100, 000		
HLA group			
High[a]	1073/100,000	**4.41 (1.16–14.7)**	**4.62 (1.55–13.8)**
Intermediate[b]	243/100,000		

Note: Significant covariates were further adjusted in multivariable analysis. Bolded numbers indicate significant values.

Abbreviation: CI, confidence interval.

[a]DQ2.5/2.5 and DQ2.5/2.2.

[b]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

serological and histological approaches.[13,22,25,26] This, together with the fact that endoscopy is often refused, supports the use of serology for a more unbiased research outcome.[17,27]

Determination of HLA-DQ2/8 could help to target re-screening, as this might allow to omit approximately 30%–40% of the relatives without the genetic risk.[6,22] Accordingly, here the IR was markedly higher (336/100,000) among those carrying either of these risk alleles. Systematic re-screening including only relatives carrying the risk HLA has not previously been performed, but Wessels et al.[23] retrospectively followed-up 341 once screening-negative relatives with HLA DQ2/8 or unknown HLA. Re-screening was offered only to those relatives who were children or adolescents at the time when the index case was diagnosed. Although no exact follow-up time was reported, they observed seroconversion in 20 children. Coeliac disease was reported to be diagnosed at later screening in 27%, the majority having been <1 year of age at the time of the index diagnosis; none were adults. In addition, Bonamico et al.[22] suggested follow-up for 193 FDRs with at-risk HLA and found three new cases in re-testing 13–25 years later. Although lack of data on follow-up time and unsystematic screening inhibit conclusions, the authors of both studies recommend the use of HLA determination for selecting at-risk relatives for serological surveillance.

By applying a more detailed risk stratification,[6] we observed a strong positive predictive role of high-risk HLA DQ2.5/2.5 or 2.5/2.2 compared to intermediate-risk HLA in re-testing. Further supporting such a targeted approach, individuals with the high-risk haplotypes may be at increased risk for coeliac disease-associated complications.[28] Here it is important to note that no detailed HLA determination is currently available for use in clinical practice and determination of high-risk HLA alone would miss the majority of affected cases carrying the more common intermediate-risk haplotype. In the future, more precise genetic risk scores also including non-HLA alleles may further help by targeting the serological surveillance of at-risk relatives.[19] It must, however, be kept in mind that awareness of the hereditary susceptibility for coeliac disease may also cause increased anxiety and influence individual health care behaviour.[29] More evidence on the cost-effectiveness of genetic testing is also needed.

We cannot determine the optimal screening frequency for at-risk relatives as they were tested only twice at an interval of approximately 10 years. Age is an important factor here, as there could be a higher incidence of coeliac disease in childhood.[8–10] Here subjects with a positive study outcome were younger than those proving screening-negative, but high-risk HLA was a stronger risk factor than age. The fact that growing children may rapidly develop permanent complications further supports their frequent screening.[30] Accordingly, Leffler et al. suggested annual or biennial screening of relatives <16 years of age and less frequent testing in adulthood depending on the HLA risk,[31] while Wessels et al. proposed annual screening before 10 years of age and even omitting re-testing thereafter.[23] Of note, Pittschieler et al. screened 86 at-risk relatives annually over a 12-year period and found five new cases, but none of these were adults.[13] For comparison, patients with type 1 diabetes are also at increased risk for coeliac disease, and their screening, for example, at diabetes diagnosis and after two and five years has been suggested,[32] but whether this applies to adults is again unclear. Large multicentre studies are likely needed to enable firm conclusions on the optimal re-screening frequency of relatives and other risk groups.

No association was found between the presence of symptoms or co-morbidities and later coeliac disease/screening positivity, which concurs with earlier studies focussing on first-time testing.[17] This supports re-screening of even asymptomatic relatives for an optimal diagnostic yield. However, it remains debatable whether the benefits of early diagnosis exceed the burden of a strict gluten-free diet in all such individuals,[2,17,33] emphasising the importance of shared decision-making before screening.

Notably, although relatives with positive outcome in the present study had been EmA negative at first screening, their median initial TGA value had been significantly higher than that among unaffected relatives. This indicates that these subjects may already have experienced the early stages of the ongoing autoimmunity process.[34]

Strengths and limitations

The main strength of the study was the systematic re-screening for a large number of at-risk relatives who had undergone their first screening in the same centre approximately 10 years earlier. Furthermore, carefully updated familial data were available on all participants and well-validated serological tests were used. As a limitation, only a moderate fraction of the once-screened relatives participated in the re-testing. In addition, a subgroup of the participants was on self-instituted gluten-free diet, which may have led to a false negative screening result. In theory, there could have also been

rare cases of seronegative coeliac disease.[35] Furthermore, the vast majority of the participants were FDRs and more studies among SDRs and more distant relatives are needed. It must also be emphasised that the ethnically homogeneous study population may impede the generalisability of the results to other countries.

CONCLUSIONS

By using a design reflecting a real-life scenario, we found an IR of 221/100,000 for all and 336/100,000 for HLA DQ2/8 positive once seronegative family members for a new coeliac disease diagnosis/screening positivity. Determination of the high-risk HLA haplotypes could be of further help in targeting those individuals who benefit most from re-screening.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICS APPROVAL

The study was approved by the Ethics Committee of Tampere University Hospital (Ethical committee code **R17043, 7 April 2017**).

AUTHOR CONTRIBUTIONS

Saana Paavola: designing the study, collecting and interpreting data, statistical analyses, writing the manuscript; Kalle Kurppa: designing the study, collecting and interpreting data, drafting the manuscript, critical review of the paper for important intellectual content; Heini Huhtala: designing the study, statistical analyses, interpreting data, critical review of the paper for important intellectual content; Päivi Saavalainen: collecting and interpreting data, critical review of the paper for important intellectual content; Katri Lindfors: designing the study, interpreting data, drafting the manuscript, critical review of the paper for important intellectual content; Katri Kaukinen: designing the study, collecting and interpreting data, drafting the manuscript, critical review of the paper for important intellectual content. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared as it contain potentially identifying patient information.

INFORMED CONSENT

Written informed consent was obtained from each participant.

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Supplementary Table 1. Characteristics of the 2,967 at-risk relatives who were screened in 2006-2010 for coeliac disease and participated/did not participate to the follow-up study in 2017-2021.

	Participants, n=640		Non-participants, n=2,327		
	Median	**Quartiles**	**Median**	**Quartiles**	**P value**
Age at first testing, years	40.0	22.1, 53.3	35.6	17.1, 56.2	0.396
Current age, years	53.6	35.6, 66.7	49.1	30.6, 69.5	0.305
Initial TGA value, U/ml	8	7, 10	8	7, 11	0.863
	n	**%**	**n**	**%**	
Females	430	67.2	1,214	52.2	**<0.001**
Age <18 years at first testing	135	21.1	606	26.3	**0.008**
Member of a multiple case family[a]	143	22.3	503	21.6	0.693
HLA haplotype[b]					0.357
High risk[c]	43	7.9	132	6.5	
Intermediate risk[d]	336	61.4	1222	60.3	
Low risk[e]	168	30.7	673	33.2	

[a]≥2 first- or second-degree relatives previously diagnosed with coeliac disease; [b]Data missing from 434 subjects; [c]DQ2.5/2.5 and DQ2.5/2.2; [d]DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive; [e]DQ2/8 negative. HLA, human leucocyte antigen; TGA, tissue transglutaminase antibody (Inova®, cut-off >20 U/ml). Bolded numbers indicate significant values.