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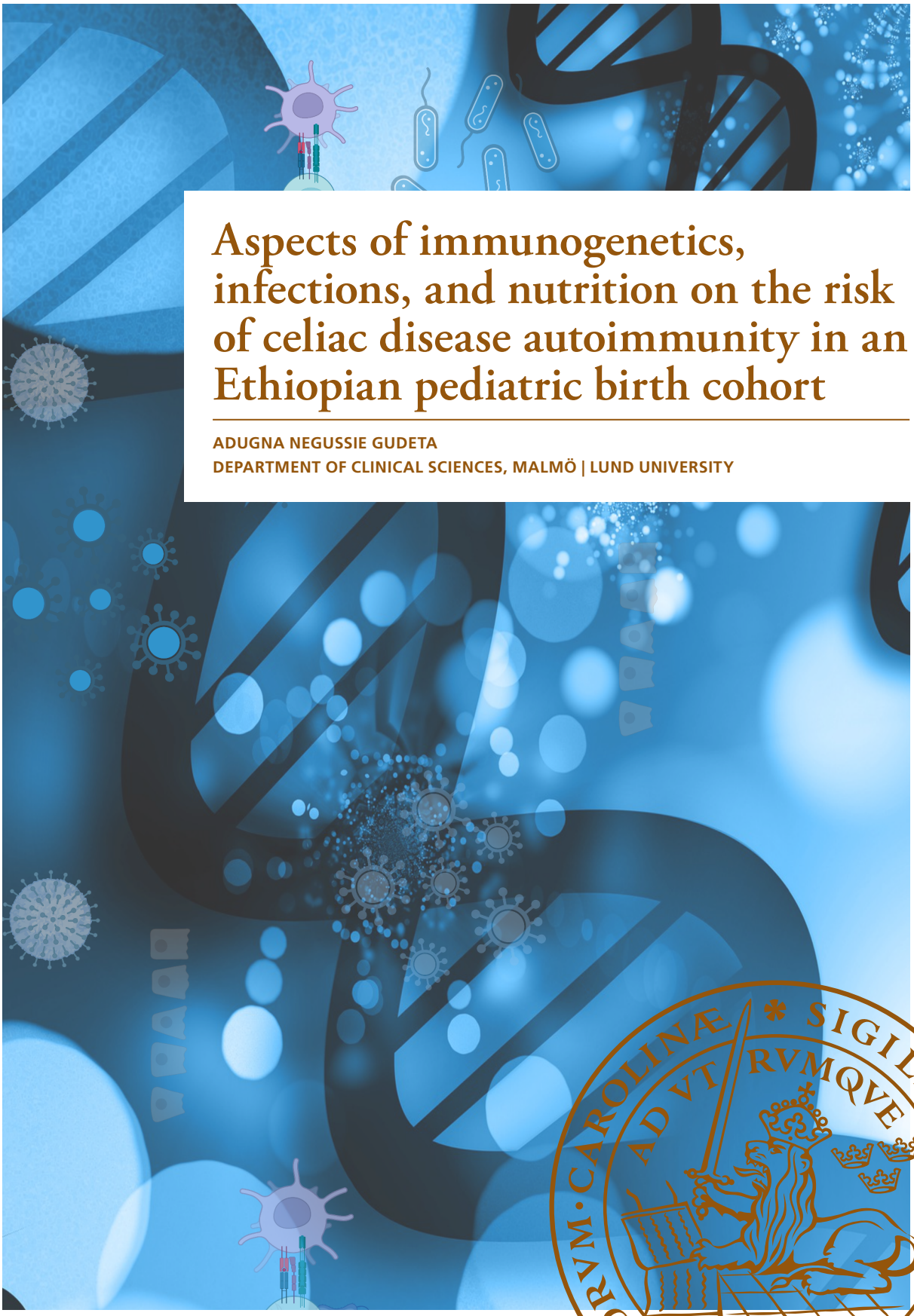

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Title and subtitle: Aspects of immunogenetics, infections, and nutrition on the risk of celiac disease autoimmunity in an Ethiopian pediatric birth cohort

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Methods: Data from the general population were used for longitudinally prospective and retrospective studies. Serum samples collected from women and kept in a repository were examined for tissue transglutaminase autoantibodies (tTGA) using radioligand binding assays (RBA). Children from a birth cohort were prospectively followed for CDA and evaluated for genetic risk factors (HLA-DQ). Screening for CDA was performed annually from age 2 years by an ELISA. Children with positive tTGA results were retested using RBA and if persistently confirmed as tTGA positive, they were defined as having CDA. Parents were interviewed to obtain information on diet and infections of the study participants. An integrated cohort provided information on maternal tuberculosis exposure. At the age of 4 years, serum samples from children were tested for serum *Helicobacter pylori* (HP) antibodies using an ELISA.

Results: The prevalence of CDA ranged from 0.05% to 0.6% (1:2000 to 1:174). Children are more likely than adults to have CDA. There were no differences among the gender of the birth cohort. The distribution of the CD associated HLA risk-haplotypes, HLA-DQ2, and -DQ8, were comparable to that of the Swedish population. CDA was not associated with either Ethiopian traditional diet or *Mycobacterium tuberculosis* and *Helicobacter pylori* infections.

Conclusions: Although prevalence of CDA in Ethiopian children had increased more than tenfold compared to a screened adult female population, it was still lower than the pooled global prevalence. Despite the differences in individual HLA-DQ2 and HLA-DQ8 predisposing risk-haplotypes for CD that were found between the Ethiopian and Swedish cohort, the distribution of CD risk-genotypes was overall the same. Neither was the frequency of consumption of cereals based on teff nor wheat among study participants associated with CDA. Moreover, neither latent *Mycobacterium tuberculosis* exposure nor *H. pylori* infections were associated with the incidence of CDA.

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To my family, friends and almighty of God!

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Abbreviations

Ab	antibody
AGA	anti-gliadin antibodies
CDA	celiac disease autoimmunity
CD	celiac disease
CI	confidence interval
CMV	cytomegalovirus
CRC	Clinical Research Center
DGP	deamidated gliadin peptide
ELISA	enzyme linked immune sorbent assay
EMA	endomysial autoantibody
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
GFD	gluten-free diet
HBV	hepatitis B virus
HCV	hepatitis C virus
HSV-1	herpes simplex virus type I
HLA	human leukocyte antigen
HP	helicobacter pylori
IELs	intraepithelial lymphocytes
IFN-γ	interferon-gamma
IgA	immunoglobulin A
IgG	immunoglobulin G
LMTB	latent mycobacterium tuberculosis
MHC	major histocompatibility complex
MTBC	mycobacterium tuberculosis complex
PCR	polymerase chain reaction
QFT	QuantiFERON-tuberculosis test
RBA	radioligand-binding assays
RSV	respiratory syncytial virus
TEF	traditional Ethiopian food
tTGA	tissue transglutaminase autoantibody
ULN	upper limit of normal
WHO	World Health Organization

List of scientific publications

The current thesis is based on the following papers, referred to by the roman numerals I to IV.

I. **Adugna Negussie Gudeta**, Charlotte Brundin, Daba Muleta Feyissa, Taye Tolera Balcha, Daniel Agardh. Prevalence of Celiac Disease Autoimmunity in Ethiopian Pregnant Women: A Cross Sectional Study from the Oromia region. International Journal of Celiac Disease (2019); 7, (3), 74-77.

II. **Adugna Negussie Gudeta**, Anita Ramelius, Taye T. Balcha, Alemayehu Girma, Jorma Ilonen, Daniel Agardh. Distribution of HLA-DQ risk genotypes for celiac disease in Ethiopian children. HLA (2020); 96: 681–687.

III. **Adugna Negussie Gudeta**, Carin Andrén Aronsson, Taye Tolera Balcha and Daniel Agardh. Complementary Feeding Habits in Children Under the Age of 2 Years Living in the City of Adama in the Oromia Region in Central Ethiopia: Traditional Ethiopian Food Study. Front. Nutr. (2021); 8:672462.

IV. **Adugna Negussie Gudeta**, Carin Andrén Aronsson, Bayissa Bekele Binagdie, Alemayehu Girma, and Daniel Agardh. Incidence of celiac disease autoimmunity and associations with maternal tuberculosis and pediatric Helicobacter pylori infections in 4-year-old Ethiopian children followed up in an HLA genotyped birth cohort. Front. Pediatr. (2022); 10:999287.

Abstract

Background: Celiac disease (CD) is an immune-mediated inflammatory disease of the intestine in genetically susceptible individuals caused by loss of tolerance to the storage proteins (gluten) in wheat, rye, and barley. Little is known about CD and associated risk factors in Sub-Saharan African countries including Ethiopia. The main aim of the present thesis was to explore how the incidence of celiac disease autoimmunity (CDA) associates with genetic and environmental factors with emphasis on the diet and infections in an Ethiopian pediatric population.

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Background

Historical overview

The significant dietary and lifestyle changes brought on by the agricultural revolution are historically linked to celiac disease (CD) (1). Aretaeus of Cappadocia, a Roman physician, published the earliest description of CD in the second century *anno domini*. Aretaeus used the Greek word "koiliakos," which originally meant "suffering in the bowels" to describe CD (2). Later, Samuel Gee provided the first comprehensive modern description of the clinical picture of CD in 1888, together with his theories on the role of nutrition in its management. Dr. Gee described CD as a kind of chronic dyspepsia that can affect people of all ages, but is more common in young children between the ages of 1 and 5 years (3). Willem-Karel Dicke, a Dutch pediatrician, confirmed for the first time that many children with the CD syndrome might be successfully treated with a diet free from wheat and rye flour. He studied and presented his theories published in 1950, that wheat proteins, not the carbohydrates, are actually the cause of the disease (4). Later, the pediatrician Charlotte Anderson and her Birmingham team performed studies which firmly established that the main protein of wheat flour, the gluten complex, was a necessary factor in the development of small intestinal mucous membrane injury in CD patients (5).

The discovery of essential human leukocyte antigens HLA-DQ2 and -DQ8, of endoscopic intestinal biopsies, and circulating antibodies contributed to today's understanding of the pathophysiology, etiology, and diagnosis of CD. The discovery of CD-specific antibodies; endomysial autoantibodies (EMA), tissue transglutaminase autoantibodies (tTGA), and antibodies against deamidated gliadin peptides (DGP), has improved the diagnosis of CD and facilitated screening for CD, enabling estimation of true prevalence in high-risk groups as well as in the general population (6–11).

Disease definition

CD is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals. It is characterized by the presence of a variable gastrointestinal and/or extraintestinal clinical manifestations, CD specific antibodies, HLA-DQ2 or -DQ8 haplotypes and small bowel enteropathy (12,13). Individuals with CD may or may not have symptoms. The European Society of Pediatric Gastroenterology, Hepatology, and Nutrition's (ESPGHAN) guidelines define the classifications of CD as classical, atypical, asymptomatic, latent, and potential CD (**Table 1**). The classical signs and symptoms include gastrointestinal problems such as diarrhea, steatorrhea, and weight loss as a result of malabsorption (14). Atypical symptoms may be considerably more common than classic symptoms. The presence of CD-specific antibodies, and the findings of small bowel biopsies are used to distinguish between these types of CD.

Table 1. Classification of celiac disease (13,15).

Classification	Definition
CDA	Persistent tTGA positivity on at least two occasions.
Potential CD	Positive serological tests and normal intestinal biopsy.
CD serology	Detecting the presence of CD-specific antibodies as a marker for diagnosis.
Classical CD	CD presenting with signs and symptoms of malabsorption; diarrhea, steatorrhea, weight loss or growth failure.
Non-classical CD	Lack of signs of malabsorption although there are still additional symptoms.
Sub-clinical CD	The presence of extraintestinal symptoms together with gluten sensitivity enteropathy.
Asymptomatic CD	Neither classical symptoms of CD nor clinical response to GFD.
Refractory CD	Persistent symptoms and villous atrophy despite gluten-free diet.

Different methods are used to diagnose CD (clinical history, serology, HLA testing, histology), but none of them have been thought to be sufficient on their own to give a reliable diagnosis (16,17). Individuals with high levels of tTGA almost always have related small intestinal enteropathy. According to ESPGHAN guidelines (13,17), the non-biopsy approach could therefore be considered for the diagnosis of CD in pediatric patients when the following criteria are met:

- Symptoms suggestive of CD.
- Serum IgA-tTG levels greater than 10 times the upper limit of normal (ULN).
- Positive for IgA-EMA.
- Parents/patients are committed to a gluten-free diet (GFD) and understand the diagnosis.

Incidence and prevalence of celiac disease

The incidence is the rate of ”new” cases with a disease during a particular period, while prevalence, is the proportion of existing disease in defined time period (12,18). Previously, it was believed that CD was a disease that mostly afflicted people with European ancestry or who were born in Europe (14,19,20).

Epidemiological studies carried out in the past few decades in Europe, Northern Africa, the Middle East, Asia, USA, and South America revealed that CD had previously gone undiagnosed but is now recognized as a common chronic disease worldwide (16,21–29). Several studies using either intestinal biopsies and/or serological tests, identified a prevalence of CD between 1:70 and 1:200 in the majority of Western populations, or an average prevalence of about 1% (30,31). Despite the fact that CD prevalence has been increasing overtime, there is a wide discrepancy between populations, age, study period, and definition of CD (32). Although the frequency of CD differs in different ethnic groups, Caucasians are more prone to develop CD compared with other ethnicities (33).

