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The Clinical Relevance of Anti-Gliadin Antibodies
in the Ageing Population



ACADEMIC DISSERTATION

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To my family

ABSTRACT

Anti-gliadin antibodies (AGA) have been used in screening for coeliac disease. There are studies indicating that AGA-positivity may be the only and first marker of gluten sensitivity and developing coeliac disease. On the other hand, while the sensitivity of AGA is good, the specificity of AGA for coeliac disease is low, as they may even be present in healthy individuals. Besides coeliac disease, AGA have been linked to other autoimmune diseases, neurological disorders, especially ataxia and neuropathy, and also to psychiatric disorders. Recently, AGA have been introduced as markers of gluten sensitivity, defined as a condition where gluten ingestion causes various symptoms not induced by allergic or autoimmune mechanisms. The purpose of the present study was to explore the clinical relevance of AGA-positivity in the older general population exposed to gluten over a long period.

This work comprised three studies. In study **I** serum IgA- and IgG-class AGA and IgA-class anti-transglutaminase-2 antibodies (anti-TG2) were determined in 2815 individuals aged 52-74 years. All AGA-positive subjects were included as cases in the study. Equal numbers of AGA- and anti-TG2-negative participants of similar age and gender, but without known coeliac disease, were randomly selected as controls. Information on clinical history was obtained from hospital records in both groups to study the association of AGA-positivity with various diseases. In studies **II-III** AGA and anti-TG2 were again measured in the same study population as in study **I** and persistently AGA-positive subjects without a prior coeliac disease diagnosis were invited for HLA testing to find individuals genetically predisposed to coeliac disease. These persistently AGA-positive subjects with coeliac-type HLA and age-and sex-matched persistently AGA-negative controls comprised the study population in studies **II-III**. All subjects in studies **II-III** were also anti-TG2-negative. In study **II** the association of persistent AGA-positivity with overt and

potential coeliac disease and gastrointestinal symptoms was explored by interview, The Gastrointestinal Symptom Rating Scale (GSRS) questionnaire and gastroduodenoscopy with small-bowel mucosal biopsies. Study **III** assessed whether persistent AGA-positivity was associated with neurological or psychiatric disorders. All study subjects were personally interviewed and underwent a thorough neurological examination. The Psychological General Well-Being (PGWB) questionnaire, the Short Form 36 Health Survey (SF-36) questionnaire and the Depression Scale (DEPS) were employed to evaluate psychological well-being. The hospital records of all subjects were analyzed for previous illnesses.

Studies **I-II** showed that AGA-positivity is common (14%) and often persistent (81%) in the older population. Ten per cent of the 381 AGA-positive subjects had coeliac disease. Additionally, among the 49 clinically studied and 36 endoscoped persistently AGA-positive cases without prior coeliac disease diagnosis, one (2.8%) was diagnosed with coeliac disease. Furthermore, many (54%) biopsies showed signs of inflammation, but without villous atrophy. Persistently AGA-positive subjects with coeliac-type HLA had more gastrointestinal symptoms than AGA-negative controls. In study **I** AGA-positivity was associated with rheumatoid arthritis and depression, but not with neurological disorders. In studies **II-III** no association with any disease entities, including autoimmune, neurological and psychiatric disorders, could be proved.

The findings demonstrated, that AGA-positivity is common and often persistent in the older population exposed to gluten for decades. However, isolated AGA-positivity is rarely clinically relevant. At population level AGA-positivity would not appear to be related to neurological disorders. Further studies are needed to explore whether small-bowel mucosal inflammatory changes and gastrointestinal symptoms detected in persistently AGA-positive subjects with coeliac-type HLA are gluten-dependent.

TIIVISTELMÄ

Keliakia on autoimmuunisairaus, jossa vehnän, rukiin tai ohran varastoproteiini, gluteeni, aiheuttaa geneettisesti alttiilla henkilöillä tulehdusreaktion ohutsuolen limakalvolle johtaen lopulta suolinukan vaurioitumiseen ja ravintoaineiden imeytymishäiriöön. Vehnän gluteenin osaa, gliadiinia vastaan kohdistuvia gliadiinivasta-aineita (AGA) on käytetty keliakian seulonnassa, ja tutkimuksissa on esitetty, että ne voisivat olla gluteeniherkkyden ja keliakian ensimmäinen ja ainoa merkki. Gliadiinivasta-aineita löytyy kuitenkin usein terveiltäkin. Keliakian lisäksi gliadiinivasta-ainepositiivisuus on yhdistetty muihin autoimmuunisairauksiin ja neurologisiin sairauksiin, erityisesti ataksiaan ja neuropatioihin sekä psykiatriisiin sairauksiin. Gluteeniherkkyden käsite on viime aikoina laajentunut keliakian ulkopuolelle. Gluteenin on todettu aiheuttavan monenlaisia oireita, vaikka varsinaista keliakiaa ei olisikaan todettu. Gliadiinivasta-aineiden on ajateltu osoittavan gluteeniherkkyttä. Tämän tutkimuksen tarkoituksena oli selvittää gliadiinivasta-ainepositiivisuuden kliinistä merkitystä vanhemmassa väestössä, joka on altistunut gluteenille ravinnossaan vuosikymmenten ajan.

Tutkimus koostui kolmesta osatyöstä. Aineistona oli 2815 52-76-vuotiasta henkilöä, joista määritettiin gliadiini- ja transglutaminaasi-2-vasta-aineet. Osatyössä **I** tutkimuksen kohteena olivat kaikki AGA-positiiviset sekä heille satunnaisesti valitut ikä- ja sukupuolivakioidut vasta-ainenegatiiviset verrokkit. Molempien ryhmien sairauskertomuksista kerättiin tiedot sairaushistoriasta ja vertailemalla tietoja selvitettiin, liittyykö AGA-positiivisuus joihinkin sairauksiin. Osatöissä **II** ja **III** vasta-aineet määritettiin uudestaan samalta väestöltä. Pysyvästi AGA-positiiviset, joilla todettiin lisäksi keliakialle altistava HLA-tyyppi, mutta ei transglutaminaasi-2 vasta-aineita, sekä näille satunnaisesti valitut toistuvasti vasta-ainenegatiiviset ikä- ja sukupuolivakioidut verrokkit muodostivat osatöiden **II-III**

tutkimusryhmät. Osatyössä **II** tutkittiin gastroskopian ja ohutsuolibiopsian avulla, onko pysyvästi AGA-positiivisilla keliakiaa ja liittyykö AGA-positiivisuuteen vatsaoireita. Tutkimuksessa molemmat ryhmät haastateltiin ja he täyttivät The Gastrointestinal Symptom Rating Scale -kyselyn. Osatyössä **III** molemmille ryhmille tehtiin perusteellinen neurologinen tutkimus ja psyykkistä hyvinvointia kartoitettiin haastattelulla ja Psychological General Well-Being (PGWB), Short Form 36 Health Survey (SF-36) ja Depression Scale (DEPS) -kyselyillä. Aiempi sairaushistoria selvitettiin lisäksi sairauskertomustiedoista.

Osatyöt **I** ja **II** osoittivat, että gliadiinivasta-aineet ovat väestössä yleisiä (14%) ja usein pysyviä. Kymmenellä prosentilla kaikista 381 AGA-positiivisista oli todettu keliakia. Kliniseen tutkimukseen osallistui 49 toistuvasti AGA-positiivista keliakialle perinnöllisesti altista henkilöä ja 52 toistuvasti AGA-negatiivista verrokkia. Yhdellä 36:sta (2.8%:lla) gastroskoopidusta toistuvasti AGA-positiivisesta perinnöllisesti keliakialle alttiista henkilöstä todettiin keliakia. Yli puolella (54%:lla) näistä gastroskoopiduista oli ohutsuolen limakalvolla tulehdusmuutoksia, vaikka keliakialle tyypillistä suolinukan atrofiaa ei todettukaan. Toistuvasti AGA-positiivisilla oli myös enemmän vatsaoireita kuin verrokeilla. Osatyössä **I** AGA-positiivisuus näytti liittyvän nivelreumaan ja masennukseen. Yhteyttä neurologisiin sairauksiin ei löytynyt. Osatyössä **III** ei todettu eroa eri sairauksien esiintyvyydessä tai psyykkisessä hyvinvoinnissa toistuvasti AGA-positiivisten ja AGA-negatiivisten verrokkien välillä.

Tämä tutkimus osoitti, että gliadiinivasta-aineet ovat ikääntyvässä väestössä yleisiä ja usein pysyviä. Niillä on kuitenkin vain harvoin kliinistä merkitystä. Yhteyttä neurologisten sairauksien ja AGA-positiivisuuden välillä ei tässä tutkimuksessa voitu osoittaa väestötasolla. Lisää tutkimuksia tarvitaan selvittämään, aiheuttaako gluteeni pysyvästi AGA-positiivisilla keliakialle perinnöllisesti alttiilla henkilöillä todetut ohutsuolimuutokset ja vatsaoireet.

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ABBREVIATIONS

AGA	anti-gliadin antibodies
anti-TG2	transglutaminase-2 antibodies
anti-TG3	transglutaminase-3 antibodies
anti-TG6	transglutaminase-6 antibodies
ARA	anti-reticulin antibodies
BMD	bone mineral density
BMI	body mass index
CD	coeliac disease
DM	diabetes mellitus
DEPS	the Depression Scale
DGP-AGA	deamidated gliadin peptide antibodies
ELISA	enzyme-linked immunosorbent assay
EMA	endomysium antibodies
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
EU	ELISA unit
GFD	gluten-free diet
GOAL	Good Aging in the Lahti region
GSRS	The Gastrointestinal Symptom Rating Scale
HLA	human leucocyte antigen
IBS	irritable bowel syndrome
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
IL	interleukin
MMSE	Mini Mental State Examination

MHC	major histocompatibility complex
ND	no data
nd	not done
PGWB	Psychological General Well-Being
QoL	quality of life
SF-36	Short Form 36 Health Survey
TGA	tissue transglutaminase antibodies
U	unit

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by the Roman numerals **I-III**:

I Ruuskanen A, Kaukinen K, Collin P, Huhtala H, Valve R, Mäki M, Luostarinen L (2010): Positive serum antigliadin antibodies without celiac disease in the elderly population - does it matter? *Scand J Gastroenterol* 45: 1197–1202

II Ruuskanen A, Luostarinen L, Collin P, Krekelä I, Patrikainen H, Tillonen J, Laurila K, Haimila K, Partanen J, Mäki M, Valve R, Kaukinen K (2011): Persistently positive gliadin antibodies without transglutaminase antibodies in the elderly: gluten intolerance beyond coeliac disease. *Dig Liv Dis* 43:772-788

III Ruuskanen A, Kaukinen K, Collin P, Krekelä I, Patrikainen H, Tillonen J, Nyrke T, Laurila K, Haimila K, Partanen J, Valve R, Mäki M, Luostarinen L (2013): Gliadin antibodies in older population and neurological and psychiatric disorders. *Acta Neurol Scand* 127:19-25

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INTRODUCTION

Coeliac disease is an autoimmune disorder in which ingestion of gluten derived from wheat, rye or barley causes an inflammatory reaction in the small-bowel mucosa, leading eventually to villous atrophy with crypt hyperplasia in genetically predisposed individuals. Elimination of gluten from the diet heals the small-bowel mucosal damage and alleviates the symptoms, and also prevents complications of the disease. The classical presentation of coeliac disease is a gastrointestinal disorder with diarrhoea, malnutrition and weight loss (Visakorpi, Kuitunen & Pelkonen 1970). Another classical form of coeliac disease is dermatitis herpetiformis, a blistering skin condition (Duhring 1884). Also a variety of other extra-intestinal manifestations and complications, for example osteoporosis, anaemia, liver disease and even psychiatric disorders, have been reported. Neurological disorders, mainly ataxia and neuropathy, have been described in coeliac patients since 1966 (Cooke, Smith 1966). During the last two decades the field of gluten sensitivity-related neurology has expanded, but the true clinical relevance of gluten sensitivity as a cause of neurological disorders in general has remained obscure.

While coeliac disease was formerly considered a rare paediatric disorder, extensive screening studies have shown it to be a common inflammatory disease of the gut which can present at any time of life, even in older age (Vilppula et al. 2009). It has been realized that the severity of coeliac disease varies from minor inflammatory small-bowel mucosal changes to a refractory disease with a risk of malignancy. Also patients' symptoms vary from inconspicuous to severe.

An immunological reaction against gliadin, the wheat gluten component, has proved in population screening to be common. Only a small proportion of these anti-gliadin antibody (AGA) -positive individuals have villous atrophy compatible

with coeliac disease. AGA-positivity is often a transient phenomenon, but some individuals still later develop coeliac disease (Collin et al. 1993, Johnston et al. 1998). New diagnostic markers, transglutaminase-2 (TG2) -targeted small-bowel mucosal autoantibody deposits and intraepithelial lymphocyte counts, have become good aids in the early diagnostics of coeliac disease.

The aim of the present study was to explore the prevalence and clinical relevance of AGA-positivity in an older population exposed to gluten over a long period. The connection of AGA-positivity to coeliac disease and associated diseases was evaluated. A special focus was on individuals persistently AGA-positive with coeliac-type genetics. They were thoroughly examined by gastroscopy and small-bowel biopsy to search for latent or potential coeliac disease. Their gastrointestinal symptoms, general well-being and quality of life as well as neurological and psychological states were evaluated and compared to AGA-negative controls.

REVIEW OF THE LITERATURE

1. COELIAC DISEASE

1.1 Clinical picture

1.1.1. Classical gastrointestinal disease

Classically coeliac disease was a severe condition of small children involving chronic diarrhoea, steatorrhoea, abdominal distension, pain and malabsorption of nutrients, vitamins and trace elements resulting in failure to thrive and short stature (Visakorpi, Kuitunen & Pelkonen 1970). Since the introduction of serological screening and routine small-bowel biopsies concurrently with gastroscopies, coeliac disease diagnosis has become common at all ages. While some quarter of paediatric and almost half of adult coeliac disease cases may nowadays be silent or subclinical (Collin et al. 1997, Bottaro et al. 1999), diarrhoea is still the most common (about 50%) presenting symptom in all age groups (Murray et al. 2003, Ludvigsson et al. 2004, Mukherjee et al. 2010, Casella et al. 2012). Other common symptoms are abdominal pain, bloating and anaemia (Murray et al. 2003, Ludvigsson et al. 2004, Savilahti et al. 2010). Also vomiting, constipation, anorexia and dyspepsia are occasionally reported (Murray et al. 2003, Ludvigsson et al. 2004, Casella et al. 2012). Malabsorption of iron and iron-deficiency anaemia has proved common even in otherwise silent disease (Bottaro et al. 1999). Anaemia affects 10–46% of coeliac disease patients at presentation (Bottaro et al. 1999, Murray et al. 2003, Rampertab et al. 2006, Mukherjee et al. 2010, Vilppula et al. 2011, Casella et al. 2012). The risk

of anaemia is related to the severity of small-bowel mucosal damage (Kurppa et al. 2010, Zanini et al. 2013) and a gluten-free diet has been shown to cure anaemia (Kurppa et al. 2009, Vilppula et al. 2011).

1.1.2. Extraintestinal disease

Dermatitis herpetiformis, the skin form of coeliac disease, typically presents with symmetrical diffuse lesions consisting of erythema, urticarial plaques, papules, herpetiform vesiculae and blisters, which evolve to erosions, excoriations and hyperpigmentation. The most common locations of the rash are the extensor surfaces of elbows (90%) and knees (30%), but it also appears on the buttocks, sacral region, shoulders and face. Intensive itching often results in scratching, secondary inflammation and lichenification (Duhring 1884). Dermatitis herpetiformis usually presents in middle age and in contrast to gastrointestinal coeliac disease is more common in males than in females. Its prevalence in Finland is eight times lower than that of the gastrointestinal form (Salmi et al. 2011). Uncommon skin manifestations include palmoplantar purpuric lesions, petechial lesions on the fingertips, chronic urticaria-like lesions and palmoplantar keratosis (Bonciolini et al. 2012). Dermatitis herpetiformis patients evince small-bowel mucosal changes compatible with coeliac disease (Marks, Shuster & Watson 1966) and both adults and children respond to a gluten-free diet (Gawkrödger et al. 1984, Reunala et al. 1984).

Neurological disorders have been reported in about 10-20% of coeliac disease patients (Volta et al. 2002, Briani et al. 2008). In 1966 Cooke and Smith (Cooke, Smith 1966) first described sixteen coeliac disease patients with severe neurological conditions not obviously associated with malabsorption of vitamins. All cases had ataxia predominantly in the lower limbs, most had sensory ataxia due to peripheral neuropathy, but three also had cerebellar-type ataxia. Loss of Purkinje cells in the cerebellum was found in six out of nine patients upon autopsy. Spinal cord pathology was found in all, demyelination being the most prominent feature.

Dementia was reported in two patients and attacks of loss of consciousness in five. Since the report by Cooke and Smith (Cooke, Smith 1966), ataxia as a manifestation of coeliac disease has been documented in many case reports (Finelli et al. 1980, Bhatia et al. 1995, Pellecchia et al. 1999b). However, when coeliac disease patients have been systematically examined for neurological symptoms, ataxia has been a rare complication (Volta et al. 2002), in fact there are many reports where none of the patients studied had ataxia (Cicarelli et al. 2003, Briani et al. 2008, Ruggieri et al. 2008). When ataxia patients have been screened for coeliac disease, an excess prevalence of the disorder compared to controls or the general population has been noted (Pellecchia et al. 1999a, Luostarinen et al. 2001, Hadjivassiliou et al. 2003b). However, in some studies coeliac disease has not proved more frequent in patients with sporadic ataxia than in controls (Combarros et al. 2000, Abele et al. 2003, Lock et al. 2005). A gluten-free diet has been reported to alleviate symptoms of coeliac ataxia in some cases (Pellecchia et al. 1999b, Hadjivassiliou et al. 2003a), but more commonly they tend to persist or progress despite dietary restrictions (Cooke, Smith 1966, Finelli et al. 1980, Bhatia et al. 1995).

Both sensorimotor axonal neuropathy (Cooke, Smith 1966, Hadjivassiliou et al. 1997, Chin et al. 2003) and small fibre neuropathy (Brannagan et al. 2005, De Sousa et al. 2006) have been reported in coeliac disease patients. Luostarinen and associates (Luostarinen et al. 2003) found electrophysiologic evidence of neuropathy in as many as 23.1% of coeliac disease patients on a gluten-free diet. Using a similar approach Briani and colleagues (Briani et al. 2008) found neuropathy in 3 out of 71 coeliac patients. A gluten-free diet alleviated neuropathy symptoms in one patient and dietary deviation worsened them in another (Briani et al. 2008). When neuropathy patients have been screened for coeliac disease, conflicting results as to the prevalence of the disorder in this patient group have emerged. Hadjivassiliou and colleagues (Hadjivassiliou et al. 2006a) reported a 9% prevalence of coeliac disease in a group of idiopathic neuropathy patients compared to 1% in controls, and a group under Chin (Chin et al. 2003) found a 2.5% prevalence of coeliac disease in 400 patients screened. On the other hand, Rosenberg and Vermeulen (Rosenberg, Vermeulen 2005) found no association

between coeliac disease and chronic peripheral neuropathy, and likewise Matà and associates (Matà et al. 2006) found none among 330 neuropathy patients in screening with tissue transglutaminase antibodies (TGA).

Epilepsy associated with coeliac disease has been noted both in case reports and larger studies, some of which have shown a positive effect of gluten-free diet on seizure activity (Chapman et al. 1978, Cronin et al. 1998, Canales et al. 2006, Peltola et al. 2009). A specific syndrome of coeliac disease, epilepsy and cerebral calcifications has mostly been described in Italy (Gobbi et al. 1992, Magaudda et al. 1993). The risk of epilepsy among coeliac disease patients in the Swedish population was recently reported 1.43 times increased (Ludvigsson et al. 2012). In contrast, in a large Finnish screening study involving 968 epilepsy patients and 584 controls no difference in the prevalence of coeliac disease was found (Ranua et al. 2005).

Other neurological disorders associated with coeliac disease are myopathy, especially inflammatory myopathy (Hadjivassiliou et al. 2007, Selva-O'Callaghan et al. 2007), restless legs (Moccia et al. 2010) and migraine (Gabrielli et al. 2003, Dimitrova et al. 2013). Encephalopathy and dementia have occasionally been considered to be due to coeliac disease (Cooke, Smith 1966, Collin et al. 1991, Hu et al. 2006). Some recent studies have shown sensorineural hearing loss to be common in coeliac disease patients (Leggio et al. 2007, Hizli et al. 2011). This association was not found in a larger series of adult coeliac patients (Volta et al. 2009). In general, children and dermatitis herpetiformis patients seem less prone to develop neurological manifestations of coeliac disease (Wills et al. 2002, Ruggieri et al. 2008), this possibly due to the shorter duration of the disease without treatment (Cicarelli et al. 2003).

In several studies coeliac disease patients have been reported to suffer from depression (10-57%) more often than controls, and it has been considered a feature of the disease (Hallert, Derefeldt 1982, Holmes 1996, Ciacci et al. 1998, Addolorato et al. 2001). Interestingly, in a large study based on medical records no difference in this respect between healthy controls and coeliac patients was noted (Garud et al. 2009). Studying Swedish coeliac disease patients on a gluten-free diet for ten years,

Roos and group (Roos, Karner & Hallert 2006) did not find them to differ from population controls in respect of psychological well-being. In prospective quality of life (QoL) studies coeliac disease patients have evinced lower QoL at diagnosis but have usually improved to normal after starting a gluten-free diet. In cross-sectional studies patients with coeliac disease, especially women, seem to have lower QoL than healthy controls. Studies of QoL in coeliac disease are presented in Table 1.

Oral manifestations of coeliac disease include dental enamel defects, clinical delayed eruption in children, recurrent aphthous stomatitis and aspecific atrophic glossitis in both adults and children (Aine et al. 1990, Campisi et al. 2007). A gluten-free diet has been shown to heal recurrent aphthous stomatitis (Campisi et al. 2007) and alleviate dental enamel defects in permanent teeth (El-Hodhod et al. 2012). Older and atypically presenting children with coeliac disease appear to have more dental enamel defects than younger or typically presenting children, this possibly due to longer duration of coeliac disease without treatment (Majorana et al. 2010).

Occasionally coeliac disease presents with rheumatic complaints (Collin et al. 1992). Lubrano and associates (Lubrano et al. 1996) found arthritis to be more common in coeliac disease patients than in controls, especially in those not dieting. A gluten-free diet has sometimes been reported to heal arthritis in coeliac disease patients (Bourne et al. 1985). Other reported extraintestinal manifestations of coeliac disease include alopecia areata (Corazza et al. 1995), elevated liver enzymes or liver dysfunction (Volta et al. 1998a, Kaukinen et al. 2002) and infertility (Collin et al. 1996, Martinelli et al. 2000), which have been reported to reverse with the institution of gluten-free diet.

Table 1. Quality of life (QoL) studies in coeliac disease

Study	Study population, n, country	QoL measured by	Main results
Hallert et al. 1998	89 CD patients and 5277 population controls, Sweden	SF-36	After 10 years of GFD women with CD had lower QoL than controls.
Fera et al. 2003	100 CD patients on GFD, 100 healthy controls, 100 DM patients, Italy	SF-36	QoL of CD patients was poorer than healthy controls` but not different from DM patients`.
Häuser et al. 2006	446 members of German CD Society, 2037 population controls, Germany	SF-36	All subscales of SF-36 except physical function were poorer in CD patients than representative German population sample.
Roos, Karner & Hallert 2006	51 CD patients on GFD for 10 years, 182 general population controls, Sweden	PGWB	Psychological well being was as good in CD patients as in controls; however, in CD group women had lower scores than men.
Nachman et al. 2009	132 newly diagnosed CD patients, 70 healthy controls, Argentina	SF-36	At diagnosis CD patients had lower QoL than controls and classical CD patients had lower QoL than clinically silent CD patients. After 1 year of GFD both CD groups were equal and comparable to controls.
Tontini et al. 2010	43 CD patients, 86 healthy controls, Italy	SF-36	At diagnosis CD women had lower QoL than controls. After 1 year of GFD all CD patients were equal to controls.
Barratt, Leeds & Sanders 2011	225 CD patients, 348 controls, United Kingdom	SF-36	CD patients had lower QoL than controls. Difficulties in adherence to GFD was associated with poorer QoL.
Paavola et al. 2012	96 screen-detected, 370 symptom-detected CD patients, 110 healthy controls, 2060 population controls, Finland	PGWB SF-36	PGWB scores were same as controls` apart from general health, which scored lower in CD patients. SF-36 scores were same as control population sample`s except in role physical subscale, which scored lower in CD patients.

CD, coeliac disease; DM, diabetes mellitus; QoL, quality of life; SF-36, Short Form 36 Health Survey; PGWB, Psychological General Well-Being survey; GFD, gluten-free diet

1.2 Associated diseases and risk groups

Increasing numbers of coeliac disease patients are nowadays diagnosed by screening populations or risk groups. About a quarter of paediatric and almost half of adult patients are asymptomatic at diagnosis (Collin et al. 1997, Bottaro et al. 1999). Since coeliac disease is markedly genetically predisposed, relatives of coeliac disease patients are at increased risk of developing the disorder. The prevalence of coeliac disease in relatives of coeliac patients in screening studies has been 5-10% (Mäki et al. 1991, Fasano et al. 2003, Bonamico et al. 2006, Dögan, Yildirmaz & Ozercan 2012). Other known risk groups are subjects with autoimmune diseases such as autoimmune thyroid diseases, type I diabetes mellitus, Sjögrens`'s syndrome, autoimmune hepatitis, primary biliary cirrhosis, Addison`s disease, IgA-nephropathy and subjects with selective IgA deficiency, Down`s syndrome and Turner`s syndrome. Likewise, coeliac disease patients often suffer from other autoimmune diseases (Collin et al. 1994, Viljamaa et al. 2005). An increased prevalence of coeliac disease has also been reported among patients with chronic urticaria (Caminiti et al. 2005, Confino-Cohen et al. 2012), sarcoidosis (Rutherford et al. 2004, Ludvigsson et al. 2007b) and psoriasis (Lindqvist et al. 2002, Ludvigsson et al. 2011). Prevalences of coeliac disease among patients with associated conditions are presented in Table 2.

Table 2. The prevalence of biopsy-proven coeliac disease in patients with associated conditions

Condition and study	Population	Antibody positivity (%)	Coeliac disease (%)
Autoimmune thyroid diseases Collin et al. 1994 Spadaccino et al. 2008	83 adults 276 adults	ARA/EMA 4.8 EMA/TGA/IgG AGA 3.6	4.8 3.6
Type I diabetes mellitus Collin et al. 1989 Leeds et al. 2011	195 adults 1000 adults	ARA 4.1 EMA 2.8, TGA 6.0	4.1 3.3
Sjögren`s syndrome Iltanen et al. 1999 Szodoray et al. 2004	34 adults 111 adults	EMA 8.8, AGA 38.2 EMA/TGA/AGA 5.4	14.7 4.5
Autoimmune hepatitis Volta et al. 1998b Villalta et al. 2005	181 children and adults 47 adults	EMA 4.4, AGA 13.8 EMA/TGA 6.4	2.8 6.4
Primary biliary cirrhosis Dickey, McMillan & Callender 1997 Volta et al. 2002	57 adults 173 adults	EMA 10.5 EMA/TGA 4.0	7.0 4.0
Addison`s disease Myhre et al. 2003 Betterle et al. 2006	76 children and adults 4 children, 105 adults	EMA/TGA 6.6, AGA 26.3 TGA 3.7	7.9 2.8
IgA nephropathy Fornasieri et al. 1987 Collin et al. 2002	121 children and adults 223 adults	AGA 2.5 EMA 1.8, TGA 3.6	1.7 3.6
IgA deficiency Meini et al. 1996 Lenhardt et al. 2004	65 children 126 children	AGA 24.6 AGA 21.4, TGA 14.3	7.7 8.7
Down`s syndrome Bonamico et al. 2001 Cerqueira et al. 2010	1202 children and adults 98 children and adults	EMA 5.4, AGA 21.5 EMA 19.4, TGA 12.2	4.6 9.2
Turner`s syndrome Bonamico et al. 2002 Frost, Band & Conway 2009	389 children and adults 256 adults	EMA 5.7, AGA 5.9 EMA 3.2	6.4 4.7

ARA, anti-reticulín antibodies; EMA, endomysium antibodies; AGA, anti-gliadin antibodies; TGA, tissue transglutaminase antibodies

1.3 Complications of coeliac disease

Coeliac disease patients have since the 1960s been reported to be at increased risk of developing malignancies (Harris et al. 1967). The lymphoproliferative malignancy

risk, especially in the gut, has been estimated to be three- to six-fold increased in recent larger studies (Askling et al. 2002, West et al. 2004, Viljamaa et al. 2006, Elfström et al. 2011, Leslie et al. 2012). Enteropathy-associated T-cell lymphoma in the jejunum is the classical presentation, which results from clonal proliferation of intraepithelial lymphocytes in a situation where gluten exposure continues or the small-bowel mucosa fails to heal despite gluten-free dieting (refractory coeliac disease) (Harris et al. 1967, Freeman 2004). Lymphoma in coeliac disease patients can also present at other sites and in the form of B-cell lymphoma and Hodgkin lymphoma (Harris et al. 1967, Freeman 2004, Leslie et al. 2012). Also a risk of carcinoma of the mouth and pharynx, oesophagus, small intestine, large intestine and liver has been reported to be increased in coeliac disease patients (Harris et al. 1967, Holmes et al. 1989, Askling et al. 2002). Interestingly, in some studies the risk of lung and breast cancer has been reduced in coeliac disease patients, which has been speculated to be related to their lower weight and less smoking (Askling et al. 2002, West et al. 2004). A strict gluten-free diet seems to reduce the risk of malignancies (Harris et al. 1967, Holmes et al. 1989). However, undiagnosed or developing coeliac disease does not appear to be related to an increased risk of malignancy (Anderson et al. 2007, Lohi et al. 2009a, Godfrey et al. 2010, Canavan et al. 2011). According to recent studies and a meta-analysis, the overall malignancy risk of coeliac patients does not seem to be increased (West et al. 2004, Viljamaa et al. 2006, Tio, Cox & Eslick 2012).

Mortality among coeliac disease patients has been reported to be increased about two- to four-fold compared to the general population in studies mostly involving symptom-detected patients (Logan et al. 1989, Cottone et al. 1999, Peters et al. 2003). This excess mortality is attributable mainly to non-Hodgkin lymphomas. However, screen-detected patients carry no increased risk of mortality (Lohi et al. 2009b, Godfrey et al. 2010, Canavan et al. 2011). The overall mortality of coeliac disease patients compared to the general population has in recent studies been only about 1.4-fold increased (Ludvigsson et al. 2009, Grainge et al. 2011). In studies involving dermatitis herpetiformis patients the risk of mortality has been equal to that in the general population (Swerdlow et al. 1993, Lewis et al. 2008, Hervonen et

al. 2012); likewise only the risk of lymphomas seems slightly increased, not that of other malignancies (Collin, Pukkala & Reunala 1996, Askling et al. 2002, Hervonen et al. 2005, Lewis et al. 2008).

Osteomalacia is a severe, nowadays rare complication of coeliac disease (Bianchi, Bardella 2008). Osteoporosis, again, defined as bone mineral density (BMD) lower than -2.5 standard deviations from mean values of healthy young adults, or osteopenia, defined as BMD lower than -1 standard deviations, is a common complication in all age groups. Reduced calcium absorption, deficiency of vitamin-D, high parathyroid hormone content, deficiency of growth factors and overproduction of cytokines are mechanisms underlying the development of bone defects in coeliac disease (Bianchi, Bardella 2008). Kemppainen and associates (Kemppainen et al. 1999a) reported osteoporosis in 26% and osteopenia in 35% out of 77 coeliac disease patients, among these newly diagnosed patients had osteoporosis/osteopenia more often than those already on a gluten-free diet. In an older screen-detected patient population osteoporosis/osteopenia was reported in 62% and low-energy fractures in 23% of patients (Vilppula et al. 2011). While patients with only mild gluten enteropathy may already have osteoporosis/osteopenia (Kurppa et al. 2010, Zanini et al. 2013), more severe small-bowel mucosal damage is related to an even higher prevalence of these disorders (Kaukinen et al. 2007, Garcia-Manzanares, Tenias & Lucendo 2012, Zanini et al. 2013). A gluten-free diet has been shown in several studies to heal bone (McFarlane, Bhalla & Robertson 1996, Corazza et al. 1996, Kemppainen et al. 1999b, Vilppula et al. 2011). When a GFD is started before puberty, bone development is normal (Mora et al. 1999). According to a meta-analysis involving eight studies and 20955 coeliac disease patients and 96777 controls, the overall estimated excess fracture risk among coeliac disease patients is 43% (Olmos et al. 2008).