Several factors are considered to be attributable to the increased incidence of CD. Environmental variables, such as the population's consumption of gluten, lifestyle choices, infections, and gut microbiome, are mostly to consider for this global rise in CD prevalence in people who are genetically predisposed to it (34–38). Access to simple, sensitive, and specific diagnostic methods as well as more awareness about CD may contribute to the increases (39–41).

Contrary to what was previously believed, it has lately been found that people of all ages and ethnic backgrounds also suffer from CD. However, studies demonstrate that children are more likely than adults to develop CD (21.3 vs. 12.9 per 100,000 person-years) (42,43) and females are at higher risk than males of developing CD (44–48).

Globally, the majority of CD patients are thought to be undiagnosed, with the ratio of diagnosed to undiagnosed cases estimated to be between 1:3 and 1:5 (49,50). Moreover, there is a paucity of information on the incidence and risk factors of CD in most African countries, with the exception of few countries in Northern Africa **(Fig. 1)**. Hence, in Africa, the real prevalence of CD is unknown and therefore likely to be underestimated for a number of reasons (51). Firstly, infectious burden is being reduced, public health is improved, and people's quality of life is improved via significant local and global effort. Secondly, there have been changes in dietary practices due to the impact of globalization, a lack of awareness about CD, a lack of diagnostic tools, and a shortage of skilled professionals in the region (52–54). According to the existing evidence, the CD burden will significantly increase in the near future, possibly even during the current decade. Geo-epidemiology mapping research is crucial for understanding the CD riddle in uncovered regions of the world and for locating universal treatment strategies.

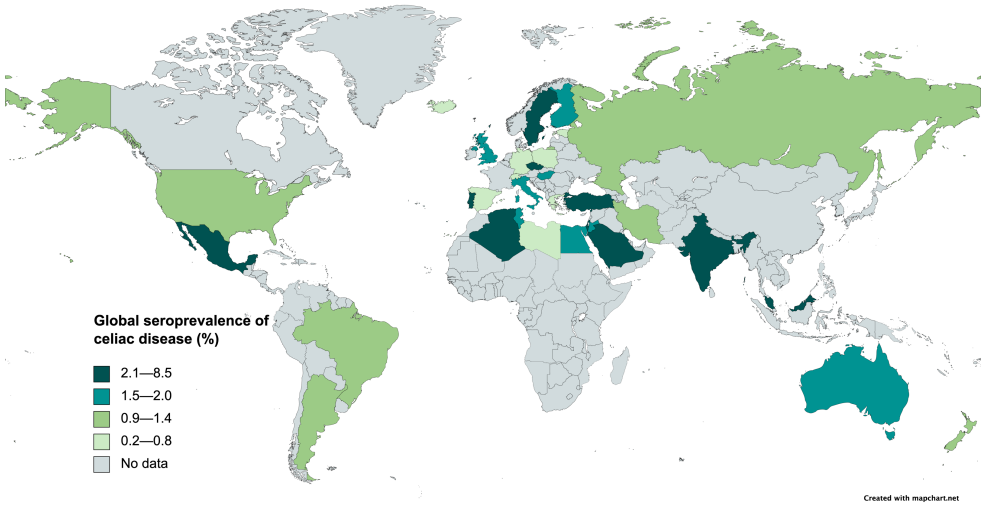

Figure 1. The map shows the global seroprevalence of CD. Higher prevalence is shown on the map by a more intense color, whilet countries without color are those where there has been no research on the prevalence of CD. Adapted from Singh et al (55,56).

Etiology of celiac disease

Immunogenetic factors

The full etiology of CD is still unclear; however, both genetic and environmental factors determine the development of CD. Several studies have shown evidence that genetics plays an important role in the pathophysiology of CD, including the high disease concordance of monozygotic twins: 70–85% compared to 10–20% among sibling pairs and family members (57–59). It is estimated that the detected genetic variability is responsible for about 54% of the heritability in CD, of which 53% is linked to the HLA-DR-DQ complex (60,61). In addition, studies revealed that the CD heritability is approximately 87%. The HLA locus has been identified as the most important genetic factor. The main genetic determinant is the HLA-DQ genes, or the genes that encode DQ2 or DQ8 haplotypes in the HLA-complex (57).

The major histocompatibility complex (MHC) is encoded by HLA, a large gene complex that spins around 4 Mbp on the short arm of chromosome 6 (6p21.3), contains more than 200 genes. The MHC encoded glycoproteins play a key role in the actions of the human immune system. It is well known that this human genetic system is the most polymorphic. The importance of MHCs in immune-mediated, autoimmune, and infectious diseases is indicated by the involvement of these

molecules in the complement system, innate and adaptive immune responses, and inflammatory control (62). There are three classes of HLA genes: class I, class II, and class III. Each region has a large number of gene loci, including expressed genes and pseudogenes. Human chromosome 6's long and short arms are depicted together with an amplified HLA region that contains the class I A, B, and C alleles as well as the class II DP, DQ, and DR alleles. HLA-DQ is the region that is usually associated with CD (63) (**Fig. 2**).

The biological role of HLA class I and class II molecules is to present peptide-based antigens to T-lymphocytes. MHC class I region contains the three classical (HLA-A, -B, and -C) and three nonclassical (HLA-E, -F and -G) loci, along with 12 non-coding or pseudogene loci (HLA-S/17, -X, -N/30, -L/92, -J/59, -W/80, -U/21, -K/70, -16, -H/54, -90 and -75). Class I proteins are expressed on the surface of all nucleated cells in different degrees. It is constituted of a single transmembrane heavy chain with three extracellular domains (α1, α2, and α3), and a β2-microglobulin light chain that anchors the heavy chain to the cell membrane. Segments α1 and α2 form a peptide-binding groove that presents viral proteins, mutant, damaged, degraded, or misfolded proteins, native proteins, and other endogenously produced peptides to CD8 + T cells **(Fig. 3)** (64,65).

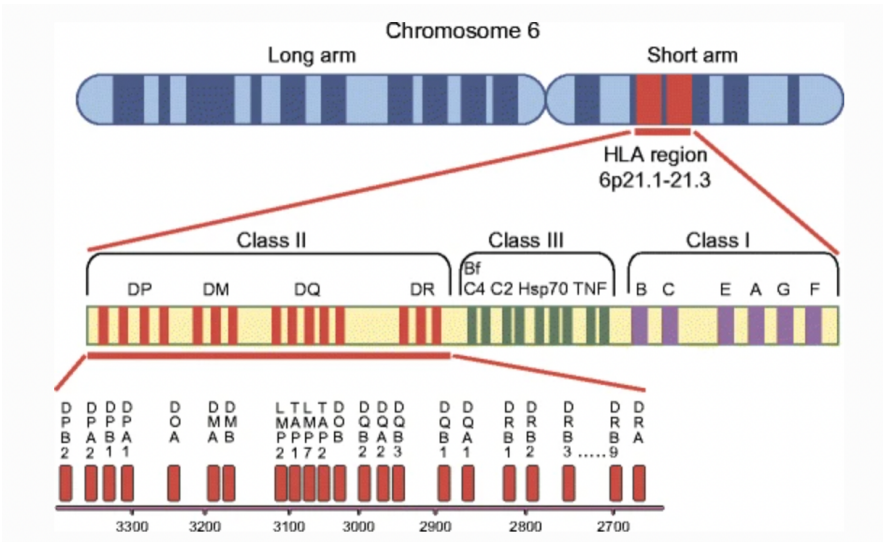

Figure 2. The HLA structure on short arm chromosome 6 with details on MHC II. Reproduced from Regnell et. al (66).

The MHC class II genes include the HLA-DP, HLA-DQ, and HLA-DR loci. HLA class II proteins are only constitutively expressed by immunologically active cells, such as B cells and other antigen-presenting cells. Different cell types may exhibit elevated expression in an inflammatory condition. Class II proteins are constituted

of alpha and beta chains and genes present in the MHC, such as HLA-DRA and HLA-DRB code for both chains. Each chain has two extracellular domains, referred to as α1 and α2 and β1 and β2, respectively. The HLA-DR the β chains are more polymorphic than the α chains. Both the α and β chains are polymorphic in HLA-DQ and -DP. The α1 and β1 segments combine to form the peptide-binding groove. MHC class II proteins present exogenously produced peptides, such as bacterial proteins, to CD4 + T lymphocytes (67–69).

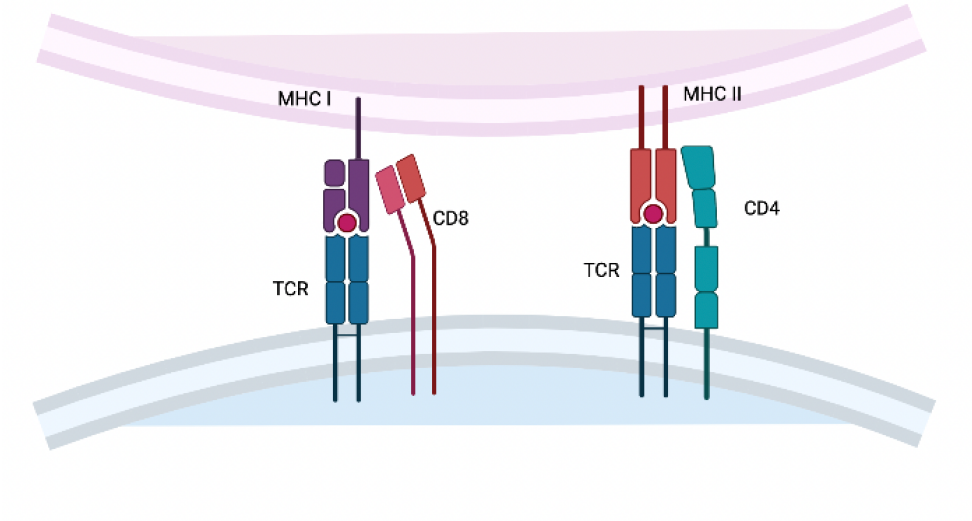

Figure 3. Molecular basis of TCR-MHC I and MHC II recognition.

The HLA class II, primarily HLA-DQ2 and -DQ8, are the most described and strongest genetic susceptibilities in CD. HLA is a necessary but not sufficient factor for the onset of CD, as evidenced by the fact that nearly all patients carry either the DQ2 or DQ8 haplotypes (70,71). On average, 35% to 40% of the world's population carries DQ2 and/or DQ8, but roughly 1% will develop CD. HLA-DQ2.5, which is encoded by the (DQA1*05:01 and DQB1*02:01) alleles, carries the highest risk for CD, particularly when two copies of the DQB1*02 allele are inherited (DQ2.5 homozygous). HLA-DQ8, encoded by the (DQA1*03 and DQB1*03:02) alleles, also confer a moderate risk. Compared to heterozygotes, there is an estimated up to five-fold greater chance of developing CD when there is homozygosity for DQ2.5 (either HLA-DQ2.5 homozygous or HLA-DQ2.5/DQ2.2) vs heterozygosity genes (72,73).

Nearly 90% or more of patients with CD carry the HLA-DQ2.5. The remaining patients who do not express HLA-DQ2.5, either display HLA-DQ2.2 (encoded by

DQA1*02:01 and DQB1*02:02 alleles of the DR7-DQ2 haplotype) or they express HLA-DQ8 (encoded by DQA1*03:01 and DQB1*03:02 alleles of DR4-DQ8 haplotypes) (74–77). Only a few patients who do not show any of these three HLA-DQ haplotypes express HLA-DQ7.5, which is often encoded by DQA1*05:05 and DQB1*03:01 alleles or the DR5-DQ7 haplotype (78). In patients with CD, the distribution of DQ2.5, DQ2.2, and DQ8 varies to some extent depending on the population (79,80).

The gene dosage effect has an impact on both the disease's phenotype and the progression of CD. HLA-DQ2.5 homozygosity may be linked to a more severe CD phenotype, which includes an earlier onset of the illness, more villous atrophy, as well as a slower rate of villous repair on a gluten-free diet (GFD) and a higher likelihood of complicated (refractory) CD. The importance of HLA-DQ2.5 homozygosity on clinical phenotype has been recognized in a CD predictive modelling tool (81–83). The risk of getting CD is higher in people who are homozygous for the HLA-DQ2.5 genotype or HLA-DQ2.2/2.5 heterozygous. HLA-DQ2.5/non-DQ2.2 heterozygotes, however, only have an intermediate risk. People who are homozygous for DQB1*02 but heterozygous for DQA1*05 (DQA1*05-DQB1*02:01/DQA1*02:01 DQB1*02:02) are at higher risk of CD. Having two copies of DQB1*02:01 predisposes to classical CD (84,85). This is also supported by experimental studies which revealed that DQ2.5 homozygotes present gluten peptides more efficiently than DQ2.5 heterozygotes and have stronger T-cell activation and pro-inflammatory responses, while heterozygotes display less pronounced activation and responses (73,86–88).

Using HLA-DQ2 and/or -DQ8 in screening for CD has a relatively weak positive predictive value; however, in uncertain situations, the lack of DQ2/DQ8 effectively rules out the diagnosis and its negative predictive value is almost 100% (12,89). On the other hand, using HLA screening is also helpful in reducing the need for repeated testing in non-risk groups (90,91). Based on the HLA-DQ gene dose effect, the HLA-DQ risk is categorized as very high, high, moderately high, low, and extremely low, if lacking any of DQ2 and DQ8 (**Fig. 4**) (74,92–96).

Non-HLA genes

Although there is strong evidence for the involvement of HLA molecules and specific genotypes in CD pathogenesis, it is not sufficient for explanations why the disease occurs in small proportion out of all carriers of the HLA-DQ2 or -DQ8 loci. Researchers studied numerous additional genetic loci outside of the HLA region and identified those that have been linked to CD. Previous studies that identified non-HLA genes which have been linked to CD, were reported (61,97). However, there is a low relative risk that appears compared with HLA gene. The majority of these non-HLA variants are single nucleotide polymorphisms (SNPs), which can be found in both the coding and non-coding regions of DNA. These are primarily located in non-coding areas, frequently within enhancers, suggesting that they have an impact

on gene expression as opposed to altering how proteins function. It was noted that the association of HLA and non-HLA favors the occurrence of CD (58,71).

Although variants found within encoding regions (e.g., MMEL1, SH2B3, IRAK1, and NCF2) play significant roles in adaptive immune response, immune cell signaling, T cell maturation, and differentiation, the pathogenic mechanisms that link to CD are not well understood (98). In addition, IL-2, which is involved in T-cell activation and proliferation, and IL-21, which promotes B-cell, T-cell, and NK-cell proliferation and other non-HLA regions, were identified as linked to CD (99). Based on the earlier studies that conducted on the link between non-HLA regions and CD, geographic variance was also reported (100).

Apart from the non-HLA gene, epigenomic studies found that CD patients' intestinal biopsies contained dysregulated miRNAs. MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression at the post-transcriptional level and play a key role in the pathogenesis of autoimmune and gastrointestinal diseases (101,102). A better understanding of miRNAs in CD will help to clarify how abnormal epigenetic regulation affects the onset and progression of the disease. Moreover, it could be used as a disease biomarkers to diagnose CD, or predict how effective a GFD will be (103).

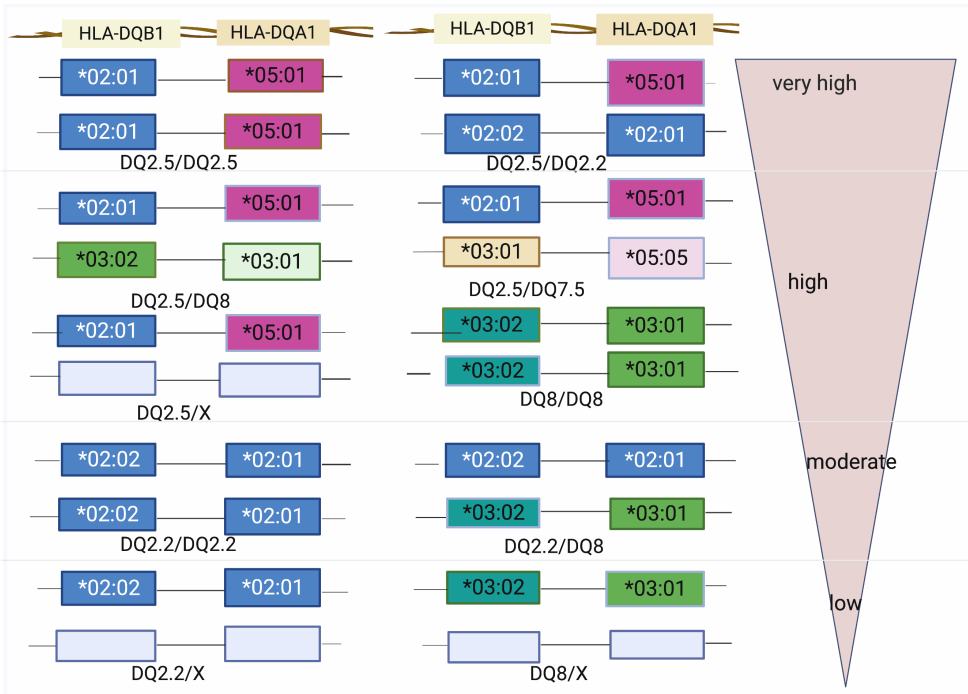

Figure 4. HLA-DQ genotype CD risk gradient per the gene dose. Illustration inspired from (92,104).

Environmental factors

Among several environmental factors, gluten has a substantial association with CD. Consuming gluten and having a genetic susceptibility are the two essential prerequisites for CD development. But only 1% of those 35% who are HLA-DQ2 and/or HLA-DQ8 positive and exposed to gluten, develop CD. A genetic predisposition is a requirement, but it is by no means the only cause of CD. CD is probably a multifactorial and polygenic disease (105–107). The development of CD is thought to be determined by a variety of environmental factors in addition to gluten (**Fig. 6**).

Strong evidence has been shown in epidemiological and clinical research indicating the involvement of environmental variables in the etiology of CD. An example of the strongest evidence is the increased incidence of autoimmune diseases in populations with stable genetic predisposing risk, for example, when individuals move from low-disease incidence to high-incidence countries, or where individuals are born in a new, high-incidence country, particularly individuals from poorer nations (108,109).

Several studies, including TEDDY, suggested that the different environmental factors contribute to the disruption of the natural state of immunological tolerance in the intestine, and lead to the development of CD. Environmental factors could account for the rise in the incidence of autoimmune diseases and allergy in the developed nations. Environmental factors that may influence CD risk, such as pre- and perinatal factors, delivery methods, parental lifestyle, infant feeding practices, quantity and quality of gluten ingested, seasonality, dietary factors, antibiotic use, childhood infections, variability in gut microbiota and the type and length of wheat dough fermentation are the most widely studied. Although for many of these external factors, the exact mechanism of action is not fully known; most of them are thought to act through disrupting the intestinal barrier, and facilitating contact between potential antigens and the immune system effector cells (36,110–113).

The "hygiene hypothesis," the "cleaner" settings present in current industrialized countries are considered as providing further evidence for an environmental role in the development of CD, in that they lead to a decline in the frequency of early childhood infections and to reduced gut microbial diversity. These changes might change the immune response and raise the risk of getting different autoimmune diseases, including CD. However, more studies are needed to determine the mechanism of how these factors can lead to an inability to tolerate gluten (114).

Dietary gluten

Gluten is the main protein used for storage in wheat, barley, and rye. Gliadin (prolamin) and glutenin (a glutelin protein), two related but distinct proteins, are the two primary proteins that make up the complex mixture known as gluten (115).

Gluten contains significant amounts of proline and glutamine residues, also referred to as prolamins (116). The various names of prolamin that are present in rye, barley, and oats, respectively, are known as secalin, hordein, and avenins. Prolamin proteins control the adaptive immune response in CD. The strongest immunogenic peptide of all gluten is gliadin. The fact that these proteins are proteolytically stable greatly enhances their immunogenicity. These proteins have unusual repeating patterns that prevent proper degradation by gastric and pancreatic enzymes (117).

The available data indicates a relationship between age of gluten consumption and CD onset. Studies discouraged introducing gluten to children either early (before 4 months of age) or late (7 months or older). Additionally, a number of studies suggested introducing gluten while the child was still breastfeeding (118–121). There is a controversy over when gluten contributes to CD. Similarly, over time researchers reported debatable results of the association of breast feeding with infant CD (122). This may indicate the need for further studies covering a wider geographic area of the world.

Traditional Ethiopian diet

The majority of Ethiopia's staple cereals include teff (*Eragrostis tef*), maize (*Zea mays*), millet (*Panicum miliaceum*), wheat (*Triticum aestivum*), sorghum (*Sorghum biocolor*), and barley (*Hordeum vulgare*), with teff predominating. Teff is an indigenous cereal consumed daily by a majority of the population in Ethiopia, is known to be gluten-free and is reported to contain more nutrients and fiber per serving than grains including wheat, rice, oats, and barley (123). Teff is available in many types. The most common types are white (nech), red (grey), and combinations of these two (sergegna).

Ethiopia is considered the place of teff origin and domestication (124). In addition to having a higher fiber content, teff has higher concentrations of potassium, magnesium, calcium, sodium, sodium chloride, iron, copper, zinc, chromium, and manganese than other cereals (125,126). Teff outperforms all other cereal grains by a wide margin due to its high concentration of a number of nutrients, the majority of which are at most readily absorbed by humans. Teff is an effective substitute for wheat flour and may be used to make a variety of baked items, including breads, cookies, and pie crusts (127). This Ethiopian national superfood is growing in popularity in many Western nations due to its nutritional characteristics, particularly its lack of gluten, and its high fiber and healthy mineral compositions (128). Teff can be part of thus be an alternative diet for the treatment of CD, a lifelong intolerance to gluten (129).

Teff is used for making a local flat bread called *injera*. Injera is an Amharic term for Ethiopian bread similar to a pan cake, made usually from teff. Injera is thin, prepared from teff flour, water, and starter (a fluid collected from previously fermented mix) after successive fermentations. The dish is a traditional, common, staple food consumed in nearly all the parts of the Ethiopia at least once a day (128). However, small portions of rice, wheat, enset, and maize-based foods are consumed as part of the staple food, along with injera, in Ethiopia.

In addition to injera, other food items made from teff have been developed. Porridges, kitta (unleavened bread), gruels (atmit or muk), and traditional alcoholic beverages such as tella and arake are some of them (130). These food items frequently naturally possess the required nutritional qualities, such as low glycaemic index, high dietary fiber content, gluten-free status, composition of balanced amino acids, healthy linoleic, significant amounts of the minerals such as calcium and iron, and contains a lot of phytochemicals. Hence, it is suggested that teff provides several health advantages when it comes to reducing anemia, diabetes, osteoporosis, and CD because of these nutritional qualities and that it is gluten-free (131–133). Due to its long-lasting health advantages, it may be found at health food stores all over the world. However, compared to wheat, it is less harvested and its production and all aspects of its cultivation are time-consuming.

A lifelong GFD is the only known treatment for CD. Gluten-free products are limited and may lead to insufficient nutrition (134). In addition, some CD patients develop clinical symptoms and mucosal damage after consumption of some gluten-free foods. Teff could be an alternative cereal for these patients (132,133).

Microbial infections

It is crucial to comprehend the complex interplay between genes, diet, and the microbiome in order to understand how CD arises and for the development of future practicable preventive or therapeutic measures (90). In people at high genetic risk of developing CD, infections may act as environmental triggers that cause or spread autoimmune or inflammatory processes and culminate in clinical CD (135–137). There is an evidence suggesting that bacteria and viruses are important environmental contributors in the development of autoimmune disease, including CD (138–141). On the other hand, growing evidence suggests that an infectious agent can counteract or mitigate autoimmunity. This mutually beneficial equilibrial relationship between two coexisting ecosystems may be represented by the protective evolutionary cross-talks between microbes/viruses and humans (142).

There are several mechanisms by which infections can contribute to the development of CD. Infections may alter gut permeability, allowing gluten peptides that are immunogenic to cross the epithelial barrier. Infections, particularly viral infections, may also change the expression of pattern recognition receptors in the mucosa, which are thought to play a crucial role in determining how the host and environment interact. Molecular mimicry, epitope spreading, bystander activation, activation of pattern recognition receptors, persistent infections, and polyclonal stimulation of B cells are other potential mechanisms (143). Through altering the gut microbiota and immune system maturation, infections are also indirectly linked to CD pathogenesis (36).

The hygiene hypothesis is supported by the fact that CD is more common in industrialized nations, affluent socioeconomic systems, and more hygienic environments. Similar to other autoimmune disorders, the number of infections are linked to CD (38,144). On the other hand, harbouring certain microbes has contributed to protect against the inflammatory response or autoimmunity. The protection provided by microbes against CD or other autoimmune disease may include reduced intestinal permeability, a shift from Th1 to Th2 immunity, induction of inflammatory immune cell apoptosis, expulsion of auto-aggressive cells from the target organ, immunosuppressive extracellular vesicles, and anti-autoreactive cell immune-regulatory proteins (38,142).

In general, numerous studies have revealed that infections caused by bacteria, viruses, or protozoa during infancy or in the later life have been linked to CD pathogenesis, though the mechanism still needs further studies. Existing published

reports have shown that some viral infections may trigger CD, while others can act as protection (36,145–149). In the same manner, a number of bacterial infections have been associated with CD induction, while others play a protective role **(Fig. 5)** (142,150–152). Developing nations like Ethiopia were previously thought to have a low prevalence of autoimmune or inflammatory diseases, despite experiencing a wide range of infectious disease. However, autoimmune disorders, like type 1 diabetes, and other non-communicable diseases are currently on the rise and may be linked to an improved way of life and a change in food types due to the influence of globalization. However, there is still insufficient data linking microbes to autoimmune diseases including CD.