1.4 Diagnosis of coeliac disease

1.4.1. Diagnostic criteria

Since 1990 the diagnosis of coeliac disease has been based on the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria for the condition, the requirements being demonstration of small-bowel villous atrophy and crypt hyperplasia while on a gluten-containing diet and relief of all symptoms of coeliac disease after starting a strict gluten-free diet. In such cases no control biopsy is needed according to the criteria. However, if the patient is symptom-free at diagnosis, a control biopsy is required to prove recovery of the small-bowel mucosa as an effect of a gluten-free diet (Walker-Smith et al. 1990). The same criteria have been applied to children and adults (United European Gastroenterology. 2001). The presence of coeliac autoantibodies supports the diagnosis. Since virtually all coeliac disease patients are either human leucocyte antigen (HLA) DQ2- or DQ8-positive, genetic testing for these alleles can be used to aid in diagnosis and to exclude patients without these risk genes from further diagnostic studies for coeliac disease (Sollid et al. 1989, Karell et al. 2003, Mäki et al. 2003). Recently, the ESPGHAN criteria have been revised, the diagnosis of coeliac disease can now be made in symptomatic children and adolescents without a small-bowel biopsy if they have high serum IgA anti-TG2 levels (above ten times the normal upper limit) verified by endomysium antibody (EMA) seropositivity and HLA DQ2- and/or DQ8- positivity (Husby et al. 2012). The diagnostic criteria of dermatitis herpetiformis are based on perilesional skin biopsy findings showing granular immunoglobulin-A (IgA) deposits in the papillary dermis under direct immunofluorescence (van der Meer 1969).

1.4.2. Small-bowel mucosal biopsy

Small-bowel mucosal biopsy samples are usually obtained by upper gastrointestinal endoscopy. A suction capsule is nowadays rarely used due to radiation risks. Since mucosal lesions can be patchy and the severity of the lesions may vary in different parts of the duodenum, multiple biopsies are recommended, at least one from the bulb and four from the second or third parts of duodenum (Husby et al. 2012). The histological abnormalities typically found in the small-bowel mucosa of coeliac disease patients include partial to total villous atrophy, decreased villus height/crypt depth ratio, increased numbers of intraepithelial lymphocytes (IELs), increased mitotic activity of IELs, infiltration of plasma cells, lymphocytes, eosinophils, basophils and mast cells into the lamina propria; also abnormalities of the epithelial cells and absence of a brush border can be seen (Marsh 1992). Small-bowel mucosal lesions develop gradually, starting from an increased number of intraepithelial lymphocytes to crypt hyperplasia and further to villous atrophy, and are classified according to Marsh (Marsh 1992) and Oberhuber (Oberhuber, Granditsch & Vogelsang 1999). Marsh 0 stands for normal villi and a normal amount of intraepithelial lymphocytes, Marsh 1 for normal villi but an increased number of IELs, Marsh 2 for hypertrophic crypts expressed as decreased villus/crypt ratio and increased IELs, Marsh 3a for partial villous atrophy, Marsh 3b for subtotal villous atrophy and Marsh 3c for total villous atrophy (Marsh 1992, Oberhuber, Granditsch & Vogelsang 1999). Villous atrophy with crypt hyperplasia compatible with active coeliac disease is determined as a villus height/crypt depth ratio below two (Kuitunen, Kosnai & Savilahti 1982). Such a condition is not, however, specific for coeliac disease but may also be caused by many other conditions such as giardiasis, autoimmune enteropathy, Crohn's disease, tuberculosis, HIV-infection, common-variable immunodeficiency, Whipple's disease, intestinal lymphoma and intolerance to foods other than gluten, e.g., milk, soy, chicken and tuna (Green, Cellier 2007). Also drugs, e.g., azathioprine (Ziegler et al. 2003), mycophenolate mofetil (Kamar et al. 2004) and olmesartan (Rubio-Tapia et al. 2012), have been reported to cause villous atrophy.

Intraepithelial lymphocytosis is a typical feature of coeliac disease, but like villous atrophy, is unspecific and can be caused for example by infections, drugs, inflammatory bowel disease, immune dysregulation and sarcoidosis (Aziz et al. 2010). Diagnostic accuracy can be enhanced by counting villous tip IELs in haematoxylin-eosin stained samples; increased values have a sensitivity of 95% and specificity of 88% in coeliac disease with villous atrophy (Salmi et al. 2010). Immunohistochemical staining for $\gamma\delta^+$ IELs and CD3⁺ IELs in freshly frozen small-bowel biopsy samples has also proved useful in the diagnostics of coeliac disease (Järvinen et al. 2003) with sensitivities of increased numbers of $\gamma\delta^+$ IELs and CD3⁺ IELs of 92% and 85% and specificities 81% and 69%, respectively (Salmi et al. 2010).

1.4.3. Coeliac disease antibodies

Antibodies against gluten peptides and tissue structures have been used to aid in the diagnosis of coeliac disease and to screen subjects for diagnostic small-bowel biopsy. The first coeliac autoantibodies discovered were those against reticulin fibres (ARA) (Seah et al. 1971). ARA can be measured in both immunoglobulin-A (IgA) and -G (IgG) -class and the sensitivity and specificity of the tests have been high (Mäki et al. 1991, Sulkanen et al. 1998). However, since the test is indirect immunofluorescence-based and thus laborious and somewhat difficult to interpret, it has been replaced by more easily performed enzyme-linked immunosorbent assay (ELISA) tests.

Antibodies against gliadin (AGA) are examined by ELISA tests and have been widely used in the screening of potential coeliac disease patients (Corazza et al. 1992, Grodzinsky et al. 1992, Weile et al. 2001, Fasano et al. 2003). However, since the sensitivity and specificity of IgA AGA in adults is only about 80% and the corresponding figures for IgG AGA are even poorer (Rostom et al. 2005), AGA tests are no longer recommended in screening for coeliac disease (Husby et al. 2012). More recently, a new ELISA test using deamidated gliadin peptides (DGP) as

antigen has been introduced (Schwartz et al. 2004). These DGP-AGA tests have proved more accurate than the conventional AGA tests as diagnostic aids and can be measured in both IgA- and IgG-class, which renders them valuable also in the screening of IgA-deficient subjects (Volta et al. 2010, Husby et al. 2012).

IgA-class endomysium antibodies (EMA) are considered sensitive and highly specific for coeliac disease (Rostom et al. 2005) and EMA tests have been used in many large population screening studies (McMillan et al. 1996, Lagerqvist et al. 2001, Fasano et al. 2003, West et al. 2003). The EMA test is an indirect immunofluorescence test and thus slow and cumbersome to perform. The main endomysial autoantigen in coeliac disease was discovered to be TG2 (Dieterich et al. 1997), a widely expressed protein with many functions, of which the most important in the pathogenesis of coeliac disease are enzymatic in trans- and deamidation processes, especially in the deamidation of gliadin peptides (Molberg et al. 1998). ELISA tests have been developed to detect IgA-class antibodies against TG2 (anti-TG2). Anti-TG2 tests have high sensitivity and specificity (Rostom et al. 2005) and are therefore recommended for coeliac screening (Husby et al. 2012). IgG-class anti-TG2 tests have lower sensitivity and are not in routine clinical use (Rostom et al. 2005). Also IgA-class anti-TG2-based rapid tests using whole blood samples have been developed. The sensitivities and specificities of these tests have been reported to be 93-97% and 93-98%, respectively (Korponay-Szabó et al. 2005, Raivio et al. 2007). Recently, two other subtypes of antibodies associated with gluten sensitivity and directed towards transglutaminases have been recognized. While anti-TG2 antibodies are found in patients with coeliac enteropathy, anti-TG3 antibodies are found in dermatitis herpetiformis patients and anti-TG6 antibodies have been reported to be associated with neurological manifestations (Hadjivassiliou et al. 2010, Stammaes et al. 2010). Studies assessing the diagnostic performance of various antibody tests in coeliac disease are collected in Table 3. It is noteworthy that seropositivity can be transient or fluctuating despite a continuing gluten-containing diet (Johnston et al. 1998, Laass et al. 2006, Simell et al. 2007) and especially gliadin antibodies are frequently also found in healthy individuals (Grodzinsky et al. 1992, Hadjivassiliou et al. 2003b). It has, however, been reported

that new seroconversion and the development of villous atrophy compatible with coeliac disease can occur later in life (Kurppa et al. 2011).

Antibodies against TG2 form in the small-bowel mucosa of coeliac disease patients and besides in the sera they can be found deposited in the small-bowel mucosa, and interestingly, also in extraintestinal tissues such as the liver and brain (Korponay-Szabó et al. 2004, Hadjivassiliou et al. 2006b). Intestinal IgA-class TG2-targeted deposits have proved 100% sensitive and specific in diagnosing untreated coeliac disease and can also be found in developing coeliac disease and in coeliac disease patients negative for serum antibodies (Salmi et al. 2010).

Table 3. Sensitivity and specificity of AGA, EMA, anti-TG2 and DGP-AGA in the diagnostics of biopsy-proven coeliac disease (CD)

Study	Study population	IgA AGA		IgG AGA		EMA		anti-TG2		IgA DGP-AGA		IgG DGP-AGA	
		Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %
Mäki et al. 1991	122 healthy first-degree relatives of CD patients	31	87	46	89	92	95	ND	ND	ND	ND	ND	ND
Dieterich et al. 1998	106 CD patients, 114 disease and healthy controls	ND	ND	ND	ND	99	100	98	94	ND	ND	ND	ND
Sulkanen et al. 1998	136 CD patients 207 disease controls	85	82	69	73	93	100	95	94	ND	ND	ND	ND
Kaukinen et al. 2007	44 CD patients, 46 disease controls	52	46	ND	ND	80	100	89	98	91	98	ND	ND
Ankelo et al. 2007	87 CD patients, 81 healthy controls	87	72	78	64	ND	ND	90	90	92	90	75	98
Niveloni et al. 2007	60 CD patients, 81 disease controls	ND	ND	ND	ND	ND	ND	95	98	98	94	97	100
Hadithi et al. 2007	16 CD patients, 447 disease controls	50	98	25	99	81	99	81	99	ND	ND	ND	ND
Volta et al. 2010	48 CD patients, 89 controls suspected of CD	ND	ND	ND	ND	92	100	94	97	84	80	82	80

AGA, anti-gliadin antibodies; EMA, endomysium antibodies; anti-TG2, transglutaminase-2 antibodies; DGP-AGA, deamidated gliadin peptide antibodies; CD, coeliac disease; sens., sensitivity; spec., specificity; ND, no data

1.5 Pathogenesis of coeliac disease

Coeliac disease is strongly genetically predisposed. The human leucocyte antigen (HLA) molecules are coded in a major histocompatibility complex on chromosome 6. The coeliac disease associated HLA genes are encoded in a region known as class II and by genes -DQ. The HLA DQ genes encode for α - and β -chains associated as heterodimers forming a cleft which binds antigens. HLA DQB1*02 and DQA1*05 constitute the heterodimer DQ2 and HLA DQB1*0302 and DQA1*0301 the heterodimer DQ8. The DQ2 genes are present in about 90% of coeliac disease patients while almost all the remainder have DQ8 (Sollid et al. 1989, Karell et al. 2003). Only less than 0.5% of coeliac disease patients are negative for DQ2 and DQ8 (Karell et al. 2003). The HLA DQ2 or DQ8 genotypes are present in about 40% of Caucasians (Mäki et al. 2003). Genome-wide association studies have identified other non-HLA gene loci associated with coeliac disease. Most of those code immune functions, for example IL2 and IL21 (van Heel et al. 2007), CCR3, IL12A, IL18RAP, RGS1, SH2B3 (Hunt et al. 2008), ETS1, RUNX3, THEMIS and TNFRSF14 (Dubois et al. 2010, Trynka et al. 2011). The contribution of these genes to the development of coeliac disease is still obscure (Kumar, Wijmenga & Withoff 2012).

Coeliac disease is triggered by ingestion of the cereal protein gluten, which belongs to the group of storage proteins, the prolamins. Gluten has a high content of glutamine, proline and hydrophobic amino acids. The main protein fractions of wheat gluten are gliadin and glutenin. Gliadin and the corresponding proteins in rye (secalins) and barley (hordeins) are central factors in the pathogenesis of coeliac disease. Ingested gluten is only partially cleaved by digestive enzymes, this resulting in the appearance of a variety of peptides, some of which are toxic, others immunogenic to coeliac disease patients (Shan et al. 2002). The most intensively studied toxic gluten-derived peptide is α -gliadin 31-34, which activates the innate immune system by inducing the production of interleukin-15 in the lamina propria cell populations and further leads to up-regulation of the MHC class I polypeptide-related molecule MICA and its receptor natural killer group 2, member A (NKG2D)

protein in epithelial and IELs, respectively (Maiuri et al. 2003, Hue et al. 2004, Meresse et al. 2004). This results in direct cytotoxic epithelial cell damage (Hue et al. 2004). Gluten peptides can also activate monocytes (Cinova et al. 2007) and cause maturation of dendritic cells (Nikulina et al. 2004) and induce enterocyte apoptosis (Maiuri et al. 2003). A damaged intestinal epithelium has weakened barrier functions, thereby allowing immunogenic gliadin peptides to enter the lamina propria. These gliadin peptides are then modified by TG2; deamidated gliadin peptides have negatively charged glutamate residues with a high affinity for predisposing HLA DQ2 and DQ8 molecules (Molberg et al. 1998). Both HLA DQ2 and DQ8 molecules have positively charged pockets where deamidated gliadin peptides collect and are then presented to T-cells, which process induces adaptive immune mechanisms such as gluten-specific CD4 T-cell activation, secretion of pro-inflammatory cytokines including interferon- γ and interleukin-21, activation of B-cells and production of anti-deamidated gliadin antibodies (DGP-AGA) and TG2-targeted autoantibodies (Kupfer, Jabri 2012). The production of TG2-targeted autoantibodies is dependent on gluten exposure (Dieterich et al. 1998, Sulkanen et al. 1998). B-cells can mediate antigen presenting to T-cells, thus enhancing the CD4 T-cell response leading to proliferation and clonal expansion of antigen-specific T-cells (Crawford et al. 2006). In in vitro studies coeliac disease patient antibodies have been shown to inhibit differentiation and induce proliferation of intestinal epithelial cells (Halttunen, Maki 1999, Barone et al. 2007). It has also been suggested that autoantibodies could promote the passage of gliadin peptides to the lamina propria (Matysiak-Budnik et al. 2008), inhibit angiogenesis (Myrsky et al. 2008, Caja et al. 2010) and modulate the enzymatic activity of TG2 (Esposito et al. 2002, Kiraly et al. 2006). In summary, both innate and adaptive immune reactions contribute to the development of small-bowel mucosal damage and also to the progression of coeliac disease.

Besides gluten exposure and genetic predisposition, environmental factors can influence the risk of developing coeliac disease. Gastrointestinal infections such as rotavirus infections (Stene et al. 2006) have been suggested to trigger coeliac disease. The mode of delivery has been reported to influence the risk of coeliac

disease: children born by caesarean section had an increased risk compared to those born vaginally in a study by Decker and associates (Decker et al. 2010). This has been held to be due to differing bacterial colonization at birth (Dominguez-Bello et al. 2010). According to a meta-analysis of five studies, breastfeeding protects from developing coeliac disease (Akobeng et al. 2006). Early feeding with gluten-containing foods, again, increases the risk of coeliac disease (Norris et al. 2005).

1.6 Prevalence of coeliac disease

Screening of populations and risk groups has revealed coeliac disease to be common. The overall prevalence of the disorder in the European population is estimated to be one per cent (Mustalahti et al. 2010), and it would appear to be increasing (Lohi et al. 2007, Vilppula et al. 2009, Catassi et al. 2010). Comparing prevalence figures in screening studies and clinically diagnosed coeliac disease patients, it has been estimated that as much as 75% of coeliac disease remains undiagnosed (Virta, Kaukinen & Collin 2009). Prevalence figures in different adult populations are presented in Table 4.