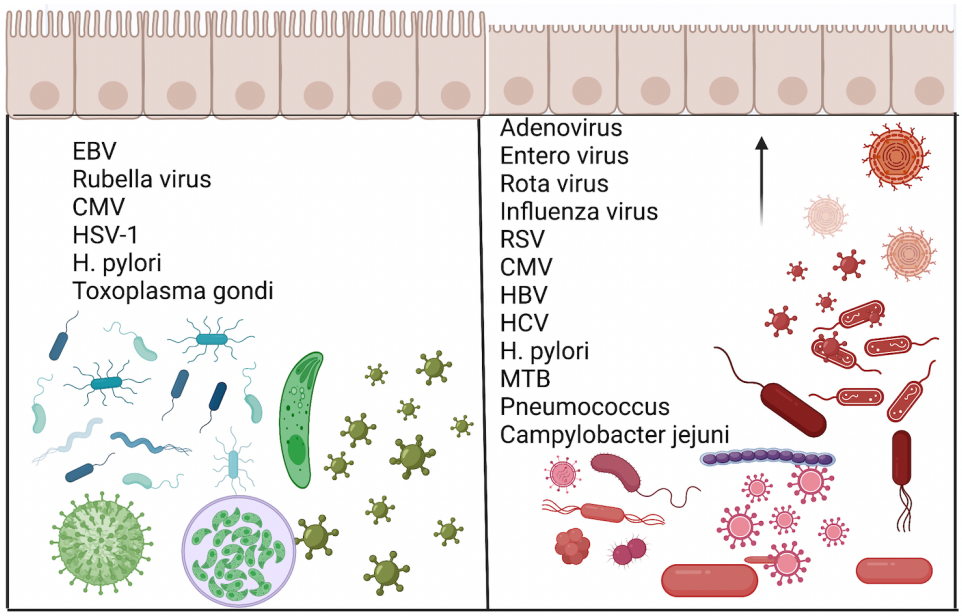

Figure 5. Infectious agents that were suggested trigger (right side) or protect against (left side) CD. Inspired from (142).

Helicobacter pylori (H. pylori) and *Mycobacterium tuberculosis* (*MTB)* are two of the most prevalent bacterial infections that infect humans (153–155). These two chronic infections are more prevalent in areas with high densities of population, in developing countries, and in countries with low socioeconomic wealth (156).

H. pylori has colonized human gastric mucosa, which mostly injures the stomach. *H. pylori* is well adapted to existing in the human stomach for the lifetime of its host. *H. pylori* infection rates can reach up to 50% worldwide, and childhood infection rates are also high. *H. pylori* prevalence varies between countries, and the highest is reported in developing countries (153,157,158). Previous studies have

demonstrated an association between *H. pylori* and duodenal intraepithelial lymphocytosis (IELs), which is compatible with the early intestinal mucosa damage in CD. On the other hand, less significant villous atrophy was reported in *H. pylori* infected CD patients. The association between *H. pylori* infections and CD is still debatable. Furthermore, it is still unclear how *H. pylori* infections may influence CD (152,159).

The *MTB* complex causes tuberculosis, often known as TB. *MTB* is one of the most prevalent human infections, affecting roughly one-third of the world's population. Ethiopia is one of the highest TB burdened countries. According to a WHO report, Ethiopia is in fact one of the world's 30 countries with the highest rates of TB, TB-HIV, and multidrug resistance (160). MTB persists in the host for long periods of time without causing any symptoms and is able to switch between an infectious phase that is clinically evident and an asymptomatic non-infectious phase. The term "latent TB infection" (LTBI) refers to a condition where there are ongoing immune responses to MTB antigens, but no sign of any clinical symptoms (161,162).

Approximately about 1.7 billion individuals have LTBI globally, with a global prevalence of 24.8% (163,164). Several prior studies in Ethiopia on the LTBI confirmed that the pooled prevalence reaches about 51% in the country and varies by regions (165–167). In tuberculosis-endemic regions, rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases are frequent. Previous research suggested that these autoimmune diseases may be brought on by the cross-reactive epitope that TB reactive T-cells may mislead for self-antigen. However, the association between TB exposure and CD is not completely understood, and therefore further studies are warranted (168,169).

Socioeconomic factors linked to multiple infections and lifestyles are considered to be associated with the incidence of CD. This is linked to diets, gut microbiota, burden of infection, and general hygiene conditions. This is also another theory suggesting that microbes may protect against autoimmune diseases as described in the “hygiene hypothesis” theory (38,109).

Change in the microbiota and antibiotic use are additional factors associated with CD. It has been documented that the gut microbiome of people with CD and healthy controls differs (170,171). It is now known that the gut microbiota is essential for the normal development and functioning of the human body, particularly for the priming and maturation of the adaptive immune system (172). In low- and middle-income countries, a high number of antibiotic prescriptions are given to children between the ages of birth and five years (173). The use of antibiotics may negatively affect the gut microbiota in a number of ways, including decreased species diversity and altered metabolic processes. Antibiotics can change the composition and functions of the microbiota, dysbiosis, and have long-lasting negative consequences on the host. Even, prenatal and peripartum antibiotics use can affect infants' resistive profiles and gut microbial colonization, upsetting the natural balance between the

diverse species of gut microbiota (174,175). The immune system is being trained to fight off infections during this infancy and later life, and it is the time when microbial colonization takes place. It has been demonstrated that any interruption to microbial colonization affects immune maturation because of this co-developmental process. These impacts on the ecological balance of the microbiota, cause a number of gastrointestinal, immune disorders and have an impact on the mucosal barrier, immune system development, and oral tolerance (36,172,176–180). The majority of those environmental factors' roles in CD are not fully understood. The association between environmental factors and autoimmune diseases, including CD in particular, in the developing countries like Ethiopia is not well studied.

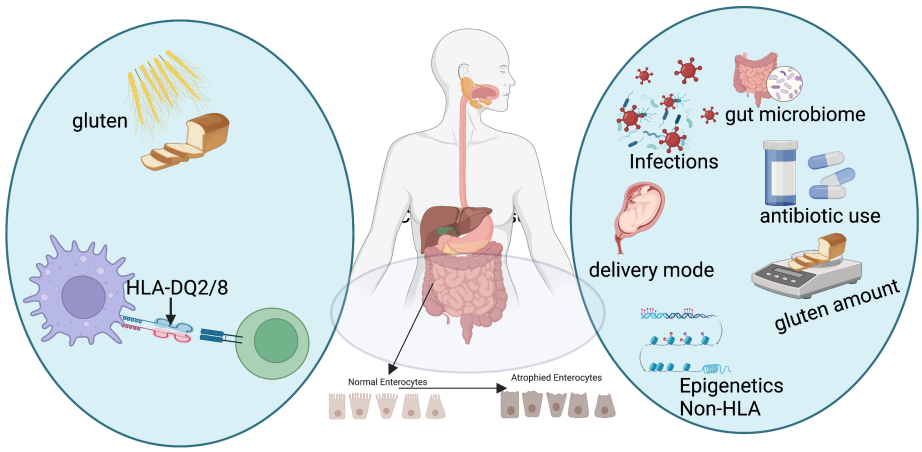

Figure 6. Necessary risk factors (left) and potential triggers (right) of CD.

Pathogenesis of celiac disease

Gluten peptides reach the lamina propria of the small intestine via transcellular or paracellular or enhanced intestinal tight junction permeability. The enzyme tissue transglutaminase (tTG), a dominant autoantigen, deamidates gliadin molecules, altering or deamidating the original peptide (181). The expression of tTG is induced as a result of tissue damage or inflammation primarily for extracellular matrix formation or damaged tissue repair (182). Deamidation enhances the gliadin's affinity for the HLA-DQ2 and/or -DQ8 class II molecules and increase the immune response. Deamidated peptides uptake and are presented to CD4+ T-lymphocytes by antigen-presenting cells that express HLA-DQ2 and/or -DQ8 molecules in the lamina propria. Dendritic cells and macrophages are the antigen presenting immune cells that express HLA-DQ at the highest levels in healthy duodenal mucosa (183–185).

HLA-DQ-2 and/or -DQ8 bound gluten peptides and T cell receptors leading to the activation of gluten-specific CD4+ T cells, which induces cytokine release (IFN-γ, IL-2, and IL-21) and T cell clonal proliferation. Activated CD4+ T cells and released proinflammatory cytokines lead the activation of tTG and gluten-specific B cells in differentiating into plasma cells that produce antibodies. Plasma cells and activated T cells that are specific for gluten then go to the lamina propria and serve as effector cells. The activated CD4+ T cells supply intraepithelial cytotoxic T lymphocytes (IE-CTLs) with signals (186–188). Importantly, people with CD continue to have gluten-specific CD4+ T cell clone types for decades (189). IELs are a diverse population of T cells that monitor the mucosal barrier and have the ability to act as effectors without antigen specific priming; they interact directly with intestinal epithelial cells and, when necessary, can trigger apoptosis. IELs are not driven by TCR-dependent antigens in the mucosa of CD patients. The expression of NKG2D, an activating receptor on the surface of IELs, rises in CD in response to IL-15. Unconventional stress-induced HLA class I molecule MICA, whose expression is upregulated in CD, is the primary ligand for NKG2D produced on intestinal epithelial cells. The intestinal epithelial cell death along with apoptotic pathway are both directly induced by the interaction of NKG2D and MICA. Activated IE-CTLs kill the epithelial cells resulting in villous blunting. The development of small intestine mucosal villous atrophy is influenced by these mechanisms (**Fig. 7**) (190–192).

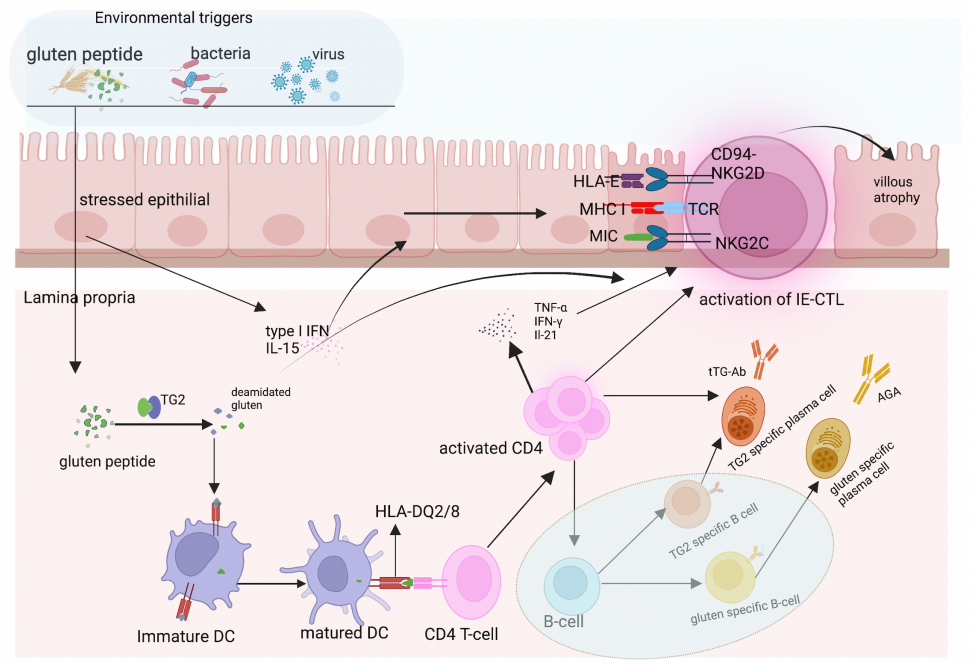

Figure 7. Pathogenesis of CD. Inspired from (77,105,188).

Diagnosis of celiac disease

Serology

In recent decades, CD serological testing has evolved significantly. The three antibodies that are most frequently used are tTGA, EMA, and DGP. These antibodies are present at the time of diagnosis, together with a characteristic small intestine mucosa, and disappear as a clinical response to a GFD and return after gluten challenge. Even though EMA have specificity, especially in adult patients, false-positive results might occur in children. According to the American College of Gastroenterology, IgA-tTG is the best single antibody suggested for identifying CD in individuals older than two years of age, but with the recommendation of using multiple CD tests in combination rather than IgA-tTG alone to enhance the sensitivity and specificity (193–195).

Serology-based techniques are currently among the most frequently used in a pediatric CD diagnosis (196). Serology may be able to detect CD in its early stages, before serious intestinal damage appears. There is general agreement that detecting IgA-tTG is the best method for screening of CD, due to its high sensitivity (up to

97%), whereas EMA is suggested as a confirmatory test in tTGA-positive cases due to its higher specificity (near 100%) (**Table 2**) (197–201).

Table 2. Sensitivity and specificity of serology tests for CD (19,194,202)

Antibody	Sensitivity	Specificity	Comment
IgA-EMA	95% (86–100)	99% (97–100)	The most specific test. To confirm tTG-IgA positive findings. More time consuming, expensive, observer dependent.
IgA-tTG	98% (78–100%)	98% (90–100%)	The most sensitive test. High values are predictive of mucosal atrophy. The best test for the initial screening of the patients. Lack of standardization.
IgG-tTG	70% (45–95%)	95% (94–100%)	Often positive in IgA deficient patients. Useful in IgA deficient patients. Variable diagnostic accuracy of commercial kits.
IgG-DGP	80% (70–95%)	98% (95–100%)	Often positive in IgA-tTG negative children. Recommended in children and in IgA deficient patients. Less accurate compared with IgA-tTG.
IgA-DGP	88% (74–100%)	90% (80–95%)	Often positive in children. Less accurate with respect to IgA-tTG and IgG-DGP.

EMA, endomysial autoantibodies; tTG, tissue transglutaminase; DPG, deamidated gliadin peptides.

Histopathology

The histological characteristics of the intestinal mucosal features in CD have been described by Michael Marsh. In order to describe the histopathologic alterations in CD patients, he developed a grading system published in 1992. Based on his descriptions, three distinctive and dynamically interrelated types of mucosal changes, infiltrative, hyperplastic, and destructive lesions, are classified into four consecutive stages of mucosal destruction (Marsh stage 0–4). In Marsh's classification, stage 0 refers to intestinal mucosa that is histologically normal and has less than 30 intraepithelial lymphocytes per 100 epithelial cells. Patients in this category may exhibit clinical silence and require only serological criteria for diagnosis (203). Stage 1 is described as having an increased number of IELs and a normal villous to crypt ratio and normal architectural mucosa features. Over 30 IELs per 100 epithelial cells are present in the mucosa. This finding is not specific but does not rule out CD. Stage 2 is characterized by an increased number of intraepithelial lymphocytes with greater than 30 IELs per 100 epithelial cells, lymphocyte infiltration of the lamina propria, and larger, hyperplastic crypts but normal villi. Stage 3 is characterized by the partial or complete blunting of the mucosal villi, while stage 4 is characterized by atrophic lesions, in which the mucosa is flattened and exhibits mild inflammation and crypt hypoplasia. The intestinal lesions of the Marsh 2–3 type is considered to be definitive diagnosis of CD (204–206).

According to the revised ESPGHAN criteria for CD, the absolute necessity of an intestinal biopsy for the diagnosis has been questioned in light of progresses in serological diagnosis. It is not necessary to consider biopsy to diagnose pediatric CD in cases where tTGA levels are more than 10 times the upper limit. On the other hand, the biopsy is still regarded as the gold standard by the majority of physicians (12,207,208).

Currently, the revised guidelines by ESPGHAN suggest a combination of approaches. The diagnosis for CD is based on IgA-tTG as the first strategy due to its high sensitivity. The sequential screening algorithm of serology tests accompanied by biopsy are the preferred strategy for effective diagnosis of CD (17,193,209,210).

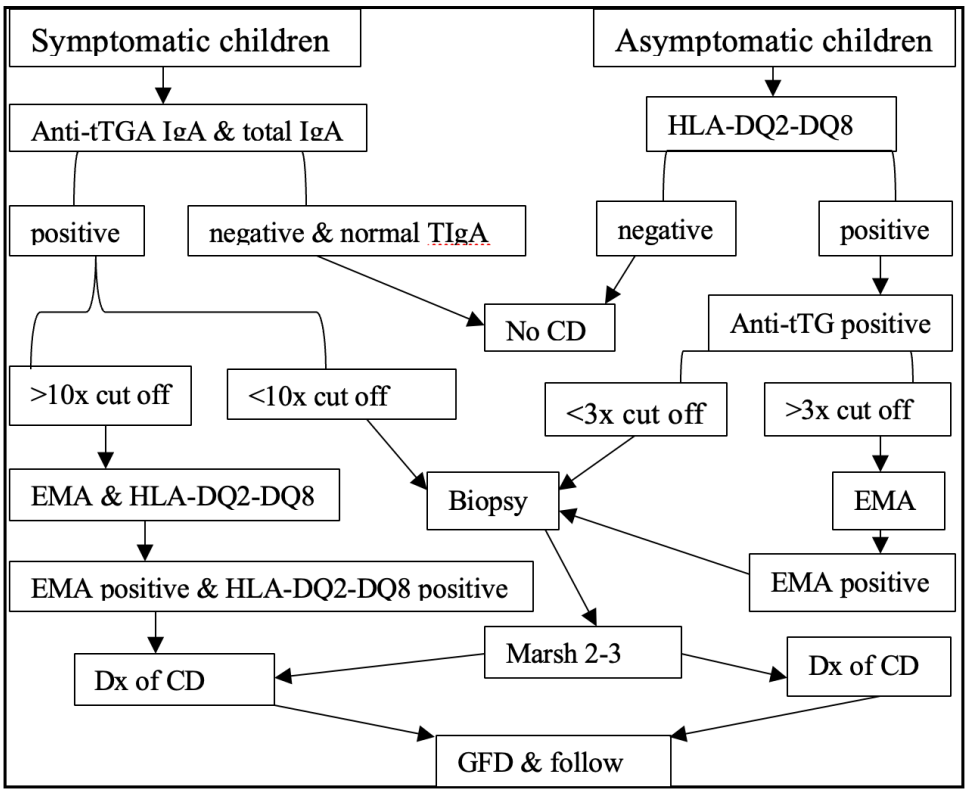

Figure 8. Algorithm proposed by the ESPGHAN to diagnose CD in symptomatic and asymptomatic children/adolescents (adopted from ESPGHAN, 2020). Dx: diagnosis.

The current thesis was motivated by the paucity of information on CD in Ethiopia and other East African countries as previously described (**Fig. 1**) (211). However, there were only two case reports from Ethiopia regarding CD up until this point,

which provide clues as to its existence and motivates further research (212,213). To fill this gap of knowledge, a screening was initiated to include two Ethiopian cohorts to estimate the prevalence of CDA using two tTGA assays with high diagnostic sensitivity and specificity, respectively (17,200).

Aims

The overall aim was to determine whether infections and dietary factors are associated with the incidence of CDA by 4 years of age in an HLA genotyped Ethiopian population.

The specific aims of the thesis were as follows:

- To determine the prevalence of CDA in Ethiopian women from the general population (**Study I**).
- To determine the distribution of HLA haplotypes and genotypes in the Ethiopian birth cohort and compare it with a Swedish birth cohort (**Study II**).
- To describe recent infant feeding practices in Ethiopian children under two years of age and how they associate with sociodemographic characteristics (**Study III).**
- To determine the incidence of CDA and its associations with LMTB and *H. pylori* infections in Ethiopian children by age 4 years in an HLA genotyped cohort study (**Study IV**)**.**

Overview of the studies

The research questions, study population, and study design are summarized below (**Fig. 9**). Cross-sectional data from a repository and retrospective data of women were used for Paper I, while longitudinal data from participants in the birth cohort were used in papers II through IV.

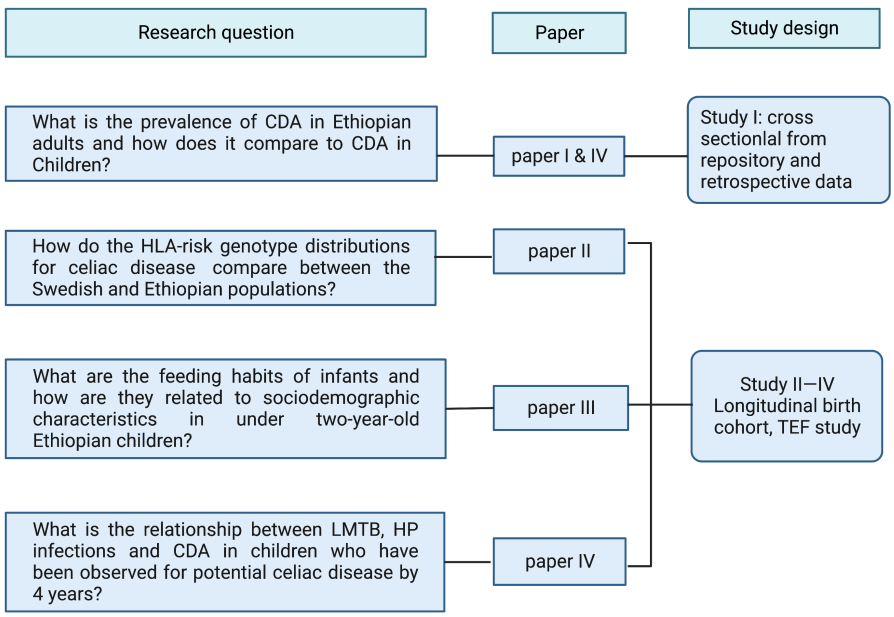

Figure 9. Overview of the thesis.

Subjects and Methods

Study populations

The thesis is based on the data from Ethiopians who were selected from the general population and enrolled to the "Traditional Ethiopian Food (TEF)" birth cohort. Out of 1389 invited, 1256 mother–child pairs who gave their agreement to participate in the study were recruited and followed for four years between 2018 and 2022. Out of this cohort, 1046 child–mother pairs completed the required number of follow-ups and were considered in the analysis. Baseline data on the mothers of the child participants were collected from the parallel cohort at their first recruitment during their antenatal care follow up. This was a cohort which had followed about 2000 women since 2015 before, and after giving birth, in order to investigate the relationship between TB infections and pregnancy outcomes, in parallel with the TEF birth cohort. Baseline data on child participants were collected by six weeks after delivery. The participants were then followed with the pre-planned timeline by the ages of 9, 18, 24, 36 and 48 months. Child–mother pairs which satisfied the completion criteria were included in the analysis for papers II to IV. For Study I, 1942 pregnant women whose serum samples were provided from the Adama Public Health Research and Referral Laboratory, were included.

The cohort study was conducted in the city of Adama at Adama Health Center, Adama Medical College Hospital, and Geda Health Center. Adama Public Health Research and Referral Laboratory was used as the center for store samples and screening. Adama is located directly in the west of the great Rift Valley in the central Oromia regional state of Ethiopia, at about 100 kilometers southeast of Addis Ababa. In Adama, there are about 600,000 inhabitants. The city serves as a central transportation hub for the surrounding area as it is situated on the road that connects the eastern city of Dire Dawa, and Djobouti, with Ethiopia's capital, Addis Ababa (**Fig. 10**).

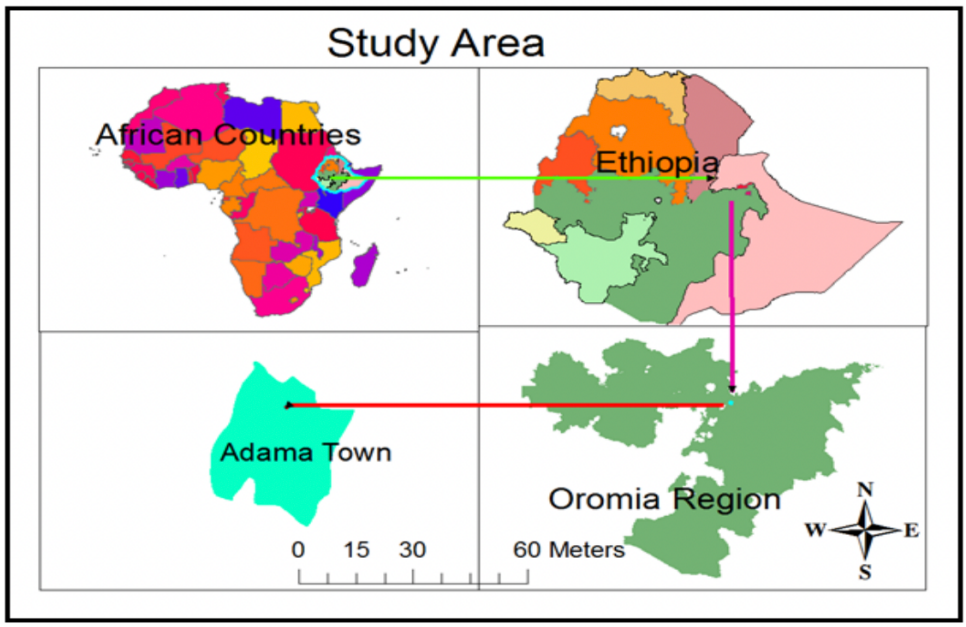

Figure 10. Geographic location of the research site. Reproduced from Siraj et al (214).

Study design

Study I was a cross-sectional study using retrospective data to explore the prevalence of CDA in an Ethiopian adult women population. Based on the prerecorded data, a total of 2000 women were randomly selected from 10 health facilities of whom 1942 with sufficient serum samples in the repository and had complete information were used in the analysis (**Fig. 11**). Although we selected this study population based on the samples and data that were already available, the study population is in line with prior researches suggesting that women are more likely than men to get CD (24,44,46,215–219).