Table 4. Prevalence of coeliac disease in different populations

Study	Country	Study population, diagnostic method	Coeliac disease prevalence %
Lohi et al. 2007	Finland	8028 adults, national health survey, previous CD diagnosis or anti-TG2- and EMA-positivity	2.0
Vilppula et al. 2009	Finland	2815 randomly selected older adults biopsy-proven CD biopsy-proven CD or anti-TG2-positivity	2.3 2.7
Mustalahti et al. 2010	Finland Germany Italy UK	Altogether 24646 adults, previously diagnosed CD or anti-TG2- and EMA-positivity	2.0 0.3 0.7 1.5
Walker et al. 2010	Sweden	1000 randomly selected adults, biopsy-proven coeliac disease	1.8
Fasano et al. 2003	USA	2845 adults not at CD risk, blood donors and other volunteers, EMA-positivity and biopsy-proven CD or EMA-positivity and HLA DQ2/DQ8	1.0
Gomez et al. 2001	Argentina	2000 adults attending prenuptial laboratory tests, AGA-positivity and EMA-positivity/biopsy-proven CD	0.6
Alencar et al. 2012	Brasilia	4000 blood donors, screened with anti-TG2 and EMA, biopsy-proven CD	0.4
Makharia et al. 2011	India	2879 adults and children invited by door-to-door visits, serological screening and biopsy-proven CD	1.4
Tatar et al. 2004	Turkey	2000 blood donors, anti-TG2-positivity	1.3
Akbari et al. 2006	Iran	2799 adults, randomly selected, gluten-sensitive enteropathy in small-bowel biopsy	1.0

CD, coeliac disease; anti-TG2, transglutaminase-2 antibodies; EMA, endomysium antibodies; AGA, anti-gliadin antibodies

1.7 Treatment of coeliac disease

The treatment of coeliac disease involves a gluten-free diet, this entailing avoidance of all gluten-containing foods, wheat, rye and barley. The molecular structure of oats avenins differs from that of other cereals, and pure oats are tolerated by most coeliac disease patients (Janatuinen et al. 2002, Cooper et al. 2013) and also by dermatitis

herpetiformis patients (Hardman et al. 1997, Reunala et al. 1998). However, occasionally coeliac patients also get symptoms from oats (Lundin et al. 2003, Peräaho et al. 2004) and in a recent study persistent small-bowel mucosal lymphocytosis was reported to be more common among subjects using oats, even though they did not have persistent villous atrophy more often than coeliac patients not using oats (Ilus et al. 2012). Wheat starch-containing gluten-free products have also proved safe in the treatment of coeliac patients (Kaukinen et al. 1999, Ilus et al. 2012). The gluten-free diet should be strict to avoid gastrointestinal symptoms and to initiate the healing of the small-bowel mucosa; even very small amounts of gluten consumed only for a couple of weeks can induce deterioration of the small-bowel mucosal structure, symptoms increasing already within days (Leffler et al. 2013).

A gluten-free diet heals the small-bowel mucosa in the majority of patients within the first years of treatment (Wahab, Meijer & Mulder 2002, Tursi et al. 2006, Hutchinson et al. 2010, Ilus et al. 2012). The recovery is dependent on dietary compliance (Tursi et al. 2006, Hutchinson et al. 2010). However, over half of patients have been reported to have persistent intraepithelial lymphocytosis despite a long gluten-free diet (Lanzini et al. 2009, Ilus et al. 2012). The correlation between mucosal recovery and the disappearance of serum coeliac antibodies is not particularly good (Sategna-Guidetti et al. 1996, Kaukinen et al. 2002, Tursi, Brandimarte & Giorgetti 2003). While a gluten-free diet heals symptoms of coeliac disease, some patients continue to have gastrointestinal symptoms (Midhagen, Hallert 2003, Murray et al. 2004). A long-term gluten-free diet prevents and alleviates the complications of coeliac disease (Harris et al. 1967, Holmes et al. 1989, Corazza et al. 1996, McFarlane, Bhalla & Robertson 1996, Kempainen et al. 1999B, Vilppula et al. 2011). Besides healing the small-bowel mucosa, the diet also cures the skin manifestations of coeliac disease (Harrington, Read 1977, Gawkrödger et al. 1984, Reunala et al. 1984). Dermatitis herpetiformis patients also respond to dapsone (Reunala et al. 1984).

In a small proportion of patients both symptoms and small-bowel mucosal damage persist despite a strict gluten-free diet. The prevalence of this so-called refractory coeliac disease has been reported to be about five per cent (Wahab, Meijer

& Mulder 2002, Roshan et al. 2011, Dewar et al. 2012). The condition is divided into type I and type II. Type II refractory coeliac disease is characterized by aberrant T-cells and is more often associated with severe malabsorption, ulcerative jejunitis, lymphocytic gastritis, a higher risk of enteropathy-associated T-cell lymphoma and poorer prognosis (Malamut et al. 2009). The treatment of refractory coeliac disease involves parenteral nutrition and immunosuppressant drugs such as corticosteroids, azathioprine, mesalazine and cladribine; in treatment-resistant cases also autologous stem cell transplantation has been used (Mooney et al. 2012).

A strict gluten-free diet is troublesome to implement and the compliance of patients is variable. In countries like Finland, where gluten-free products are readily available and general knowledge of coeliac disease is wide, patients adopt the dietary restrictions well and their quality of life does not worsen on the diet (Vilppula et al. 2011, Paavola et al. 2012). However, patients have expressed a desire for alternative treatments and extensive research is ongoing. Proposed strategies include enzyme therapy to promote complete digestion of gluten peptides, correction of intestinal barrier defects by inhibiting zonulin, blockage of the binding of gluten epitopes to HLA-DQ2 molecules, TG2 inhibitors, gluten vaccination and blockage of the cytokine response (Fasano 2012). Most studies are in phase I-II and it remains to be seen whether these treatments will provide coeliac disease patients with clinically relevant help.

2. GLUTEN SENSITIVITY

According to a recent consensus statement from coeliac disease experts, gluten sensitivity is defined as a condition where gluten ingestion causes symptoms not attributable to allergic or autoimmune mechanisms (Sapone et al. 2012). In gluten sensitivity no autoantibodies or increased permeability of the gut are present and the duodenal villous morphology is normal, even though some inflammation may be present (Sapone et al. 2011). Antibodies against native gliadin (AGA) as well as coeliac-type HLA have been reported in about half of such cases (Carroccio et al. 2012, Volta et al. 2012). The clinical symptoms include abdominal pain, eczema or rash, headache, fatigue, diarrhoea or constipation, depression, anaemia, numbness in the legs, arms or fingers and joint pain (Sapone et al. 2012). The symptoms resolve with a gluten-free diet and reappear when gluten is again ingested (Biesiekierski et al. 2011, Sapone et al. 2012).

AGA has been studied as a marker of gluten sensitivity in many clinical conditions. AGA-positivity without coeliac disease has been associated with arthritis (Pellegrini et al. 1991, Paimela et al. 1995), psoriasis (Michaëlsson et al. 1993), Sjögren`s syndrome (Iltanen et al. 1999), systemic lupus erythematosus (Rensch et al. 2001), autoimmune liver (Volta et al. 1998b) and thyroidal disease (Collin et al. 1994) and inflammatory bowel disease (Tursi et al. 2005). In studies of psychiatric patients, AGA-positivity has been found to be more common in patients with schizophrenia (Dohan et al. 1972), bipolar disorder (Dickerson et al. 2011) and acute mania (Dickerson et al. 2012) than in controls. There are reports showing that a gluten free diet may alleviate symptoms of arthritis (Hafström et al. 2001) and psoriasis (Michaëlsson et al. 2000). Prevalences of AGA-positivity in selected disorders are presented in Table 5.

Table 5. Prevalences of AGA-positivity in selected disease groups

Study	Disorder	Study population, n	AGA + in cases %	AGA + in controls %
O'Farrelly et al. 1988	Rheumatoid arthritis	93 cases, 25 controls	47	8
Paimela et al. 1995	Rheumatoid arthritis	78 cases, 25 controls	37	12
Lepore et al. 1993	Juvenile chronic arthritis	53 cases	34	
Rensch et al. 2001	Systemic lupus erythematosus	103 cases	23	
Iltanen et al. 1999	Sjögren`s syndrome	34 cases	44	
Michaëlsson et al. 1993	Psoriasis	302 cases	16	
Collin et al. 1994	Autoimmune thyroid diseases	83 cases	27	
Cuoco et al. 1999	Autoimmune thyroid diseases	92 cases	14	
Volta et al. 1998b	Autoimmune liver diseases	243 cases	18	
Tursi et al. 2005	Crohn`s disease	27 cases	30	
Kull et al. 1999	Ulcerative colitis	50 cases, 53 controls	34	4
Dohan et al. 1972	Schizophrenia	229 cases, 261 controls	20	11

AGA, anti-gliadin antibodies

Most attention has, however, been directed to gluten sensitivity-related neurological disorders, especially ataxia. Gluten ataxia has been defined as idiopathic sporadic ataxia with serological evidence of gluten sensitivity (Hadjivassiliou et al. 2003b, Sapone et al. 2012). When ataxia patients have been screened with coeliac antibodies, even 41% AGA-positivity has been reported (Hadjivassiliou et al. 2003b). Antibodies against TG6 have recently been proposed as a specific marker in gluten ataxia (Hadjivassiliou et al. 2013). Deposition of transglutaminase antibodies has been reported around brain vessels in gluten ataxia patients (Hadjivassiliou et al. 2006b). Furthermore, serum from gluten ataxia patients has been shown to cause ataxia in mice when injected intraventricularly (Boscolo et al. 2007). However, in one recent study no ataxia or cerebellar damage developed in HLA DQ2-transgenic mice immunized with gliadin despite high antibody production (Tarlac et al. 2013). Also in other studies involving ataxia patients, the association between AGA-positivity and ataxia has not been

consistently proved (Sivera et al. 2012), as shown in Table 6 presenting prevalences of AGA in ataxia patients in different studies (Table 6).

Previous studies have mainly involved selected patient populations in specialist care centres. General population screening studies have shown that AGA-positivity is much more common than coeliac disease. The clinical relevance of AGA-positivity at population level has remained obscure.

Table 6. Gluten sensitivity in ataxia patients

Study	Study population (n)	Screening methods	Main results
Pellecchia et al. 1999a	Ataxia without definite diagnosis (24), hereditary ataxia (23)	AGA, EMA	3/24 (12.5%) AGA-positive, 1/24 (4.2%) EMA-positive, 3/24 (12.5%) had coeliac disease, none of the patients with hereditary ataxia seropositive
Combarros et al. 2000	Idiopathic cerebellar ataxia (32)	AGA, ARA, EMA, anti-TG2	All seronegative
Bushara et al. 2001	Sporadic ataxia (26), autosomal dominant ataxia (24)	AGA, ARA, EMA, anti-TG2	7/26 (27%) with sporadic ataxia AGA-positive, none had coeliac disease 9/24 (37%) with hereditary ataxia AGA-positive, none had coeliac disease
Bürk et al. 2001	Sporadic ataxia (104)	AGA, EMA	11/104 (10.6%) AGA-positive, 1/104 (1%) EMA-positive, 2/104 (1.9%) had coeliac disease
Luostarinen et al. 2001	Ataxia of unknown origin (24)	AGA, EMA, anti-TG2	16.7% had coeliac disease
Abele et al. 2003	Sporadic ataxia (32), hereditary ataxia (63), healthy controls (73)	AGA, EMA	Antibody-positive: sporadic ataxia 19%, recessive ataxia 8%, dominant ataxia 15%, controls 8% The groups did not differ statistically
Hadjivassiliou et al. 2003b	Sporadic idiopathic ataxia (132), familial ataxia (59), normal controls (1200)	AGA	AGA-positive: sporadic ataxia 41%, familial ataxia 14%, controls 12% Coeliac disease diagnosed in 12/51 (24%) of biopsied AGA-positive with sporadic ataxia
Lock et al. 2005	Idiopathic ataxia (20), hereditary ataxia (7)	AGA, anti-TG2	AGA-positive: idiopathic ataxia 40%, hereditary ataxia 43%, None were anti-TG2-positive

AGA, anti-gliadin antibodies; ARA, antireticulin antibodies; EMA, endomysium antibodies; anti-TG2, transglutaminase-2 antibodies

3. THE PRESENT STUDY

3.1 The aims of the present study

Earlier screening studies have shown serum AGA-positivity to be common in the population. In small case series AGA-positivity has been connected to various disorders, also in the absence of coeliac disease. There are findings indicating that AGA-positivity may be the only and first marker of gluten sensitivity and developing coeliac disease. On the other hand, AGA without coeliac disease has been suggested to be a common denominator of neurological disorders and also related to psychiatric complaints. The purpose of the present study was to explore the clinical relevance of AGA-positivity in the older general population exposed to gluten over a long period of time.

The specific aims were:

1. To study the prevalence and persistence of serum AGA-positivity in the older population (**I-II**)
2. To establish whether AGA and especially persistent AGA discovered in population screening is related to overt, potential or developing coeliac disease or gastrointestinal symptoms (**I-II**)
3. To establish whether serum AGA-positivity discovered in population screening is related to co-morbidities (**I-II**)
4. To establish whether serum AGA-positivity discovered in population screening is related to neurological or psychiatric disorders (**I, III**)

3.2 The study population

The original study cohort comprised 4272 randomly selected individuals born in the years 1926–1930, 1936–1940, 1946–50 and living in the Päijät-Häme Central Hospital district in southern Finland. Equal numbers of women and men and of each age group were invited to participate in a research project on aging and well-being (Good Aging in the Lahti region = GOAL) (GOAL). Altogether 2815 persons participated. All filled in questionnaires on their health condition, present and past diseases and special diets. In 2002, sera were collected for analysis of IgA- and IgG-class AGA and IgA anti-TG2; altogether 2722 samples were available. Three years later, in 2005, all eligible subjects were asked to undergo a new serological test, and 2216 consented. Serum samples were available in both 2002 and 2005 for altogether 2089 subjects.

3.2.1. Study I

In study I, all serum IgA and/or IgG AGA-positive cases in 2002 comprised the cohort. This AGA-positive group was further divided according to anti-TG2-positivity (defined in study I as the AGA+ anti-TG2+ and AGA+ anti-TG2- groups). Equal numbers of AGA- and anti-TG2-negative participants in 2002 of similar age and gender, but without known coeliac disease, were randomly selected to serve as a control group (defined as the AGA- anti-TG2- group) in study I (Figure 1 in original publication I).

3.2.2. Studies II and III

All persistently (in 2002 and 2005) AGA-positive but anti-TG2-negative subjects without known coeliac disease were offered HLA DQ2 and DQ8 testing. Those genetically predisposed to coeliac disease were invited to participate as cases in

studies II and III. Equal numbers of randomly selected persistently AGA- and anti-TG2-negative subjects of the same age and gender were invited to participate in studies II and III as a control group (Figure 1).

STUDY POPULATION IN STUDIES II-III

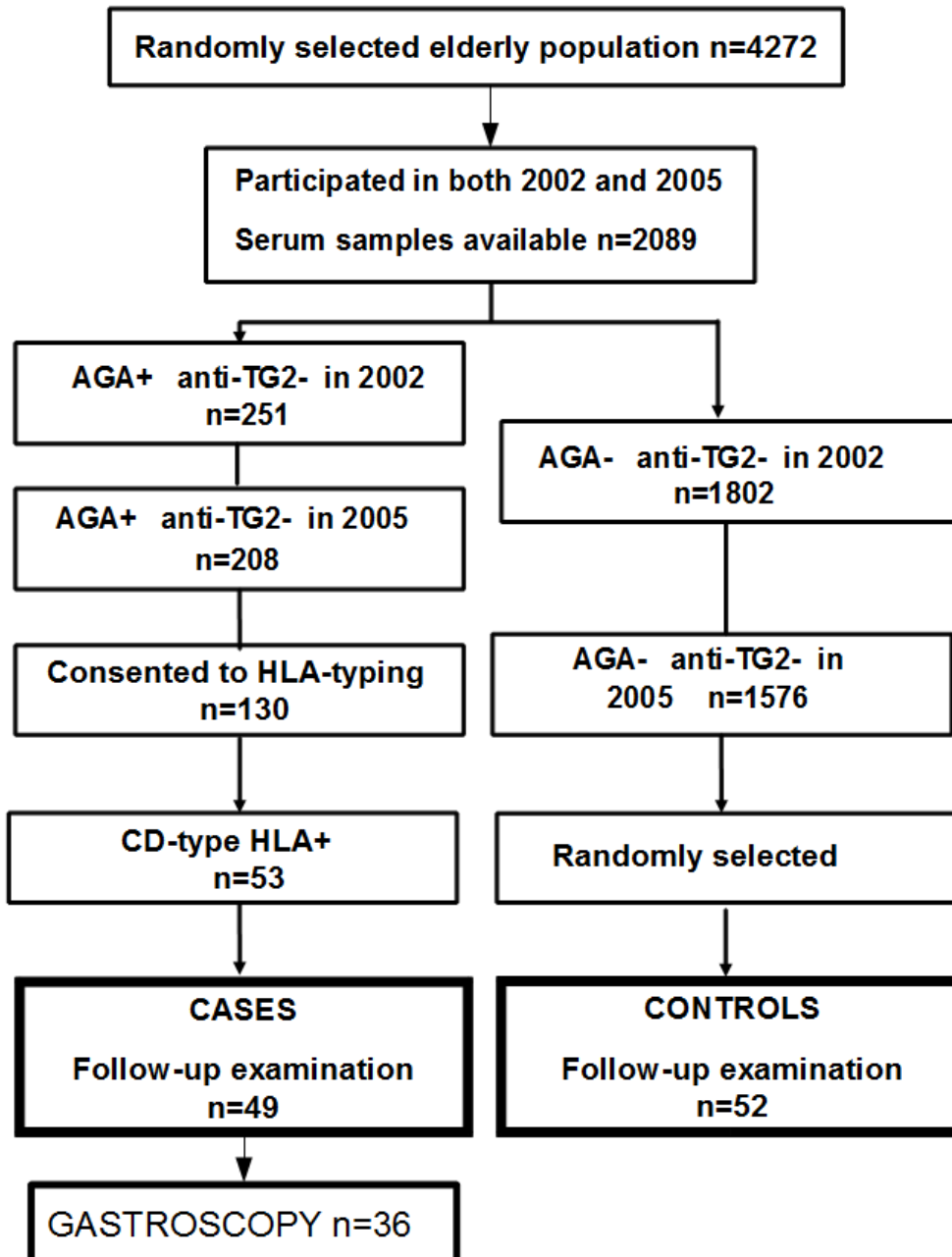


Figure 1. Flowchart of studies II-III

3.2.3. Ethical considerations

The studies were accepted by the research ethics committee of Päijät-Häme Central Hospital. All participants gave written informed consent.

3.3 Methods

3.3.1. Serology (I-III)

Serum IgA- and IgG-class AGA were investigated by enzyme-linked immunosorbent assay (ELISA) (Vainio et al. 1983); the results were obtained from the standard curve established according to dilutions of positive reference serum and converted to concentrations of arbitrary ELISA units per millilitre (EU/ml). The limits of positivity were set at 0.20 EU/ml and 20 EU/ml, respectively. Serum IgA-class anti-TG2 was investigated by ELISA according to the manufacturer's instructions (Celikey; Phadia, Freiburg, Germany), a unit value ≥ 5 U being positive (I-III). DGP-AGA testing was performed in persistently AGA+ anti-TG2- and AGA- anti-TG2- serum samples collected in 2005. An ELISA kit recognizing composites of IgA- and IgG-class DGP-AGA (QUANTA Lite™ Gliadin DGP Screen; Inova Diagnostics, San Diego, CA, USA) was applied, and unit values ≥ 20 U were considered positive as advocated by the manufacturer (II-III). IgA- and IgG-class antibodies against TG6 were detected by ELISA using human recombinant TG6 (E003 and E004 Neuronal transglutaminase ELISA kits, Zedira, Darmstadt, Germany) as antigen according to the manufacturer's instructions. Concentrations over 21 U/ml and over 38 U/ml were considered positive for IgA- and IgG-class anti-TG6 antibodies, respectively, as recommended by the manufacturer (III). Frozen sera stored at -20 °C were used in all serological tests.

3.3.2. HLA-testing (II-III)

The persistently AGA-positive study subjects were genotyped for HLA-DQB1*02, DQB1*0302 and DQA1*05 alleles using the DELFIA Coeliac Disease Hybridization Assay (Perkin-Elmer Life and Analytic Sciences, Wallac Oy, Turku, Finland). The genotypes DQB1*02 and DQA1*05 correspond to the serological HLA type DQ2 and DQB1*0302 to HLA-DQ8 (Sollid et al. 1989, Karell et al. 2003, Mäki et al. 2003). These genotypes are referred as coeliac-type HLA in studies II and III.

3.3.3. Clinical history (I-III)

The medical files of all cases and controls recorded by the Päijät-Häme Central Hospital were systematically analyzed. Weight, height and blood haemoglobin values were gleaned and body mass indexes (BMI) calculated as weight/height² (kg/m²). Malabsorption was defined as low levels of blood or serum folic acid, iron, vitamin-D or -E recorded in medical files. Endocrinological, gastrointestinal, immunological, cardiovascular, neurological, psychiatric and malignant diseases were assessed from the hospital records. Miscarriages, chromosomal disorders and histories of tuberculosis and sarcoidosis were registered. The numbers of both low- and high-energy fractures and data on osteopenia or osteoporosis (not associated with use of steroids) were recorded. In addition, in studies II and III, all cases and controls were personally interviewed by the same investigator on current and past diseases, gastrointestinal, neurological, psychiatric and other physical symptoms and family history of coeliac disease.

3.3.4. Questionnaires (II-III)

The Gastrointestinal Symptom Rating Scale (GSRS) questionnaire (Svedlund, Sjödin & Dotevall 1988) was used to assess current gastrointestinal symptoms. It

comprises altogether 15 items within five subdimensions describing diarrhoea, indigestion syndrome, constipation, abdominal pain and gastro-oesophageal reflux. Each item is graded from one to seven, a higher score indicating more gastrointestinal symptoms. The questionnaire has previously been validated (Dimenäs et al. 1996) and applied in the assessment of gastrointestinal symptoms in coeliac disease (Midhagen, Hallert 2003).

Psychological well-being and quality of life were evaluated by the Psychological General Well-Being (PGWB) questionnaire, the Short Form 36 Health Survey (SF-36) questionnaire, and the Depression Scale (DEPS). The PGWB questionnaire contains 22 items comprising six sub-dimensions: anxiety, depression, well-being, self-control, general health and vitality, scoring being based on a 6-grade Likert scale, higher scores indicating better psychological well-being (Dimenäs et al. 1993). The SF-36 questionnaire, containing eight sub-dimensions (mental health, physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning and role limitations due to emotional problems), is used to assess health-related quality of life. The raw scores on all 36 items were re-scored from 0 to 100, higher scores indicating better health and quality of life (Hallert et al. 1998). These questionnaires have been widely used in coeliac disease and are of proven validity and reliability (Dimenäs et al. 1993, Hallert et al. 1998, Kurppa et al. 2010). The Depression Scale (DEPS) has been widely applied as a self-rating depression questionnaire and is suitable in recognizing depression in primary care patients (Poutanen et al. 2010). The DEPS includes ten items covering the core symptoms of depression with four alternatives in scoring: 0 = not at all, 1 = a little, 2 = quite a lot and 3 = extremely. A higher score indicates more severe symptoms (Salokangas, Poutanen & Stengård 1995) and the cut-off level for depressive symptoms is 9/10 (Poutanen et al. 2010).

3.3.5. Clinical examination (II-III)

In addition to a general clinical examination, weight and height were measured and BMI calculated (normal range: 18–25 kg/m²). In neurological examination, the focus was especially on finding previously undiagnosed and subclinical disorders. Gait, balance, muscle strength, deep tendon reflexes, vibration, pin-prick and light touch senses and the functions of cranial nerves were evaluated. Cognition was assessed by clinical interview and a combination of the Mini Mental State Examination (MMSE) and the clock-drawing test (Cacho et al. 2010). All evaluations were made by the same, non-blinded investigator, but in borderline cases another (blinded) investigator co-evaluated the subject. If any relevant clinical symptoms or signs were detected, further studies such as electroneuromyography (ENMG), skin biopsy, lumbar puncture, radiological scans, neuropsychological and laboratory tests were performed according to clinical needs and good clinical practice. When neuropathy was suspected, ENMG using a structured study protocol and a three-mm punch skin biopsy 5-10 cm above the lateral malleolus to detect small-fibre neuropathy (Koskinen et al. 2005) were carried out. In cases where neuropathy was diagnosed, full blood count, liver enzymes and thyroid function tests, blood glucose, serum creatinine, vitamin-B12, -B6, -B1, folic acid levels, serum and urine protein fractions, antineuronal and antiganglioside antibodies were measured and if possible, cerebrospinal fluid analyzed for cells, glucose, protein, albumin, immunoglobulins, IgG index, antineuronal antibodies and antibodies against *Borrelia burgdorferi*. In addition, when ataxia was detected, vitamin-E, gene tests for MIRAS/POLG1, Friedrich's ataxia and FragileX were applied.

3.3.6. Upper gastrointestinal endoscopy and small-bowel mucosal biopsies (II-III)

Upper gastrointestinal endoscopy with small-bowel mucosal biopsies was offered to all clinically examined persistently serum IgA- and IgG-class AGA-positive cases.

Upon oesophago-gastroduodenoscopy, four small-bowel mucosal forceps biopsies were taken from the distal duodenum. Three samples were fixed in formalin, processed and stained with haematoxylin and eosin and small-bowel mucosal morphology was studied under light microscopy in several well-oriented biopsy sections. Poorly oriented sections were discarded, and if necessary, the samples were dissected again until they were of good quality. Villous height and crypt depth ratio (Vh/CrD) <2.0 was considered compatible with villous atrophy and overt coeliac disease (Salmi et al. 2006b, Kurppa et al. 2009). One sample was snap-frozen and embedded in optimal cutting temperature compound (OCT, Tissue-Tec, Miles Inc, Elkhart, IN) and stored at -70 °C. Immunohistochemical stainings were carried out on 5- μ m-thick frozen sections. CD3⁺ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA), and $\gamma\delta$ ⁺ IELs with TCR γ antibody (Endogen, Woburn, MA). Positive IELs were counted at a magnification of one hundred throughout the surface epithelium and at least 30 fields were counted; results were indicated as IEL density cells/mm of epithelium as previously described (Järvinen et al. 2003, Kurppa et al. 2009). The reference values were set at 37 cells/mm for CD3⁺ IELs and at 4.3 cells/mm for $\gamma\delta$ ⁺ IELs (Järvinen et al. 2003).

Intestinal TG2-targeted autoantibody deposits were studied in unfixed small-bowel mucosal frozen sections. The sections were stained by direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (Dako AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline (PBS). To establish whether there is co-localization of IgA and TG2, sections were double-stained with anti-IgA antibody as described above and using monoclonal mouse antibodies against TG2 (CUB7402, NeoMarkers, Fremont, CA, USA) followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (Dako) diluted 1:200 in PBS. In coeliac disease subepithelial deposition can be found along the villous and crypt epithelium and around vessels, whereas in contrast, in normal samples IgA is found only inside plasma cells and epithelial cells (Korponay-Szabó et al. 2004, Salmi et al. 2006b). The occurrence of TG2-targeted IgA deposits was graded semi-quantitatively

according to their intensity along the basement membrane in the villous-crypt area as follows: negative, weak (+), moderate (++) and strong positive (+++).

All biopsy specimens were evaluated by the same investigator without prior knowledge of the history or findings. Biopsy findings were considered indicative of overt coeliac disease if villous atrophy with crypt hyperplasia was found. Potential coeliac disease was suspected when the small-bowel mucosal villous morphology was normal but an increased density of CD3+ or $\gamma\delta$ + IELs was verified or small-bowel mucosal TG2-targeted IgA deposits were present. Mucosal biopsy samples from the stomach (corpus and antrum) were taken for routine histological assessment and *Helicobacter pylori* staining.

3.3.7. Statistical analyses (I-III)

Quantitative data were expressed as medians or means and ranges. Statistical differences between study groups were evaluated using Pearson's χ^2 test, Fisher's exact test or Mann–Whitney U test, as appropriate. Two-tailed values of $p < 0.05$ were considered significant. The statistics were calculated with SPSS (SPSS Inc., Chicago, IL, USA) 14.0 (I) or 15.0 (II-III). AGA-negative controls were age- and sex-matched and randomly selected from the original study population.

3.4 Results

3.4.1. Prevalence and persistence of AGA-positivity (I-II)

In 2002 altogether 381 (14%) out of 2722 participants were positive for AGA; 342 in IgA-class only, 13 in IgG-class only and 26 in both IgA- and IgG-class. Thirty-

eight (10%) out of the 381 AGA-positive subjects were also positive for anti-TG2 (AGA+ anti-TG2+ group) (I). Altogether 2089 subjects gave serum samples both in 2002 and in 2005. In 2002, 251 of them were AGA-positive but anti-TG2-negative, and three years later 208 (81%) of them were still positive for AGA; 185 proved positive in IgA-class only, four in IgG-class only and 19 were positive in both IgA- and IgG-classes (II). In addition, five out of these 208 (2.4%) persistently AGA-positive cases were DGP-AGA -positive. For comparison, seventeen (1.1%) out of 1576 persistently AGA- and anti-TG2-negative were DGP-AGA-positive, but none of them belonged to the randomly selected control group (II).

3.4.2. AGA-positivity and prevalence of overt, potential and developing coeliac disease and gastrointestinal symptoms (I-II)

According to medical files, available for 361 out of 381 (95%) AGA-positive subjects, biopsy-proven coeliac disease had been verified in 39 (10%) subjects, out of whom 34 belonged to the AGA+ anti-TG2+ group and five to the AGA+ anti-TG2- group. Gastroscopy and small-bowel biopsy had been carried out in altogether 92 AGA-positive cases (I).

During the follow-up, one persistently AGA-positive subject had developed anti-TG2-positivity and was subsequently diagnosed with coeliac disease. Five persistently AGA-positive subjects had died after giving the second blood sample in 2005. An invitation to attend for HLA DQ2 and DQ8 screening was sent to 203 persons, of whom 130 consented, and 53 were found to be either HLA DQ2- or HLA DQ8-positive. Forty-nine of these 53 agreed to clinical examination and furthermore, 36 of them underwent gastroscopy and small-bowel mucosal biopsy. Among the 36 biopsied persons, one had villous atrophy and thus overt coeliac disease. He also had deficiencies of vitamin-B12 and vitamin-D, which reversed during a gluten-free diet. Fresh frozen biopsy samples were available for 35 study subjects; nineteen (54%) of them showed minor small-bowel mucosal inflammatory

changes and were considered to have potential coeliac disease (Table 7, Figure 2 in original publication **II**). One of them was DPG-AGA-positive. *H. pylori* gastritis was found in seven and atrophic gastritis in four subjects. Ventricular mucosal findings did not correlate with duodenal biopsy findings (**II**).

Persistently AGA-positive subjects reported more gastrointestinal symptoms in their past history than persistently negative controls upon interview (Table 9). Current abdominal complaints as rated by GSRS total score were similarly more common in the AGA-positive group. The same trend was seen in all subdimensions, although only the reflux score differed statistically significantly between the groups (Figure 2, Table 2 in original publication **II**). The gastrointestinal symptoms did not correlate with ventricular or small-bowel mucosal biopsy findings (Table 7) (**II**).

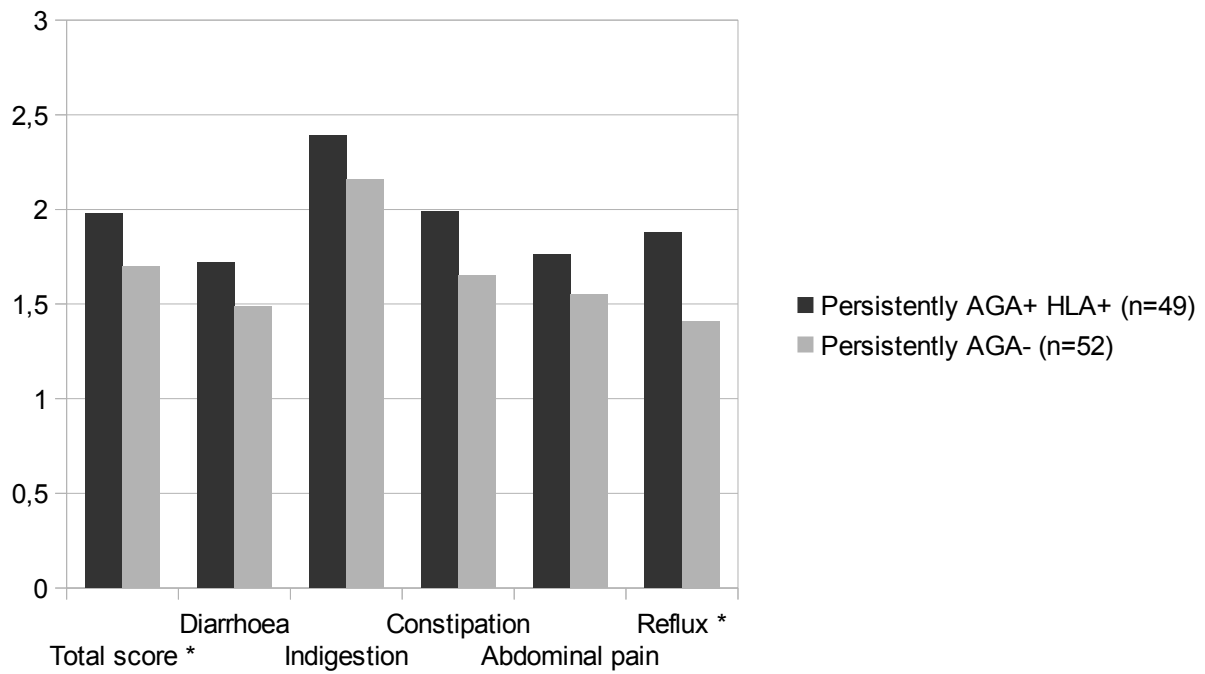


Figure 2. The Gastrointestinal Rating Scale (GRS) scores in persistently AGA-positive subjects with coeliac-type HLA and persistently AGA-negative controls.

* p<0.05

Table 7. Coeliac disease-related small-bowel mucosal biopsy results in 20 persistently AGA-positive and anti-TG2-negative subjects carrying coeliac-type HLA. Final diagnosis indicates either overt coeliac disease (Coeliac) or potential coeliac disease (Potential).