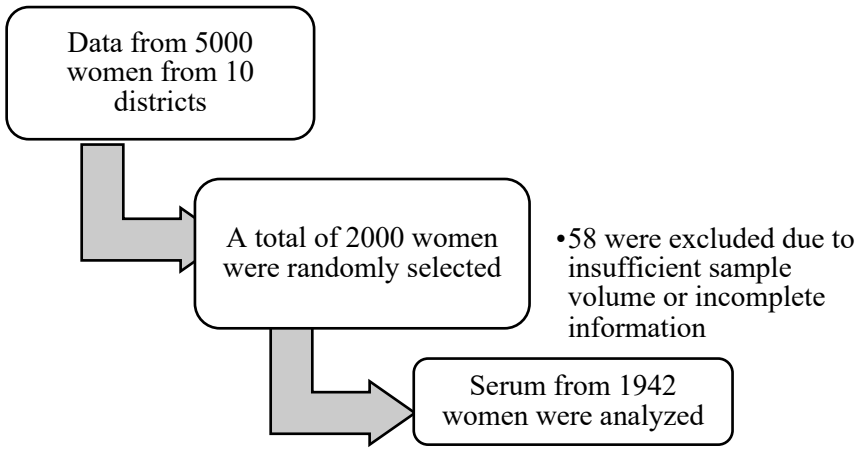

Figure 11. Flow chart of the study population in Study I.

Study II investigated the genetic propensity of CD by HLA-genotyping of an Ethiopian birth cohort and compared it with 2000 previously HLA genotyped Swedish children (220,221). A total of 1389 children were invited to participate, of whom 1256 accepted. Blood samples were successfully collected from, and genotyped in, 1193 children (**Fig. 12**).

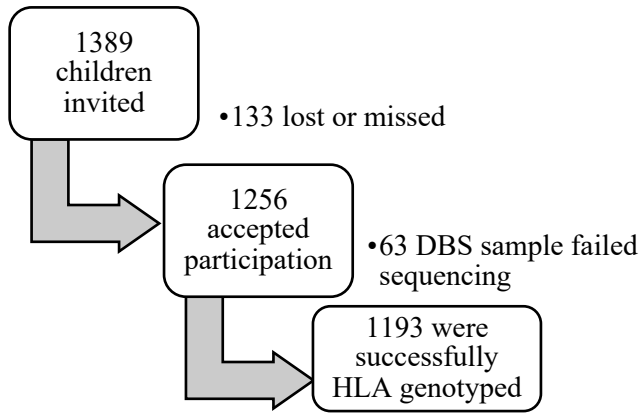

Figure 12. Flow chart of the study population in Study II.

Study III included data from 1054 children up to age 24 months. Baseline information on their mothers was obtained during pregnancy in the TB pregnancy study (165). The children's baseline data collection began six weeks after delivery, when mothers were brought up to the study site for 45-day immunization. The birth cohort of this study had subsequent follow-up at 9, 18 and 24 months (**Fig. 13**).

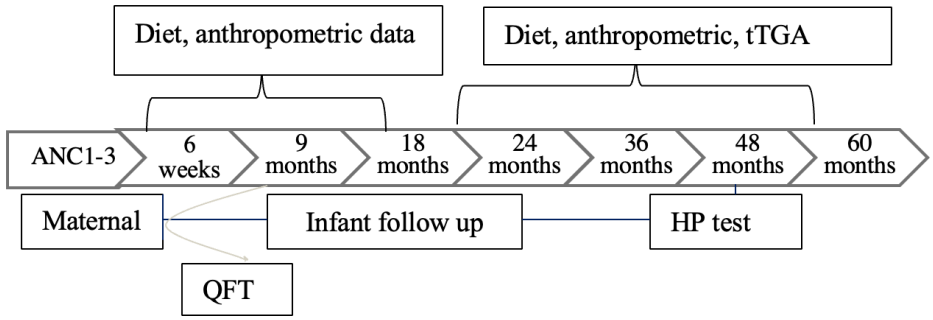

Figure 13. Flow chart of the study population of Study III.

Study IV determined the incidence of CDA and its association with TB and *H. pylori* infections in HLA-genotyped children. Blood samples were taken from the child starting at the age of two years and continuing until the child was four years old. The first screening with IgA-tTG was performed using ELISA method at the Adama Public Health Research and Referral Laboratory. IgA-tTG positive samples were shipped to the CRC in Malmö, Sweden for confirmation using RBA. To investigate the association of CDA with TB exposure and child *H. pylori* infection, 752 children whose mother had a QFT finding for latent tuberculosis were involved (**Fig. 14**).

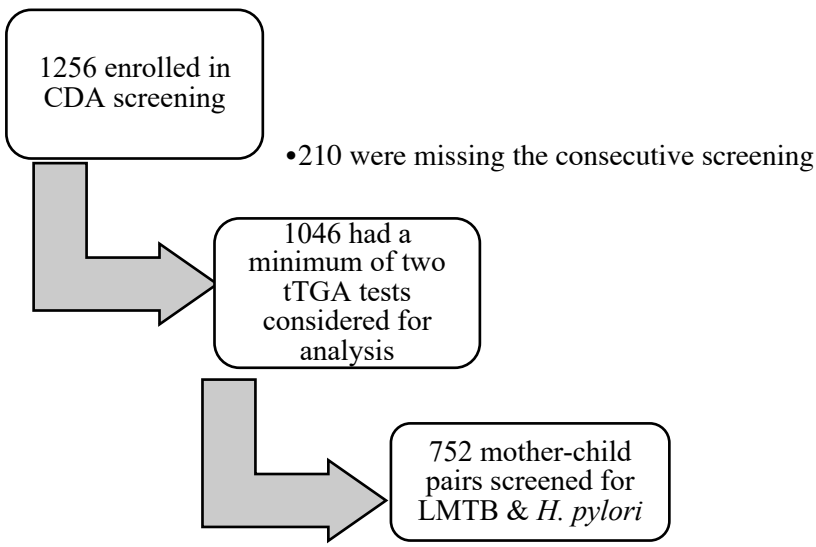

Figure 14. Flow chart of the study population in Study IV.

Laboratory methods

Tissue transglutaminase autoantibody assays

Both ELISA and RBA were used in the present study to detect tTGA. The ELISA technique was done as the initial screening and RBA was used to confirm IgA-tTG positives from the birth cohort. Both IgA-tTG and IgG-tTG were assessed in the RBA as previously noted (222) for the screening of women in Study I. *In vitro* transcription and translation were employed to synthesize human tTG in the presence of ^{35}S-methionine (Perkin Elmer LifeSciences Inc, Boston, MA). IgA-tTG antigen/antibody complexes were isolated using 10% goat anti-human IgA agarose (Sigma, St. Louis, MO), and IgG-tTG antigen/antibody complexes were separated using 30% protein A sepharose conjugate 4B (Zymed Laboratories Inc., San Francisco, CA) (223).

The ELISA IgA-neoepitopes against tTG were used as described previously (AESKULISA tTg-A New generation, AESKU. DIAGNOSTICS GmbH & Co. KG Mikroforum Ring 2 55234 Wendelsheim, Germany) (224–226). The levels of tTGA determined by both assays were expressed as U/mL and calculated from standard curves based on the manufacturer's guideline. Findings above the highest calibrator range were diluted as appropriate and re-assayed as described previously (222,227,228). Antibody levels were interpreted based on the cutoff level of the manufacturer's guideline as positive and negative.

HLA genotyping

Direct blood spot (DBS) samples collected from the study participant during the early follow-up by either the 9 or 18 months visit were shipped for HLA typing at the Immunogenetics Laboratory at the University of Turku, Finland. HLA-DQB1 genotyping was carried out by polymerase chain reaction (PCR) followed by hybridization with allele specific probes. After a "full-house" HLA-DQB1 typing presence of DQA1*02:01, DQA1*03, and DQA1*05, they were genotyped when informative for deducing common haplotypes as described in the previous studies (229,230). Previously HLA-genotyped DiPiS cohort was used as reference (231).

Screening for LMTB and *H. pylori* infections

Data on LMTB and related sociodemographic factors were collected during postnatal care after delivery from mothers in the integrated Pregnancy-TB cohort. LMTB was confirmed by measuring IFN-γ using ELISA according to the QFT-Plus protocol (165). *H. pylori* infection was examined in the leftover serum samples collected at an average age of four years and maintained in the repository. All

samples were analyzed for the anti-*H. pylori* antibody by using the commercially available ELISA kit (IBL International GmbH, Hamburg, Germany) method as previously described (232). The analysis included 752 children for data on the colonization of *H. pylori,* and mother–child pairs for LMTB exposure.

Study outcome

The primary study outcome was CDA, which was defined as being persistently confirmed tTGA positive in two consecutive measurements. The secondary outcome was CD, which was defined as an average level of tTGA ≥ 10 x ULN.

Food questionnaire data

At the research clinic, a parent or legal guardian of the study participants was interviewed using standardized questionnaires addressing details on infant feeding practices. The questionnaire, which was written in English and subsequently translated into the local languages, was used to gather information about breastfeeding duration, infant formula feeding and age at introduction to solid foods. A 24-hour diet recall was a part of the interview. Baseline maternal sociodemographic data were acquired for the pregnant tuberculosis cohort during the mother's antenatal care (ANC) follow-up and at the six-week post-delivery visit (233,234).

Anthropometrics

Anthropometric measurements were done by trained nurses according to the WHO manual. Children were measured while lying down without wearing shoes using a calibrated length board for height. The weight of the children was measured with lightweight clothing and recorded to the nearest 0.1 kg using an infant weight scale. A Z-score <-2 SD in height-for-age was defined as stunted, <-2 SD in weight-for-height was defined as wasted, <-2SD in weight-for-age was defined as underweight, and <-2 SD in body mass index (BMI) for age was defined as low BMI (235).

Statistical methods

Study data were collected and managed using REDCap (Research Electronic Data Capture), a secure, web-based software platform designed to support data capture for research studies (236,237). All data were analyzed by using Statistical Package for the Social Sciences (SPSS) software for Windows (version 25 & 27; SPSS Inc. Chicago, IL). Anthropometric data were converted to z-score by using WHO Anthro (Version 3.2.2) prior to being analyzed by SPSS. For graphs in all studies, graphic pad prism and Microsoft Office Excel were used.

The distribution of the parameters was evaluated using the Shapiro–Wilk and Kolmogorov–Smirnov test. In normally distributed quantitative variables, student's t test, one way ANOVA, Fisher's t test, and Spearman correlations were used to test the associations. For non-normal distributions, Kruskal-Wallis or Mann-Whitney U test were used to test the association. The binary logistic regression analysis was used for comparisons of dependent and independent variables (Study III and Study IV). Bonferroni's test was used for multiple comparisons (Study II). The Chi-square test was used for comparison of categorical data. Odds ratio was used in Study III and Study IV. For all studies, a p-value < 0.05 was considered statistically significant.

Ethical approval

All the studies were conducted according to good clinical practice and based on the Declaration of Helsinki, a statement describing ethical standards for medical research involving human subjects, including study of identifiable human material and data (238). The TEF study was approved by the institutional review board of Armauer Hansen Research Institute (P028/17), National Research Ethics Committee of Ethiopia (Ref. No. 3.10/16/2018), and the ethical committee of Lund University (Pro.No.2017/3). A supportive letter to Adama Health Offices was obtained from Oromia national regional state health offices of Ethiopia. A support letter to each health facility was obtained from Adama Health Offices together with the approval letter. Written informed consent was obtained from mothers or legal guardians of children in each study before study participants were enrolled. All samples and related questionnaires were processed and kept by codes without reference to personnel identities. The DiPiS study was approved by the ethical committee of Lund University (LU 490-99). Under the guidelines set forth by the Ethiopian Ministry of Health, blood samples were taken by trained healthcare workers participating in routine medical deliveries. The screening results were kept confidential and were only shared with the pediatricians in the study team for the follow-up. All children who tested positive for tTGA in at least two consecutive

measures, were taken to the pediatric clinic for further follow-up and consultation with a GI expert.

Results

Prevalence of CDA in Ethiopian women (Paper I)

Of the 1942 women included in this study, only one was tested positive for both IgA-tTG and IgG-tTG, giving an overall prevalence of CDA estimated at 0.05% (1 in 2000) in the general female population. The median age of the study participants was 20 by the time of data collection, and three-fourth of them were from rural districts. In this study, we defined CDA as the presence of both IgA-tTG and IgG-tTG that was confirmed by using RBA. The individual antibody screening revealed that three (0.2%) tested IgG-tTG positive alone and one (0.05%) of them tested positive for IgA-tTG only.

Distribution of HLA-DQ2 and -DQ8 in Ethiopian children (Paper II)

Out of 1256 children screened for HLA-DQ, 1193 were fully sequenced for HLA-DQ of whom 15.3% carried HLA-DQ2.2 (HLA-DQA1*02:01-DQB1*02), 12% DQ7.5 (HLA-DQA1*05-DQB1*03:01), 9.7% DQ2.5 (HLA-DQA1*05:01-DQB1*02:01), 6.8% DQ8 (DQA1*03:01-DQB1*03:02) and 4.7% DQ2.3 (HLA-DQA1*03-DQB1*02).

The comparison of HLA-DQ distribution between Ethiopian and Swedish children showed that the Swedish children had a higher frequency of DQ2.5/DQ2.5 (12.8% vs. 9.7%) and DQ8 (13.1% vs. 6.8%) compared with the Ethiopian children, respectively ($p<0.05$). On the other hand, the DQ2.2 (15.3% vs. 6.7%), DQ7.5 (12.0% vs. 9.1%) and DQ2.3 (4.7% vs. 0.1%) were more frequent among Ethiopian children than the Swedish references, respectively ($p<0.05$).