No	Sex	Age	Small-bowel biopsy			γσ+ IELs	IgA-deposit	Gastrointestinal* symptoms/ diseases		GSRs total score	Final diagnosis
			Atrophy	CD3+ IELs	γσ+ IELs						
1	M	57	+	-	-	+	No	Constipation	1.13	Coeliac	
2	M	57	-	+	+	+	Constipation		2.67	Potential	
3	F	80	-	+	+	+	No		1.07	Potential	
4	F	61	-	+	+	-	Flatulence		1.40	Potential	
5	M	69	-	+	+	-	No		1.60	Potential	
6	M	67	-	+	+	-	No		1.13	Potential	
7	F	58	-	+	-	-	Constipation, diarrhoea, flatulence		2.33	Potential	
8	F	57	-	+	-	-	Diarrhoea		2.00	Potential	
9	M	69	-	+	-	-	No		2.07	Potential	
10	M	68	-	+	-	-	No		1.07	Potential	
11	F	58	-	-	+	+	No		1.20	Potential	
12	M	68	-	-	-	+	No		1.00	Potential	
13	F	60	-	-	+	-	Flatulence		1.93	Potential	
14	M	79	-	-	+	-	Constipation, diarrhoea, flatulence		4.00	Potential	
15	M	69	-	-	+	-	Lactose intolerance		2.53	Potential	
16	M	69	-	-	+	-	No		1.87	Potential	
17	F	71	-	-	+	-	Constipation		ND	Potential	
18	F	69	-	-	+	-	Lactose intolerance		2.53	Potential	
19	F	69	-	-	+	-	Dyspepsia		3.67	Potential	
20	M	68	-	-	+	-	No		1.13	Potential	

F, female; M, male; +, positive result; -, normal value; IELs, intraepithelial lymphocytes; ND, no data; GSRs, The Gastrointestinal Symptom Rating Scale

*None had a family history of coeliac disease

3.4.3. AGA-positivity and co-morbidity (I-II)

Medical files were equally available for AGA-positive study subjects and AGA-negative controls, 361 out of 381 (95%) and 358 out of 381 (94%), respectively. The AGA-positive and -negative groups were comparable in respect of age, body mass index and blood haemoglobin levels. 58% were female in the AGA+ anti-TG2+ group and 46% in other groups (Table 8).

AGA-positive subjects suffered more often from malabsorption, pernicious anaemia, osteopenia/osteoporosis, autoimmune hyperthyroidism, stomach cancer, sarcoidosis and rheumatoid arthritis than AGA-negative (Table 8). The overall risk of malignancy did not differ between the groups (Table 8). Two in the AGA-positive group had intestinal lymphoma, one of B-cell origin (in the AGA+ anti-TG2- group, without coeliac disease) and the other of T-cell origin (in the AGA+ anti-TG2+ group with coeliac disease). None in the AGA- anti-TG2- control group had lymphoma. In addition to the data presented in Table 8, there was no difference in the occurrence of diabetes mellitus, fibromyalgia, cardiovascular and liver diseases, inflammatory bowel disease, psoriasis, tuberculosis, atopy or allergy.

When AGA+ anti-TG2- subjects were separately compared with AGA-negative controls, significantly more cases were found suffering from rheumatoid arthritis even when known coeliac disease patients were excluded from the analysis (Table 8). Furthermore, there was a trend for AGA-positivity without anti-TG2 to be related to osteopenia/osteoporosis ($p=0.064$) (I).

Persistently AGA-positive subjects ($n=49$) and persistently AGA-negative controls ($n=52$) had similar family and disease histories, including autoimmune diseases, malignancies and bone disease (Table 9). The median BMI in the AGA-positive group was 26.9 kg/m^2 (range 17.4 – 42.7) and 26.7 kg/m^2 (range 19.7 – 51.5) in the AGA-negative control group ($p=0.724$) (II).

Table 8. Study subject characteristics and morbidity in anti-gliadin antibody (AGA)-positive subjects with and without transglutaminase-2 antibodies (anti-TG2), and in AGA- and anti-TG2-negative controls

Study subject characteristics. Reported morbidity.	AGA+ anti-TG2+ (n=38)		AGA+ anti-TG2- (n=343)		AGA- anti-TG2- (n=381)		p-value*
	IgA or IgG AGA-positive		IgA and IgG AGA-negative				
	n	† %	n	† %	n	† %	
Female; n (%)	22	58	154	45	176	46	
Age; median (range), years	66 (56 - 81)		69 (54 - 81)		69 (53 - 81)		
Haemoglobin; mean (range), g/l	138 (108 - 164)		140 (68 - 174)		140 (69 - 176)		
Body mass index; mean (range), kg/m ²	25.4 (18.4 - 37.5)		27.0 (17.0 - 56.0)		26.9 (16.7 - 47.5)		
Coeliac disease	34	89.5	5	1.6	0	0	<0.001
Rheumatoid arthritis	1	2.7	10	3.1 §	2	0.6	0.025
Depression	3	8.1	19	5.9 §	9	2.5	0.031
Osteopenia/osteoporosis	17	45.9	20	6.2 #	11	3.1	<0.001
Malabsorption	11	29.7	2	0.6	1	0.3	<0.001
Vitamin-B12 deficiency	7	18.9	9	2.8	3	0.8	<0.001
Autoimmune hyperthyroidism	4	10.8	3	0.9	2	0.6	0.001
Stomach cancer	2	5.4	3	0.9	1	0.3	0.019
Sarcoidosis	3	8.1	4	1.2	4	1.1	0.024
At least one low-energy fracture	2	5.4	47	14.6	40	11.1	0.187
Malignancy, any site	9	24.3	46	14.3	64	17.8	0.193
Polynuropathy, all causes	0	0	6	1.9	5	1.4	0.868
Dementia, all causes	1	2.7	9	2.8	9	2.5	0.930
Autoimmune hypothyroidism	2	5.4	14	4.4	12	3.3	0.615

*comparison between all three groups, †denominator varies depending on the available data, §differences between AGA+ anti-TG2- and AGA- anti-TG2- groups statistically significant (p=0.016 and p=0.032, respectively), # difference between AGA+ anti-TG2- and AGA- anti-TG2- groups borderline significant (p=0.064)

Table 9. Demographic data and disease history in persistently AGA- and coeliac-type HLA-positive study subjects and in AGA-negative controls. All were also anti-TG2-negative and did not have previously diagnosed coeliac disease.

	Persistently AGA-positive and HLA-positive n=49	Persistently AGA- negative n=52
Female, n (%)	22 (45)	23 (44)
Median age (range), years	69 (57 - 80)	69 (57 - 81)
Family history of coeliac disease, n (%)	3 (6)	3 (6)
Associated immunological disorders, n (%)		
Autoimmune thyroid disorder	2 (4)	2 (4)
Rheumatoid arthritis, juvenile rheumatoid arthritis	3 (6)	2 (4)
Sjögren's syndrome	1 (2)	1 (2)
Psoriasis	1 (2)	3 (6)
Sarcoidosis	2 (4)	0 (0)
Primary osteopenia or osteoporosis, n (%)	2 (4)	3 (6)
Low-energy fractures, n (%)	11 (22)	11 (21)
Malignancy, any site, n (%)	5 (10)	8 (15)
History of gastrointestinal diseases or † symptoms, n (%)		
Lactose intolerance	3 (6)	7 (13)
Dyspepsia	5 (10)	5 (10)
Irritable bowel syndrome	1 (2)	0 (0)
Constipation	6 (12)	5 (10)
Diarrhoea	5 (10)	2 (4)
Flatulence*	9 (18)	1 (2)
Neurological symptoms, n (%)	18 (37)	17 (33)
Neurological disease, n (%)	8 (16)	14 (27)
Polyneuropathy	4 (8)	3 (6)
Small-fibre neuropathy	3 (6)	1 (2)
Ataxia	1 (2)	0 (0)
Nerve root lesion	1 (2)	5 (10)
Migraine	2 (4)	1 (2)
Essential tremor	1 (2)	2 (4)
Ischaemic stroke	3 (6)	7 (13)
Epilepsy	0 (0)	1 (2)
Intracranial haemorrhage	1 (2)	0 (0)

† data based on interview

* p=0.006

3.4.4. AGA-positivity and neurological and psychiatric disorders (I, III)

According to the medical files neither AGA-positivity nor persistent AGA-positivity was associated with neurological diseases (I, III). Based on the interview and follow-up examination persistently AGA-positive but anti-TG2-negative older subjects carrying HLA DQ2 or DQ8 did not have more neurological diseases or symptoms than AGA-negative control subjects (Table 9). Furthermore, based on the hospital records, persistently AGA-positive subjects without coeliac-type HLA did not have neurological diseases (18 out of 77, 23%) significantly more often than persistently AGA-positive cases with coeliac-type HLA (16%, Table 9) or persistently AGA-negative cases (27%) (difference between the groups, $p=0.430$). Four AGA-positive and three AGA-negative subjects suffered from polyneuropathy (Table 10). Only one AGA-positive subject, a 79-year-old male, had ataxia. He had suffered an ischaemic stroke six years previously with residual mild aphasia and mild right-sided hemiparesis. In brain magnetic resonance imaging, profound cerebral and cerebellar atrophy was found along with cerebral ischaemic white matter lesions. In addition, his erythrocyte folic acid concentrations were low. He refused duodenal biopsy and a treatment trial with a gluten-free diet. In the clinical examination mild cognitive problems were suspected in three AGA-positive and two AGA-negative subjects, but none evidenced dementia in further neuropsychological evaluations. In the AGA-positive group neurological findings were not related to small-bowel mucosal coeliac disease-type morphological or inflammatory changes (data not shown). In addition, according to medical files three persistently AGA-positive subjects who did not have coeliac-type HLA suffered from polyneuropathy; none had ataxia. (III)

Altogether 12 (25%) out of the 48 persistently AGA-positive but anti-TG2-negative subjects with coeliac-type HLA had antibodies against TG6; seven were anti-TG6-positive in IgA-class, three in IgG-class, and two were positive in both IgA- and IgG-classes. Two of the anti-TG6-positive cases had polyneuropathy and

small-fibre neuropathy (Table 10), while the rest did not evince neurological dysfunction. **(III)**

In the first study based on medical records comparing AGA-positive and AGA-negative subjects, AGA-positivity seemed to be related to depression, but not to other psychiatric disorders (Table 8) **(I)**. In the clinical study comparing persistently AGA-positive subjects with coeliac-type HLA with persistently AGA-negative controls only one subject in the latter group reported depression in her past history; no other previously diagnosed psychiatric disorders were reported in the study or control groups. There were no statistically significant differences between AGA-positive and AGA-negative subjects in respect of psychological well-being or quality of life as measured by PGWB and SF-36 questionnaires (Table 3 in original publication **III**). Furthermore, persistently AGA-positive subjects with coeliac-type HLA did not suffer from depression more often than persistently AGA-negative controls as assessed by DEPS score (Figure 3). There were no differences in DEPS scores between the persistently AGA-positive subjects who evinced coeliac-type small-bowel mucosal minor changes, those who had entirely normal small-bowel mucosa or those who did not undergo small-bowel biopsy ($p=0.898$). In addition, according to medical files AGA-positive but coeliac-type HLA-negative subjects did not have depression more often (8%) than the AGA-negative control group ($p=0.241$). **(III)**

Table 10. Clinical details of persistently AGA-positive cases and AGA-negative controls evincing polyneuropathy.

Sex Age	Small-bowel mucosal biopsy	Type of neuropathy	Clinical findings	Medical history and laboratory findings
<i>Persistently AGA-positive with coeliac-type HLA</i>				
F 78	nd	Axonal polyneuropathy and small-fibre neuropathy	Absent achilles tendon reflexes, mild balance problem	High vitamin-B6 IgA anti-TG6-positive
M 80	Normal	Axonal polyneuropathy and small-fibre neuropathy	Ostostatic hypotension, absent achilles tendon reflexes, reduced sensation of touch	Antiganglioside antibodies positive, prostate cancer IgG anti-TG6-positive
F 80	Minor mucosal inflammation	Axonal polyneuropathy	Absent achilles tendon reflexes, mild balance problem, reduced sensation of touch, mild muscle weakness	Deficiency of vitamin-D and folic acid Anti-TG6-negative
M 79	Normal	Axonal polyneuropathy and small-fibre neuropathy	Absent achilles tendon reflexes, mild balance problem	Deficiency of vitamin-E and -B12, DM II, prostate cancer Anti-TG6-negative
<i>Persistently AGA-negative</i>				
M 79	nd	Polyneuropathy*	Mild balance problem, reduced sensation of vibration and touch, absent achilles tendon reflexes	Prostate cancer, polymyalgia rheumatica
M 69	nd	Axonal polyneuropathy and small-fibre neuropathy	Mildly unsteady gait, mildly reduced sensation of vibration	
M 59	nd	Axonal polyneuropathy	Mild balance problem, reduced sensation of vibration, absent achilles tendon reflexes	Primary hyperparathyreosis, DM II

F, female; M, male; nd, not done; anti-TG6, transglutaminase-6 antibodies; DM II, diabetes mellitus type-II

* diagnosed 2 years before entering the study; skin biopsy not performed

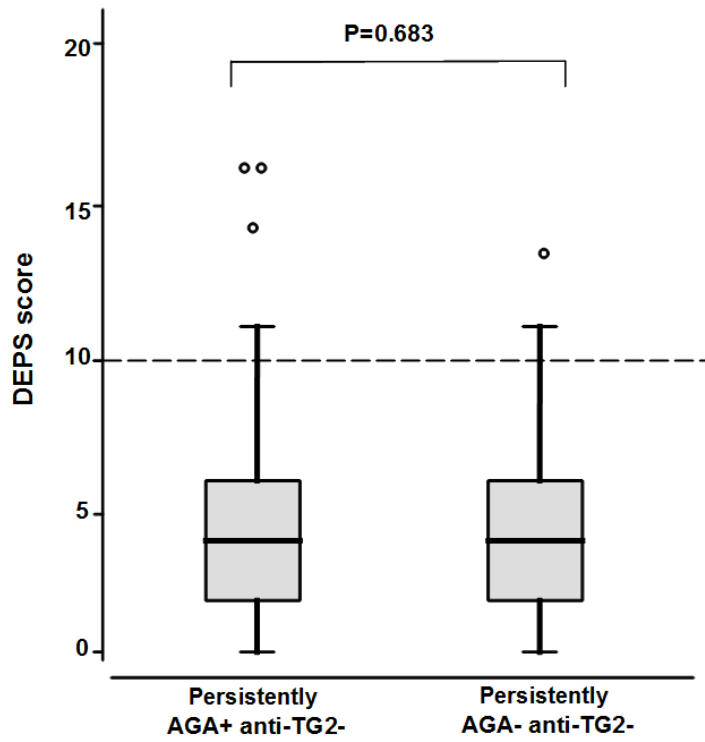


Figure 3. The Depression Scale (DEPS) values in persistently AGA-positive but anti-TG2-negative subjects and persistently AGA- and anti-TG2-negative controls. Dotted horizontal line indicates cut-off level for any level of depression.

4. DISCUSSION

4.1 AGA-positivity in the older population

This study showed that AGA-positivity is common (14%) in the older population and often persists. In previous studies, likewise AGA-positivity has been common, even in healthy adult blood donors (Grodzinsky et al. 1992, Hadjivassiliou et al. 2003b). In this study AGA-positivity was more constant than in earlier studies, which have reported that 40-60% of AGA-positive subjects convert to seronegative in follow-up (Johnston et al. 1998, Laass et al. 2006, Simell et al. 2007). The study populations in the earlier studies have been younger (Johnston et al. 1998, Laass et al. 2006, Simell et al. 2007); the long exposure to gluten in this study population may have made for the difference. Only 10% of 381 AGA-positive subjects here also had anti-TG2-antibodies. The correlation of AGA with DGP-AGA was even poorer, since only 2.4% of persistently AGA-positive subjects were also DGP-AGA-positive.

4.2 AGA-positivity, coeliac disease and gastrointestinal symptoms in the older population

In this study population only ten per cent of AGA-positive subjects had coeliac disease, which underlines the poor specificity of AGA tests in screening for coeliac disease. AGA-positivity without anti-TG2 did not predict overt coeliac disease despite long gluten exposure. Among persistently AGA-positive subjects with a genetic predisposition to coeliac disease and no previous coeliac disease diagnosis only one was discovered to have overt coeliac disease. Interestingly, over half of those who agreed to endoscopy and small-bowel mucosal biopsy had inflammatory changes in the duodenal mucosa similar to those described in coeliac disease (Järvinen et al. 2003), but also in other conditions (Aziz et al. 2010). It is noteworthy that small-bowel mucosal inflammation in these study subjects was not associated with ventricular inflammation or any other medical conditions reported by the study subjects. Whether these individuals will develop coeliac disease when continuing on a gluten-containing diet remains an open question (Vande Voort et al. 2009, Aziz et al. 2010). The prevalence of mild inflammatory changes in the duodenal mucosa in the older general population is not known and for ethical reasons biopsies could not be performed on AGA-negative healthy controls having no suspicion of coeliac disease. However, in a Swedish population-based study the prevalence of duodenal mucosal inflammation in seronegative (anti-TG2- and EMA-negative) adults was 3.8% (Walker 2010). This suggests that persistently AGA-positive subjects with coeliac-type HLA in the present study really had excessive small-bowel mucosal inflammation.

In this study persistently AGA-positive subjects with coeliac-type HLA had more gastrointestinal symptoms than AGA-negative subjects irrespective of small-bowel biopsy findings. *H. pylori* gastritis could have been the cause of the symptoms, but it did not correlate with symptoms in this study population and was not more common in AGA-positive individuals than in the Finnish population at large (Salomaa-

Räsänen et al. 2010) and thus does not explain the gastrointestinal symptoms. It has been hypothesized that gluten can induce functional bowel symptoms (Kaukinen et al. 2000, Wahnschaffe et al. 2001, Verdu, Armstrong & Murray 2009, Biesiekierski et al. 2011). A previous study of irritable bowel syndrome (IBS) patients has shown that AGA-positive IBS patients with coeliac-type HLA may benefit from a gluten-free diet (Wahnschaffe et al. 2001). IBS patients may also evince mild inflammation in their morphologically normal small-bowel mucosa and a link between potential coeliac disease and IBS has been speculated (Wahnschaffe et al. 2007, Verdu, Armstrong & Murray 2009, Aziz et al. 2010). An abnormal immunological reaction to gluten and increased gut permeability has been shown in IBS patients and is thought to cause their gastrointestinal symptoms (Cinova et al. 2007, Zhou et al. 2010). AGA-positivity has also been linked to increased gut permeability, although results have been controversial (Bonamico et al. 1997, Wolters et al. 2010). Interestingly, in an animal model of gluten sensitivity, AGA- and HLA DQ8-positive transgenic mice have been shown to develop neuromotor, permeability and low-grade inflammatory changes in the small-intestinal mucosa, but no atrophy (Verdu et al. 2008). Since a gluten-free diet was not tested in this study, it remains obscure whether the gastrointestinal symptoms in persistently AGA-positive subjects with coeliac-type HLA in this study population were gluten-dependent or not.

4.3 AGA-positivity and co-morbidity

According to medical files AGA-positivity was associated with malabsorption, pernicious anaemia, osteopenia/osteoporosis, autoimmune hyperthyroidism, stomach cancer, sarcoidosis and rheumatoid arthritis. With the exception of rheumatoid arthritis, the marked prevalence of these conditions could be explained by co-occurrence of coeliac disease in the AGA-positive study population. The finding of an increased prevalence of rheumatoid arthritis in AGA-positives without evidence of coeliac disease is in fact supported in the literature. AGA has been found in rheumatoid arthritis patients having normal small-bowel mucosal villous

morphology (O'Farrelly et al. 1988, Paimela et al. 1995), and interestingly, a gluten-free diet has been reported to alleviate symptoms in rheumatoid arthritis patients without coeliac disease in a randomized trial (Hafström et al. 2001). AGA has also been found in the intestinal fluid of rheumatoid arthritis patients more frequently than in healthy controls, suggesting a link between ingested gliadin and autoimmune joint disease (Hvatum et al. 2006).

There was a trend towards an increased prevalence of bone disease in this AGA-positive older population. Osteomalasia, osteopenia/osteoporosis and an increased risk of fractures are well known complications of coeliac disease and a gluten-free diet has a beneficial effect on bone (Bianchi, Bardella 2008). In this study AGA-positivity seemed to be related to bone disease even when coeliac disease and anti-TG2-positive subjects were excluded from the analysis. This could be associated with inflammation in the gut, leading to a deficiency of growth factors and cytokine production and a resulting imbalance in bone metabolism with subsequent osteoporosis, as has been proposed in the case of coeliac disease (Taranta et al. 2004, Bianchi, Bardella 2008).

However, when persistently AGA-positive were compared to AGA-negative controls in the clinical examination study, no difference in the occurrence of bone disease or any other diseases, including rheumatoid arthritis, other autoimmune diseases and malignancies, was verified. Since the AGA-positive and -negative study groups were otherwise comparable, the difference in results between the medical files-based study and the clinical study may be due to smaller study groups in the latter, where small differences in the prevalences of diseases do not become statistically significant.

4.4 AGA-positivity and neurological and psychiatric well-being

In this Finnish older population AGA-positivity was not associated with neurological diseases. No difference in the occurrence of neurological disorders was noted between AGA-positive and AGA-negative subjects whether they were coeliac-type HLA-positive or not. In thorough clinical examination neurological conditions were discovered equally in persistently AGA-positive and AGA-negative study subjects, which would indicate that AGA is unspecific for coeliac disease and gluten-related neurological conditions. This is in contrast to what has been proposed in the context of previous more selected patient materials (Hadjivassiliou et al. 2010). Gluten sensitivity-related neurological diseases appear to be very rare in the general population.

Although this study did not show persistently AGA-positive subjects to have more neurological disorders than controls, it is interesting that some AGA-positive subjects with neurological disorders yielded laboratory results previously reported to be associated with coeliac disease and its neurological manifestations (Table 10). One neuropathy patient had antiganglioside antibodies (Volta et al. 2006) and two were anti-TG6-positive (Hadjivassiliou et al. 2008, Hadjivassiliou et al. 2013). Altogether three had malabsorption of vitamins and one of these three evinced minor small-bowel mucosal inflammation. Although other conditions such as diabetes, hypervitaminosis-B6 or cancer may have caused the neurological disorders in these individuals, it cannot be ruled out that gluten sensitivity may have played a role and predisposed the patients to develop neuropathy or ataxia more easily.

While in other studies anti-TG6 has appeared to be specific for gluten sensitive-neurological manifestations (Hadjivassiliou et al. 2008, Hadjivassiliou et al. 2010), here it was not associated with neurological symptoms or disorders. Methodological differences might partly explain this, since a commercial test was used in this study while others have used in-house assays (Hadjivassiliou et al. 2008) and it is conceivable that the antigens used in the different tests may vary (Lindfors et al.

2011). Similarly, minor variability has previously been reported between commercial anti-TG2 ELISA tests from different manufacturers (Van Meensel et al. 2004). In coeliac disease anti-TG2-positivity often predicts or anticipates the development of small-bowel mucosal atrophy and full-blown coeliac disease (Salmi et al. 2006a). It remains to be seen whether the anti-TG6-positive subjects found in this study develop gluten-dependent neurological disorders during the follow-up.

Depression and mood alterations have been linked to coeliac disease (Hallert, Derefeldt 1982, Ciacci et al. 1998, Addolorato et al. 2001, Ludvigsson et al. 2007a) and small-bowel mucosal inflammation (Kurppa et al. 2010). In study **I** a link between AGA-positivity and depression was shown based on the hospital records of AGA-positive and -negative controls. However, when persistently AGA-positive and -negative subjects were personally interviewed and assessed by questionnaires, no difference in the occurrence of depression or in psychological well-being emerged (**III**). There was also no difference in the past history of depression between persistently AGA-positive but coeliac-type HLA-negative and AGA-negative subjects. Psychiatric disorders associated with AGA might not be coeliac-type HLA-dependent (Samaroo et al. 2010). Indeed, it has recently been shown that gluten sensitivity extends beyond coeliac-type HLA (Biesiekierski et al. 2011, Sapone et al. 2011). Moreover, in study **III** the participants were asked to contact the investigator personally and attend for laboratory and clinical examination. This may have left depressed and poorly functioning subjects out of the study, which could explain the difference in results in studies **I** and **III**.

4.5 Limitations and strengths of the study and future objectives

This study material represents well the Finnish older population. The original study cohort was large and randomly selected with equal numbers of both sexes and representatives of all age groups invited. Sixty-four per cent of the original

population invited is represented in this study. Controls came from the same study population and were randomly selected from a large group of seronegative subjects and were age- and sex-matched to cases. Päijät-Häme Central Hospital is the only secondary referral centre in the area with over 210 000 inhabitants. Practically all residents requiring specialist care are remitted to this hospital. Medical files were available in the hospital records for almost all (95%) study subjects and equally for cases and controls. All records were thoroughly analyzed using a structured data-collecting formula. All study subjects attending the clinical examination were interviewed and examined by the same investigator in a structured and consistent manner. All study subjects in studies II-III filled in the same validated and widely used questionnaires. In a word, the different groups in this study were comparable.

Since participation in this study was based on the consent and personal initiative of the study subjects, it is probable that physically or mentally poorly functioning and institutionalized persons did not take part. This leaves the most seriously ill individuals out of the study population, which explains why such disease entities as dementia, mental retardation, severe ataxia and severe psychiatric diseases were rarely reported. This selection bias was relevant especially in studies II-III.

Medical records were used to collect data in study I. Whether similar diagnostic criteria for various diseases have been applied to all study subjects cannot be guaranteed. This uncertainty, however, concerns both cases and controls, and thus has no effect when the groups are compared with each other.

Due to the study design and the concern to focus on the clinical relevance of persistent AGA-positivity in individuals genetically predisposed to coeliac disease, the sizes of the study groups in studies II-III were relatively small. This lowers the statistical power of the study to detect small differences between the groups.

The study population was old and since gastroscopy with biopsies is an invasive and burdensome investigation, it was for ethical reasons decided that gastroscopy and small-bowel mucosal biopsies would be offered only to persistently AGA-positive subjects with a genetic predisposition to coeliac disease. It was therefore impossible to compare biopsy results between AGA-positive and negative subjects. Duodenal mucosal lymphocytosis has been associated with consumption of non-

steroidal anti-inflammatory drugs (Vande Voort et al. 2009, Aziz et al. 2010). Though the use of these drugs could not be reliably traced through the study subjects, nevertheless, the study groups had equal histories of musculo-skeletal and other chronic diseases (except gastrointestinal complaints), and it is thus unlikely that the consumption of non-steroidal anti-inflammatory drugs would explain the results in persistently AGA-positive subjects. Small-bowel mucosal biopsies were taken from the distal duodenum, as previously recommended. However, there is recent evidence that in some anti-TG2-positive individuals villous atrophy can be found only in the duodenal bulb while the more distal duodenum is normal (Gonzalez et al. 2010). Whether such a phenomenon could also have been found in AGA-positive cases remains obscure.

As no intervention with a gluten-free diet was carried out in this study, it is not clear whether small-bowel minor mucosal changes or gastrointestinal symptoms were gluten-dependent in the persistently AGA-positive study population with coeliac-type HLA. A gluten-free diet was offered to the single ataxia case in the AGA-positive group, but he refused. Thus in this study the effect of a gluten-free diet on neurological disorders in AGA-positive subjects was not assessed.

At the time of sera collections in 2002 and 2005, analysis of anti-TG6 was not available and therefore the prevalence of anti-TG6 could not be compared between AGA-positive and -negative subjects. Extra serum samples were collected from AGA-positive subjects attending clinical examination for scientific purposes. These sera were used in analysis of anti-TG6 when the test came into use. For this reason data on anti-TG6 were not available for all study subjects. It remains to be seen whether the anti-TG6- and AGA-positive subjects found in this study develop gluten-dependent neurological disorders in the follow-up.

The present study revealed that immune reactivity against gliadin is common in the population. It rarely indicates overt coeliac disease but seems to be related to gastrointestinal symptoms in coeliac-type HLA-positive subjects. AGA-positivity seemed also to be associated with rheumatoid arthritis. This study could not prove that gluten sensitivity is related to neurological or psychiatric disorders at population level. Further researches are needed to assess the value of AGA analysis in the

diagnostic work-up of patients with neuropathy, ataxia or psychiatric problems. The therapeutic effect of a gluten-free diet should be evaluated in these disorders and also in the context of the small-bowel mucosal inflammatory changes discovered in this study.

5. SUMMARY AND CONCLUSIONS

This large population-based study evaluated the clinical relevance of AGA-positivity in an older population exposed to gluten for decades. The findings showed that AGA-positivity is common in the general older population, but rarely indicates overt coeliac disease when other coeliac antibodies are negative. Only 10% of all AGA-positive and 2.8% of those persistently AGA-positive who had coeliac-type HLA but no previous coeliac disease diagnosis or TG2-antibodies were found to have coeliac disease. Interestingly, over half of the persistently AGA-positive subjects who were genetically predisposed to coeliac disease evinced inflammatory small-bowel mucosal changes similar to what has been described in coeliac disease. Persistently AGA-positive subjects with coeliac-type HLA also had more gastrointestinal symptoms than persistently AGA-negative controls. However, there was no correlation between gastrointestinal symptoms and small-bowel mucosal findings.

AGA without other coeliac antibodies was associated with rheumatoid arthritis and depression in the first study comparing medical records-based data on AGA-positive subjects and AGA-negative controls. Osteoporosis or osteopenia was also more common in AGA-positive subjects, though the difference was not statistically significant. Persistently AGA-positive subjects and AGA-negative controls did not differ in respect of family or medical history, body mass index or the occurrence of neurological diseases. Only one subject with AGA-positivity and ataxia was found in this large study population. Neuropathy was found in four persistently AGA-positive subjects but also in three controls. Psychological well-being measured by PGWB, DEPS and SF-36 questionnaires was equal in persistently AGA-positive and persistently AGA-negative controls.

To conclude, AGA-positivity is common and often persistent in the older population exposed to gluten for decades. However, isolated AGA-positivity is rarely clinically relevant. At population level AGA-positivity would not appear to be related to neurological disorders. According to the literature, coeliac disease should be screened for in selected cases with neuropathy or ataxia, especially if other coeliac disease-related symptoms such as malabsorption or gastrointestinal symptoms are present. However, more studies of gluten sensitivity and neurological symptoms in unselected patient materials are needed before screening with AGA or anti-TG6 in neurological patients can be recommended. Further studies are also needed to explore whether small-bowel mucosal inflammatory changes and gastrointestinal symptoms detected in persistently AGA-positive subjects with coeliac-type HLA are gluten-dependent.

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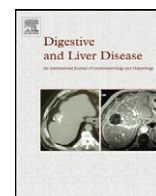
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Alimentary Tract

Persistently positive gliadin antibodies without transglutaminase antibodies in the elderly: Gluten intolerance beyond coeliac disease

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ABSTRACT

Background: The specificity of the conventional gliadin antibody test is considered low.

Aims: We explored whether gliadin antibody (AGA)-positivity without tissue transglutaminase antibodies (tTGA) is persistent in the elderly population and whether such positivity indicates overt or potential coeliac disease in genetically predisposed individuals.

Methods: AGA and tissue transglutaminase antibody were measured in 2089 elderly individuals twice with a three-year interval. AGA-positive but tissue transglutaminase antibody-negative subjects with coeliac-type human leucocyte antigen (HLA) were examined and underwent gastroduodenal endoscopy (cases). Small-bowel mucosal villous morphology and densities of CD3+ and $\gamma\delta$ + intraepithelial lymphocytes and the occurrence of tissue transglutaminase-specific IgA deposits were analysed. Randomly selected persistently AGA-negative age- and sex-matched subjects served as controls.

Results: AGA-positivity was persistent in 81% of those initially positive. Amongst the 49 clinically studied and 36 endoscoped cases only one (2.8%) had coeliac disease. Many (54%) showed signs of inflammation in the biopsy, without villous atrophy. Coeliac-type HLA was not over-represented in the persistently AGA-positive compared to the general population. Persistently AGA-positive coeliac-type HLA-positive subjects had more gastrointestinal symptoms than AGA-negative controls.

Conclusions: AGA-positivity is often persistent. Overt coeliac disease is seldom found behind persistent AGA-positivity, but this characteristic is associated with mucosal inflammation and gastrointestinal symptoms at least in HLA-positive individuals.

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1. Introduction

The diagnosis of coeliac disease is challenging, as patients may present with a wide range of gluten-triggered symptoms. Serological markers have been used in screening to select individuals for diagnostic small-bowel biopsy. Antibodies against the triggering agent wheat gluten, gliadin, have traditionally been used, but are of low sensitivity and specificity, and more accurate autoantibody tests, tissue transglutaminase (tTGA) and endomysial antibody (EMA) assays, have since been adopted [1]. Moreover, intensive screening policy now also detects subjects with positive antibodies

but without diagnostic small-bowel mucosal villous atrophy. In coeliac disease mucosal damage evolves gradually from initial minor inflammatory and morphological changes, and it may take years before diagnostic villous atrophy develops [2]. Positive tTGA and EMA in subjects with normal small bowel mucosal morphology may help in predicting forthcoming villous atrophy and coeliac disease [3–5]. Meanwhile, the relevance of positive gliadin antibodies (AGA) has remained obscure.