The DQ2.5/DQ8 (1.2% vs. 3.5%) and DQ2.5/X (12.1% vs. 17.1%) genotypes were significantly less frequent in Ethiopian children than in Swedish children, respectively ($p<0.05$). On the other hand, DQ2.2/DQ2.2 (3.3% vs. 0.2%) and DQ2.5_trans (3.6% vs. 1.3%) were found more frequent in Ethiopian children compared with the Swedish references, respectively ($p<0.05$). Overall, 51.6% (95% CI, 44.7–55.9) of the Ethiopian children in the study had HLA-DQ CD risk genotype

and, when compared with the Swedish reference children, showed no difference (51.6% vs. 55.9%) (**Fig. 15**).

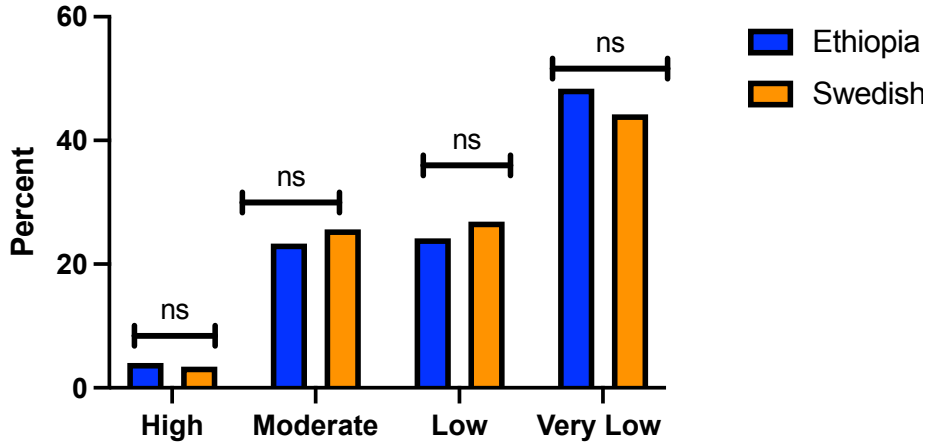

Figure 15. Comparison of merged HLA-DQ CDA risk genotypes between Ethiopian and Swedish children. ns: not significant.

Complementary feeding habits in children under the age of 2 years (Paper III)

Study results showed that of the 1054 children enrolled, 84.7% (95% CI, 82.5, 86.8) were introduced to supplemental foods by the age of six months, as recommended by WHO (239). The majority (86.1%) of infants up to six months of age were breastfed and only a very small percentage of children (3.5%) were given supplemental foods before six months old. One of the most common foods introduced by the study population who initiated before the recommended period is an infant formula made with cow's milk. More than half of the study participants were consuming teff (a gluten-free grain from Ethiopia) and wheat-based foods more than two times per day (**Fig. 16**). These dishes are based on a homemade mixture known as "mitin," which is made of a variety of foods such as teff, corn, wheat, and barley as well as sorghum and maize. Gruel, porridge, fetfet (shredded injera with butter or sauce), kitta (traditional bread made from wheat flour without fermentation), chechebsa (from teff flower and butter), and bread have been the most popular ways to serve the children from those preparations.

Regarding factors influencing the timing of the introduction of supplemental meals, our study revealed maternal age (younger mothers) and employment (women who work from home), when compared to the other categories, were better at initiating complementary food at the appropriate time.

Based on anthropometric measurements, the growth of the children was also studied. In comparison to the national nutritional survey and prior studies in the country, the study found a low percentage of children to have wasting (4.5%), stunting (16.9%), were underweight (2.5%), or had low BMI (6.3%). The variances may be the result of selection bias, socioeconomic variation, research design, or differences in feeding culture (240,241).

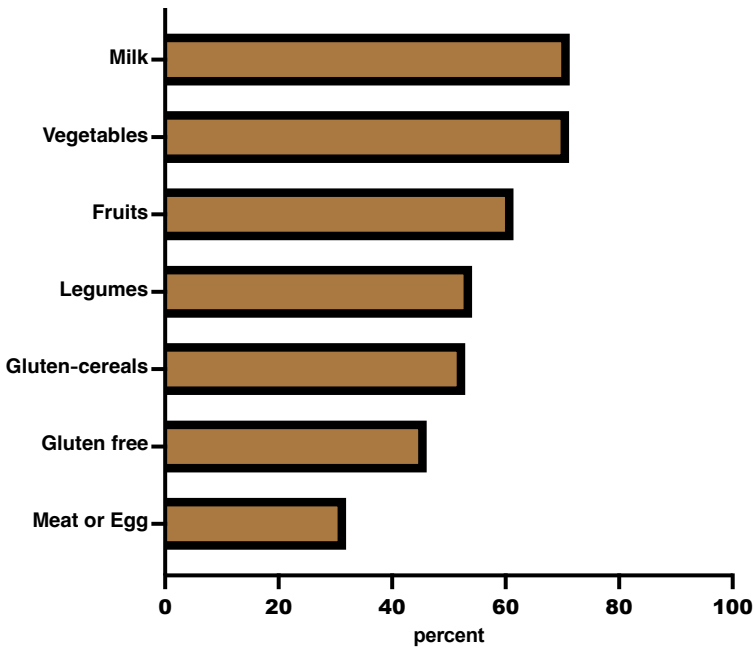

Figure 16. Type of complementary solid foods commonly introduced in early childhood.

Incidence of celiac disease autoimmunity and associations with maternal tuberculosis and paediatric *helicobacter pylori* infections (Paper IV)

Screening identified 38 (3.6%) children out of 1046 to be tTGA positive. Of those, only 10 (1.0%) were confirmed positive by RBA, of whom six children were persistently confirmed as tTGA positive, resulting in a CDA prevalence of 0.6% (95% CI; 0.15–1.23%) incidence of 1.2 per 1000 person-years.

The prevalence of CDA was 12 times higher in children under five years of age compared with the adult Ethiopian women population (0.6% vs. 0.05%) (Paper I), but lower when compared to the global pooled seroprevalence of CD (0.6% vs. 1.4%).

Five of the six children with CDA positive results carried the HLA-DQ2 and/or HLA-DQ8 risk haplotypes. However, the findings of the HLA test for one of these children were not available. **Table 3** summarizes the variation of IgA-tTG concentrations among the six children who tested CDA positive.

Table 3. Levels of IgA-tTG in children with celiac disease autoimmunity (CDA).

Child	Gender	HLA	Age: 24 months IgA-tTG (U/mL)	Age: 36 months IgA-tTG (U/mL)	Age: 48 months IgA-tTG (U/mL)
#1	Female	DQ2.3/X	68.90	73.60	67.69
#2	Male	DQ2.5trans	0.00	762.91	77.80
#3	Male	NA	139.00	95.55	116.60
#4	Female	DQ2.5trans	380.90	55.80	0.00
#5	Male	DQ2.5/X	0.00	67.50	57.40
#6	Female	DQ2.3/DQ8	70.40	98.80	3.66

Foot note: Cut off level for IgA-tTG ELISA is 18 IU/ml; NA: Not available.

Samples that had tested positive or borderline during the initial ELISA screen were repeated by RBA. The correlation between the two assays (RBA vs ELISA) showed a moderate correlation, that is, higher levels of IgA-tTG in ELISA are more likely to be confirmed positive by RBA (**Fig. 17**).

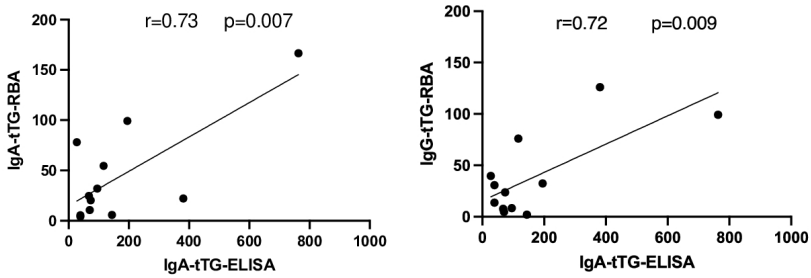

Figure 17. Correlation between ELISA and RBA assays in tTGA positive children defined as having CDA.

Associations of LMTB and *H. Pylori* with incidence of celiac disease autoimmunity

LMTB was found in 4/6 (66.7%) mothers with CDA children compared with 340/734 (46.3%) mothers of children without CDA (p=0.424). *H. pylori* was found in 3/6 (50.0%) CDA children compared with 315/746 (42.2%) children without CDA (P=0.702). Neither of the two chronic infections were associated with CDA.

Anthropometrics of the study participants

Children in this cohort were observed for their development and feeding habits, and it was found that nearly all of them had fed on injera, bread and teff/wheat-based dishes. The majority (94%) of the children was exclusively breastfed at the six-week study visit. Nearly half of the women breastfed their infants up to the age of 18 months, yet there was no association between breastfeeding duration and CDA. By the age of 48 months, 4.4%, 9.2%, and 5.9% of the children in this study population were wasted, stunted, and underweight, respectively. The malnutrition status in this cohort is low compared with the national nutrition survey and other research (Paper III).

Discussion

This thesis explores how immunogenetics, infections, and diets associate with CDA in two Ethiopian study populations. The study is the first population-based cohort on CD in one of the largest highly populated countries from East Africa. The primary goal was to study the incidence of CDA in a pediatric population and how genetic and environmental factors influence it. The main findings demonstrated that the genetic risk of CD in the Ethiopian population was as high as in Sweden, a country with one of the highest prevalence of CD in the world. This may pave the groundwork for future research in CD in Ethiopia to determine what environmental factors are associated with the risk of disease.

The prevalence of CDA in the Ethiopian population has evidently increased over time, as the screening of adult women yield a prevalence of 0.05% (95% CI, 0.001%–0.3%) as compared with 0.6% (95% CI, 0.2%–1.3%) of children by age 4 years in the birth cohort. The current findings give the prevalence rate of 1:174 (95% CI, 1:475 to 1:80) of the populations. This finding is close to the prevalence in several Western population. The finding is also in line with the seroprevalence from other African-Arabian countries (242): 0.53% Egypt (243), 0.79% Libya (244), and 0.60% Tunisia (245). However, the prevalence is lower than that of reported from North Africa, where the only screening study conducted in the general population involved refugee children of Western Sahara origin who were Saharawi of Arab-Berber descent and had the highest prevalence of CD in the world, reaching 5.6% (1/18) (25). However, the prevalence is lower than that reported in Sweden (246–248) and the pooled global seroprevalence of CD (1.4%) (55).

The findings of this study and prior case series reports (174, 206, 207) from the region were against the notion that Subs-Saharan region was a CD free region (32,251), which was based on a study from Burkina Faso (252) and research from immigrants with African origin (253).Though the number of positive children is low, the findings showed similar frequency in both sexes, contrary to several previous studies that reported the predominance in females rather than males (42,44).

The current study supports the notion that CD is a global public health problem (55,254) and calls for more research on CD in the East African region. It demonstrates that CD is underdiagnosed, probably due to a lack of access to diagnostic tools, limited awareness of clinical polymorphism, and a belief that CD

does not exist that gives emphasis to other causes of intestinal damage, such as infections (21). Although the reason for the discrepancy with frequency is unclear, it is speculated that it is due to geographic and sociodemographic factors, gluten consumptions, diagnostic methods, the research period, burden of infections and genes other than the primary HLA-DQ distribution.

The thesis further revealed that the majority of Ethiopian children adhere to the WHO guidelines for supplemental foods, as 84.0% infants started the recommended supplemental food at the median age of six months (239). In the study, the occupation (women who work from home) and age (younger mothers) of infants' mothers had a significant impact on how closely participants followed the instructions on when to introduce complementary foods. The practice of breastfeeding while introducing complementary foods was shown to be better, because the majority of mothers, 84.4%, breastfed during their first six months. This is in line with the WHO recommending exclusive breastfeeding during the first six months of life (255,256). There is a controversial report over time on the time of gluten introduction and risk of CD. The recent report from TEDDY, and other studies confirmed that the time of gluten introduction and the duration of breast feeding confer no increased risk of CD (179,257–259), although earlier studies have revealed that introducing gluten before 4 months, or after seven months, can increase the risk of developing CD (94, 95, 214–216).

This study found that the most common types of food given to the children were included wheat, barley, and teff bases, which were usually introduced at around seven months. As presented in papers III and IV, more than half of the subjects were served at least two portions with (wheat or barley) or without (teff) gluten food groups daily; and the feeding proportion seems to be high for both. Our findings show that children are regularly introduced to gluten when they turn six months old and later, despite the fact that this usually starts in Sweden at the age of four months (263).

A study on the genetic susceptibility to CDA in Ethiopian children found that the proportion of HLA-DQ2 and/or -DQ8 in the community is comparable with high-burden CD population around the globe. HLA-DQ is a necessary risk factor but does not alone sufficient to develop CD (188). The findings demonstrate the existence of HLA-DQ, which is associated with CD development in the Ethiopian population. Paper II revealed that children from Ethiopia have lower proportions of the HLA-DQ2.5 and -DQ8 haplotypes compared with Swedish children. However, there were no overall differences in HLA risk between Ethiopian children and Swedish references. In fact, the highest frequency of HLA-DQ2.2 and -DQ2.3 haplotypes were found in Ethiopian children. According to several previous studies, 90% of CD patients have the HLA-DQ2.5 haplotype; however, individuals without it frequently express HLA-DQ2.2 or -DQ8, and very few have HLA-DQ7.5 or -DQ2.3 (264). Although the contribution of HLA-DQ2.3 in the pathogenesis of CD is not well studied, previous studies showed that in *trans* position HLA-DQ2.3 can have

a presentation of deamidated gluten peptide epitopes efficiently (218, 219). This study revealed that almost all Ethiopian children with CDA had either the HLA-DQ2 or -DQ8 haplotypes.

This study, furthermore, looked at the association between CDA and two low grade chronic infections, LMTB and *H. pylori*. These two were chosen for because they were expected to be found in a higher proportion of the population than the other infectious agents. In this study, we confirmed that there was a 42.3% frequency of *H. pylori* infections in children and a 46.5% prevalence of LMTB among mothers of children in the study. Both prevalence rates were in line with those from past studies and the country's survey report (157,166). Although the statistical analysis showed no association, the study observed that the prevalence of exposure to *H. pylori* and LMTB infections is high, while CDA incidence is low in the Ethiopian population.

Overall, the current study showed that there is a similar gluten consumption by the majority of Ethiopian children, close to the CD high-burden global population. Ethiopians also carry the HLA-DQ risk genes for CD. Moreover, in the study region, there is a high infection burden, and the additional practice of eating a gluten-free traditional food, teff. The cumulative thought is clearly useful for future studies which will be focused on the environmental factors of CD in the region.

This is the first prospective Ethiopian population-based study on CDA. The prospective birth cohort was approached to reduce the probability of participant selection bias. The study looked both at genetic and environmental factors related to CDA. The study involved both adults and children at various time points. This information may be useful for expanding research on, and developing environmental-genetic risk models in, the area where there was a prior paucity of information and knowledge regarding CD.

The thesis has some limitations that are need to be taken into account before drawing conclusions. The screening was conducted on a birth cohort in a single city despite the fact that Ethiopia's population is ethnically and culturally diverse. Therefore, a more robust effort covering a larger geographic area will be needed. Moreover, tTGA-positive children were not confirmed by EMA or biopsy; therefore, diagnosis of CD could not be done according to the ESPGHAN´s criteria. The information on LMTB was based on the previous maternal data. Even though Ethiopians are prone to a number of infectious diseases, only two of them, TB and *H. pylori,* were studied in relation to CDA.

Conclusions

- The prevalence of CDA in Ethiopian children is higher than for adult women, but lower than the pooled global prevalence.
- HLA-DQ2.5 and -DQ8 are less prevalent whereas HLA-DQ2.2 and -DQ2.3 are more prevalent in the Ethiopian population compared with Swedish references.
- Overall, the distribution of HLA-DQ predisposing risk-genotypes for CD is the same in the Ethiopian population compared with Swedish references.
- Ethiopian infants were often given supplemental foods while they were breastfed between the ages of six and seven months, in accordance with the WHO recommendation.
- Neither the frequency of teff-nor wheat-based cereal consumption among study participants were associated with CDA.
- Neither LMTB exposure nor *H. pylori* infections were associated with the incidence of CDA.

Future perspectives

The multifaceted interactions between genetic and environmental factors that underlie CD can be better understood with information about the geographic distribution of CD. Although there are several reports on various aspects of CD, the majority of previous studies were either restricted to specific geographical locations or largely originated from those same places. As a result, there is a lack of population-based multicenter epidemiological investigations in many countries around the world, in particular from developing countries such as those in Sub-Saharan Africa (55). This suggests the necessity for extensive population-based research globally to better understand epidemiology and, if any, unknown contributory factors.

Studies included in the present thesis were only conducted in one city of one region of Ethiopia, but the population is quite diverse and has a wide range of ethnic backgrounds. We expected that there would be variations in the genetics and diet of people, and the types of microbes that inhabited their surroundings. Hence, we are highly motivated to conduct a multicenter population-based study on the true epidemiology of CD and its associations with the environment in the future.

The development of sensitive and specific serological tests revealed previously unrecognized suspected CD in young children. Numerous studies have suggested that CD can substantially increase childhood morbidity and mortality in many developing nations (49), with the majority suffering from a paucity of CD diagnostic tools. As a result, numerous tests must be considered. Future work aims to support in validating various CD tests that developing nations will use.

Several studies showed that different chronic inflammatory disorders and autoimmune diseases are linked to an imbalance of the gut microbiota composition, which results in gut dysbiosis. Diet has a dramatic effect on the composition of the intestinal microbiota in children with CD and on GFD treatment (266). The disparity in nutrition and environment may be a factor for the imbalance. However, in this study, less was considered in this regard. Ethiopia is rich in a wide range of less-explored grain species, like teff. Less is known about the composition and quantity of microbiota or yeast present in the fermented teff's dough or starter. Hence, in order to develop potential preventive or therapeutic treatments as well as understand how CD develops, it is important to understand the intricate interactions between genes, nutrition, and the microbiome (90). However, there is still a controversy as

to whether dysbiosis found in CD patients causes an autoimmune response or is a secondary effect of the damaged intestinal mucosa. It would be interesting to study more about the gut as well as teff dough or starters microbiota in the surroundings.

According to health authority reports and few existing studies, the emergence of non-communicable diseases is currently becoming a challenge for Ethiopia (267). CD and type 1 diabetes mellitus are a distinct autoimmune disorder with related pathogenic mechanisms or share of common pathogenic etiologies (268). Despite this, there are few population-based studies on the incidence of these autoimmune disorders and their underlying environmental causes. I'm interested in future study on these disorders as well as various related genetic and environmental factors.

Popular science summary

Autoimmune disease is one of the collective names given for diseases caused when one's own tissue is damaged by the self-protective immune system. Celiac disease is one of the autoimmune diseases in which the sharp finger like absorptive portion of the small intestine is damaged by one's own immune system, which is activated by a stimulatory protein named gluten in people who have a risk gene. Celiac disease is not seen in all population but usually occurs in people who have a risk gene named HLA-DQ who are exposed to the gluten protein. Gluten is usually found in a cereals such as wheat, barley, and ryes. Gluten and genetics are the key players in the occurrence of the celiac disease. However, these two factors alone are not enough to cause the disease. This is implicating that another several environmental factors contributing the disease development.

The disease was previously thought to appear in limited geographical areas of the world and reported only from some developed countries of the world. Those past studies focused on genetic factors and limited environmental contributors of the disease. However, now the study coverage has increased, the disease looks to be emerging in wide areas of the world. According to researchers' reports over time, now a day the disease affects at least one individual out of hundreds of the world's populations. The disease affects all age and sexes of the population. However, the higher occurrence of the disease is observed in children and females, according to several previous reports. For celiac disease, the peak age for the incidence assumed between 2 and 5 and the third decade of life.

Remarkably, the disease's effect has not been limited to the small intestine's tissue damage; the effect also includes body parts other than the intestine. The primary effect is seen on the abdomen, and symptoms include diarrhoea, abdominal bloating, constipation, weight loss and mainly malabsorption syndrome. In children with malabsorption syndrome the condition is usually very serious, in that it is linked to malnutritional problems which affect a normal growth and has several subsequent consequences. Celiac disease's clinical features are varied; hence it is frequently named a chameleon, and the majority of people are known about the illness when tested in clinics or asked about it in population-based surveys.

Despite several efforts, the true frequency of celiac disease is unknown in most of the developing nations; as a result, it is frequently regarded as an uncommon disease and lacking in structured research. In particular, there were no data from the Sub-

Saharan African nations, where the burden of infectious disease has remained high despite improvements following several initiatives by numerous partners. This research, which was carried out in Ethiopia, attempted to narrow the knowledge gap regarding the genetics and complete absence of the disease. Children in the study were followed from birth to age five through screening, by following their typical dietary habits, and through infection incidents studied. The study provides a chance to investigate whether or not traditional foods usually consumed in Ethiopia are linked to the emergence of celiac disease.

Uniquely, the average Ethiopian dish consists of a variety of vegetable dishes, spicy meat stews, and curries, all of which are topped with a large flatbread called injera. It is an Ethiopian ethnic and traditional main dish. Teff flour, water, and starter (a liquid gathered from previously fermented mix) are combined to make injera, which is thin. Teff is a gluten-free grain with a high nutritional value. Teff is also well known for having more bio-available iron than wheat. Teff can be used to make a variety of foods such as injera, bread, porridge, and cake. Teff might be a good alternative to the modified gluten-free diet usually used for the treatment of celiac disease

On the other hand, in Ethiopia, there is a propensity to switch to a Western diet, as many individuals have access to burgers, and pizza, among other frequently eaten items, particularly when people are away from home. Due to urbanization, improved economic status, diaspora, and globalization, more people are consuming a wheat-base Western meals. In addition, with an expected improved and cleaner environment in Ethiopia, it will be important to follow these factors in relation to the incidence of celiac disease.

Overall, this study highlights that the population's genetic risk is comparable to that of previously studied populations around the world where celiac disease is common. This may suggest that undiscovered environmental factors contribute to the development of, or protection against, the disease. Additionally, the study stresses that celiac disease is spreading and becoming more prevalent over time in regions that had previously appeared to be free of this disorder.

Populärvetenskaplig sammanfattning

Autoimmun sjukdom uppstår när den egna vävnaden skadas av det självskyddande immunsystemet. Celiaki är en av de autoimmuna sjukdomarna där den tunntarmens slemhinna skadas av det egna immunförsvaret. Celiaki förekommer hos endast en del av personer som bär på en uppsättning riskgener som heter HLA-DQ och som exponeras för glutenproteinet. Gluten finns vanligtvis i spannmål som vete, korn och råg. Gluten och HLA-DQ är nyckelaktörerna vid uppkomsten av celiaki. Dessa två faktorer ensamma är dock inte tillräckliga för att orsaka sjukdomen. Detta innebär att ytterligare miljöfaktorer bidrar till sjukdomsutvecklingen.

Sjukdomen ansågs tidigare förekomma i begränsade geografiska områden i världen och rapporterades endast från vissa utvecklade länder. Studier från olika delar av världen visar att celiaki ökat och förekommer på de allra flesta ställen i världen med en genomsnittlig förekomst av en på 100 individer. Sjukdomen drabbar alla individer i alla åldrar och av båda kön i befolkningen. Den högre förekomsten av sjukdomen observeras dock hos yngre barn och kvinnor i trettioårsåldern.

Vanliga symtom och tecken på celiaki är diarré, uppblåsthet i buken, förstoppning, viktminskning och undernäringsproblem som påverkar en normal tillväxt och har flera efterföljande konsekvenser. Celiaki kan dock uttrycka sig på många andra varierande sätt; därför kallas den ofta för en kameleont. Majoriteten av individer med celiaki upptäcks med enkla blodprov genom screening i befolkningsbaserade undersökningar. Den verkliga frekvensen av celiaki okänd i de flesta utvecklingsländer och betraktas därför som en ovanlig sjukdom. I synnerhet fanns det inga uppgifter från de afrikanska länderna söder om Sahara.

Detta projekt, som utfördes i Etiopien undersökte förekomsten av celiaki bland barn och vilka genetiska och omgivningsfaktorer som kunde kopplas till sjukdomen. Barn i studien följdes från födseln till fem års ålder genom årlig screening för celiaki. Information om kostvanor och infektioner samlades in och studerades. Studien syftade också till att undersöka om traditionell mat som vanligtvis konsumeras i Etiopien är kopplad till uppkomsten av celiaki.

Den traditionella etiopiska kosten består av en mängd olika grönsaksrätter, kryddiga köttgrytor och curryrätter, som alla toppas med ett stort tunnbröd som kallas injera som utgörs av teffmjöl, vatten och starter (en vätska som samlats från tidigare fermenterad blandning). Teff är ett glutenfritt spannmål med ett högt näringsvärde. Teff är också välkänt för att ha mer biotillgängligt järn än vete. Teff kan användas

för att göra en mängd olika livsmedel som injera, bröd, gröt och kakor. Teff kan vara ett bra alternativ till den modifierade glutenfria dieten som vanligtvis används för behandling av celiaki.

I Etiopien har man under de senaste 20 åren haft en benägenhet att byta till en västerländsk diet. På grund av urbanisering, förbättrad ekonomisk status och globalisering, äter allt fler människor västerländska rätter baserade på vete. De ändrade matvanorna tillsammans med en alltmer renare miljö är Etiopien med sin varierande och folkrika befolkning ett intressant land för att förstå vilka miljöfaktorer som är involverade i uppkomsten av celiaki.

Sammantaget visar denna studie att den etiopiska befolkningens genetiska risk är jämförbar med den för tidigare studerade populationer runt om i världen där celiaki är vanligt. Detta kan tyda på att tidigare oupptäckta miljöfaktorer är av betydelse för utvecklingen eller skyddande av sjukdomen. Dessutom visar studien att celiaki verkat ökat över tid i Etiopien, ett land där sjukdomen tidigare ansetts som mycket ovanlig.

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