When AGA-positive individuals with normal small-bowel mucosal villous architecture have been followed up with repeated biopsies, some have developed villous atrophy and coeliac disease whilst continuing on a gluten-containing diet [6,7]. Although some studies amongst AGA-positive subjects genetically predisposed to coeliac disease have shown small-bowel mucosal morphological changes similar to those found in early-stage coeliac disease [8,9],

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others hold positive AGA to be merely an unspecific immunological reaction to a food antigen, not reflecting intestinal damage [10]. Furthermore, follow-up studies have suggested that AGA-positivity is often transient and rarely indicative of subsequent coeliac disease [11,12].

As the development of coeliac disease takes time, the question arises, whether the presence of AGA especially in the elderly could indicate coeliac disease even without positive tTGA. In a recent study we found AGA-positivity to be common (14%) in the elderly Finnish population [13]. Positive AGA was indicative of coeliac disease when tTGA coexisted, but according to data extracted from the medical records this was not the case with isolated AGA-positivity. However, positive AGA without tTGA was related to coeliac disease-associated conditions such as depression, arthritis and bone disease [13]. This prompted us to explore whether AGA-positivity without positive tTGA is a permanent phenomenon in the elderly population and whether such persistently AGA-positive subjects, who have been exposed to gluten for decades, have signs of overt or early stage coeliac disease. The present study is a follow-up study of the previously described elderly cohort three years after the initial screening [13]. Persistently AGA-positive subjects with coeliac-type human leucocyte antigen (HLA) were further invited to attend for a follow-up examination and upper gastrointestinal endoscopy. We focused especially on assessing self-perceived abdominal symptoms and coeliac-type small-bowel mucosal minor inflammatory changes as recent studies have shown that coeliac disease is not restricted to villous atrophy, and mild mucosal changes may predict the future development of villous atrophy [3–7]. The correlation of persistently positive conventional AGA with deamidated gliadin peptide antibodies (DGP-AGA) was

also explored, as this test has recently emerged as a more specific screening approach than conventional AGA [14,15].

2. Methods

2.1. Patients, controls and study design

Originally altogether 4272 randomly selected individuals born in the years 1926–1930, 1936–1940 and 1946–1950 and living in the Päijät-Häme Central Hospital district were invited to participate in a research project on ageing and well-being (Good Aging in the Lahti region=GOAL)[16]; the study cohort was representative of the general population in the respective age groups. Of those approached 2815 agreed to participate; they filled in questionnaires on their health condition, present and past diseases and special diets. In 2002, sera were collected for the analysis of IgA- and IgG-class AGA and IgA-class tTGA [13]. Three years later, all eligible subjects were asked to undergo a new serological test, and 2216 consented (Fig. 1). Serum samples were available in both 2002 and 2005 for altogether 2089 subjects. As coeliac disease is strongly linked to HLA DQ2 and DQ8 haplotypes [17], it was hypothesized that those persistently AGA-positive subjects who have also coeliac-type HLA might have overt or potential coeliac disease. Therefore, all persistently (in 2002 and 2005) AGA-positive but tTGA-negative subjects without known coeliac disease were offered HLA-testing. Those genetically predisposed to coeliac disease were invited for a clinical examination and upper gastrointestinal endoscopy with small-bowel mucosal biopsies (study group, cases). Equal numbers of randomly selected persistently AGA- and tTGA-negative subjects of the same age and gender were

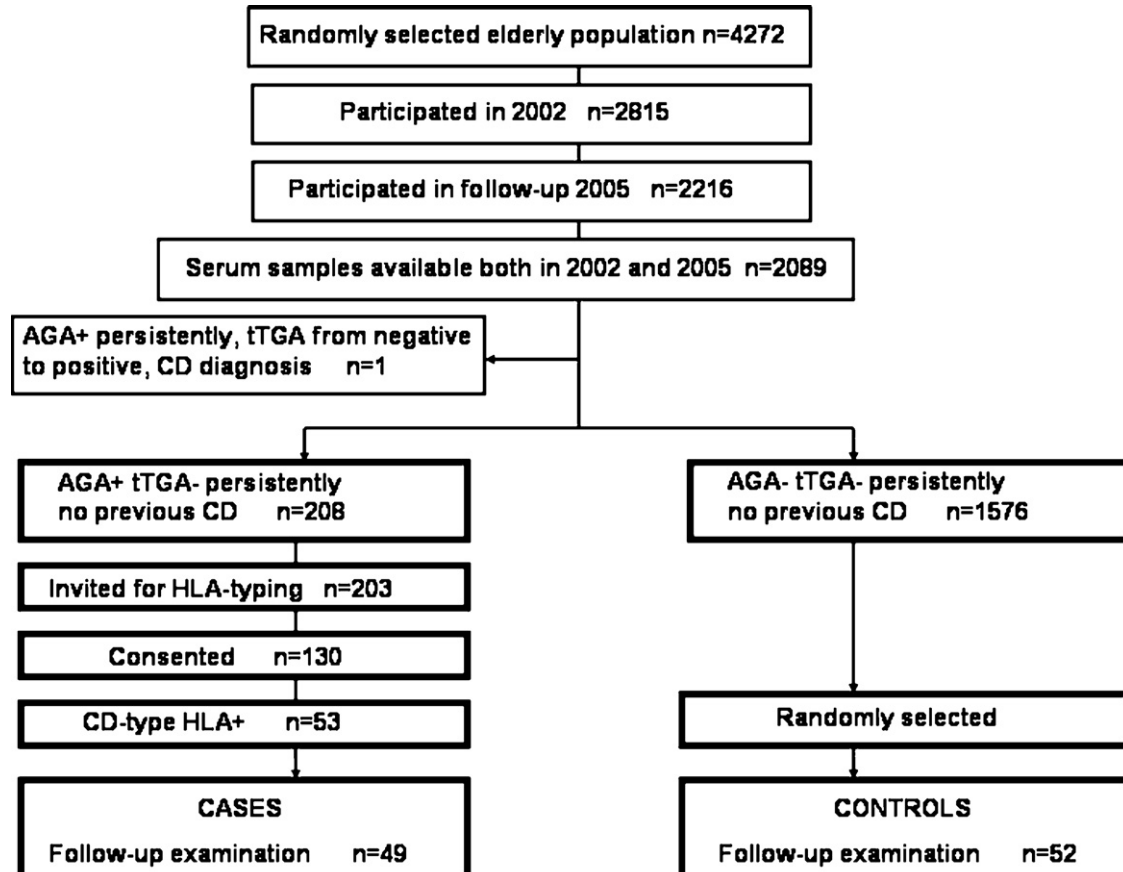


Fig. 1. Flowchart of the study.

invited for clinical examination (control group). For ethical reasons, HLA-typing and endoscopy were not performed on control subjects. Furthermore, DGP-AGA testing was performed retrospectively in persistently AGA+ tTGA– and AGA– tTGA– serum samples collected in 2005.

2.2. Serology

Serum IgA- and IgG-class AGA were investigated by enzyme-linked immunosorbent assay (ELISA) [18]; the results were obtained from the standard curve established according to dilutions of positive reference serum and converted to concentrations of arbitrary ELISA units per millilitre (EU/ml). The limits of positivity were set at 0.20 EU/ml and 20 EU/ml, respectively. Serum IgA-class tTGA was investigated by ELISA according to the manufacturer's instructions (Celikey; Phadia, Freiburg, Germany), a unit value ≥ 5 U being positive. An ELISA kit recognizing composites of IgA- and IgG-class DGP-AGA (QUANTA Lite™ Gliadin DGP Screen; Inova Diagnostics, San Diego, CA) was applied, and unit values ≥ 20 U were considered positive as advocated by the manufacturer.

2.3. HLA-testing

The study subjects were genotyped for HLA-DQB1*02, DQB1*0302 and DQA1*05 alleles using the DELFIA Coeliac Disease Hybridization Assay (PerkinElmer Life and Analytic Sciences, Wallac Oy, Turku, Finland). The genotypes DQB1*02 and DQA1*05 correspond to the serological HLA type DQ2 and DQB1*0302 to HLA-DQ8. These genotypes together or DQB1*02 alone are found in 96–100% of coeliac disease patients [17], and in this study are considered to constitute coeliac-type HLA.

2.4. Clinical examination

All cases and controls were examined and interviewed in similar manner by the same investigator. Previous medical conditions, gastrointestinal symptoms and a family history of coeliac disease were recorded. Weight and height were measured and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres (normal range: 18–25 kg/m²).

The Gastrointestinal Symptom Rating Scale (GSRS) questionnaire [19] was used to assess current gastrointestinal symptoms. It comprises altogether 15 items within five subdimensions describing diarrhoea, indigestion syndrome, constipation, abdominal pain and gastro-esophageal reflux. Each item is graded from one to seven, a higher score indicating more gastrointestinal symptoms. The questionnaire has previously been validated [20] and applied in the assessment of gastrointestinal symptoms in coeliac disease [21].

2.5. Upper gastrointestinal endoscopy and small-bowel mucosal biopsies

At esophago-gastroduodenoscopy, four small-bowel mucosal forcep biopsies were taken from distal duodenum. Three samples were fixed in formalin, processed and stained with haematoxylin and eosin and small-bowel mucosal morphology was studied under light microscopy in several well-oriented biopsy sections. Poorly oriented sections were discarded, and if necessary the samples were dissected again until they were of good quality. Villous height and crypt depth ratio (Vh/CrD) was determined as previously described, and Vh/CrD < 2.0 was considered compatible with villous atrophy and overt coeliac disease [4,5].

One sample was snap-frozen and embedded in optimal cutting temperature compound (OCT, Tissue-Tec, Miles Inc., Elkhart, IN) and stored at -70°C . Immunohistochemical stainings were carried

out on 5- μm -thick frozen sections. CD3+ intraepithelial lymphocytes (IELs) were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA), and $\gamma\delta$ + IELs with TCR γ antibody (Endogen, Woburn, MA). Positive IELs were counted at a magnification of 100 throughout the surface epithelium and at least 30 fields were counted; results were indicated as IEL density cells/mm of epithelium as previously described [5,22]. The reference values were set at 37 cells/mm for CD3+ IELs and at 4.3 cells/mm for $\gamma\delta$ + IELs [22].

Intestinal tissue transglutaminase-targeted autoantibody deposits were studied in unfixed small-bowel mucosal frozen sections. The sections were stained with direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (Dako AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate buffered saline (PBS). To establish whether there is co-localization of IgA and tissue transglutaminase, sections were double-stained with anti-IgA antibody as described above and using monoclonal mouse antibodies against tissue transglutaminase (CUB7402, NeoMarkers, Fremont, CA, USA) followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (Dako) diluted 1:200 in PBS. In coeliac disease subepithelial deposition can be found along the villous and crypt epithelium and around vessels, whereas in contrast, in normal samples IgA is found only inside plasma cells and epithelial cells [23,24]. It has previously been shown that these mucosal IgA deposits are targeted specifically against tissue transglutaminase and they have been shown to possess excellent specificity for coeliac disease even whilst the mucosal villi are still morphologically intact [23,24]. The occurrence of IgA deposits was graded semi-quantitatively according to their intensity along the basement membrane in the villous-crypt area as follows: negative, weak (+), moderate (++) and strong positive (+++).

All biopsy specimens were evaluated by the same investigator without prior knowledge of the history or findings. In this study biopsy findings were considered indicative of overt coeliac disease if villous atrophy with crypt hyperplasia was found. Increased density of small-bowel mucosal CD3+ or $\gamma\delta$ + IELs and the presence of tissue transglutaminase-targeted IgA deposits were considered as minor mucosal changes.

Mucosal biopsy samples from the stomach (corpus and antrum) were taken for routine histological assessment and *Helicobacter pylori* staining.

2.6. Statistics

Quantitative data were expressed as medians or means and ranges. Statistical differences between study groups were evaluated using Pearson's χ^2 test, Fisher's exact test, *t*-test or Mann–Whitney *U* test, as appropriate. Values of $p < 0.05$ were considered significant. The statistics were calculated with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

2.7. Ethics

The study was accepted by the research ethics committee of Päijät-Häme Central Hospital. All participants gave written informed consent.

3. Results

Altogether 2089 subjects gave serum samples both in 2002 and in 2005. In 2002, 251 of them were AGA-positive but tTGA-negative, and three years later 208 (81%) of them were still positive for AGA. In the follow-up analysis, 19 proved positive in both IgA- and IgG-class, 185 in IgA-class only, and the remaining four subjects in IgG-class only. In addition, one persistently AGA-positive subject developed tTGA-positivity at follow-up and

coeliac disease was subsequently diagnosed (Fig. 1). Five persistently AGA-positive and tTGA-negative subjects had died soon after the second serum samples were analysed. Eventually 203 persistently AGA-positive and tTGA-negative subjects were invited for HLA-typing and 130 consented and were tested. Fifty-three (41%) were found to have coeliac-type HLA, and 49 of them agreed to undergo further studies. Fifty-two persistently AGA-negative subjects participated in the clinical examination as controls (Fig. 1).

Persistently AGA-positive subjects and AGA-negative controls had similar family and disease histories, including autoimmune diseases, malignancies and bone disease (Table 1). Median BMI in the AGA-positive group was 26.9 kg/m² (range 17.4–42.7) and 26.7 kg/m² (range 19.7–51.5) in the AGA-negative control group ($p=0.724$). AGA-positive subjects reported more gastrointestinal symptoms in their past history than controls upon interview (Table 1). Current abdominal complaints as rated by GSRS total score were similarly more common in the AGA-positive group. The same trend was seen in all subdimensions, although only the reflux score differed statistically significantly between the groups (Table 2).

Thirty-six persistently AGA- and HLA-positive study subjects agreed to undergo gastroscopy and duodenal biopsy. One (2.8%) of these 36 had villous atrophy compatible with coeliac disease. He had no gastrointestinal symptoms, but markedly low serum vitamin B12 and vitamin D values. The vitamin levels normalized on a gluten-free diet. Frozen small-bowel mucosal biopsies were available for 35 study subjects. Nineteen (54%) of these evinced minor mucosal inflammatory changes in biopsy (Table 3, Fig. 2). Biopsy findings did not correlate with gastrointestinal symptoms. *H. pylori* gastritis was found in seven and atrophic gastritis in four subjects. Ventricular mucosal findings (*H. pylori* gastritis or atrophic gastritis) did not correlate with symptoms, duodenal villous atrophy or inflammatory changes (data not shown).

The DGP-AGA test results were positive in five out of 208 (2.4%) persistently AGA-positive and tTGA-negative subjects. Three of the five were HLA-tested and two had coeliac-type HLA, one having minor mucosal inflammatory changes in biopsy (no. 2 in Table 3), the other one did not undergo gastroscopy. Seventeen (1.1%) out of 1576 persistently AGA- and tTGA-negative were DGP-AGA-positive, but none of them belonged to our randomly selected control group.

4. Discussion

In this elderly population AGA-positivity was a constant (81%) finding corresponding to that in a previously published small-scale study involving patients with suspected coeliac disease [6]. By contrast, in larger screening studies also comprising younger adults and children only about 40% of AGA-positive subjects have proved persistently positive [11,12,25]. The long exposure to gluten in our study population may have made for this difference.

The positive predictive value of AGA testing in many populations has been low [1], but it has been suggested that prolonged AGA response might be a better indicator of coeliac disease than transient antibody positivity [11]. The development of small bowel mucosal deterioration in coeliac disease may take time and the disease may manifest only later in life, even at the age of 50 years or more [26]. In our study, persistent AGA-positivity without tTGA did not predict overt coeliac disease despite long gluten exposure. Persistent AGA-positivity was also not associated with more coeliac disease-specific DGP-AGA-positivity [14,15]. This is in accord with the observation that persistent AGA without tTGA is rarely a sign of overt coeliac disease.

Notably, mild mucosal inflammatory changes were found in over half of the biopsies in persistently AGA-positive subjects with coeliac-type HLA. Increased density of CD3+ IELs is not a specific finding for coeliac disease, but the presence of $\gamma\delta$ + IELs and tissue transglutaminase-specific IgA-deposits may be predictive markers for the condition [4–7]. Whether these individuals have potential coeliac disease and will develop overt disease when continuing on a gluten containing diet remains an open question [27,28]. The prevalence of mild inflammatory changes in the duodenal mucosa in the elderly general population is not known and for ethical reasons we could not perform biopsies on AGA-negative healthy controls having no suspicion of coeliac disease. However, a recent Swedish population-based study found that the prevalence of duodenal mucosal inflammation in seronegative (tTGA- and EMA- negative) adults is 3.8% [29]. This suggests that persistently AGA positive subjects with coeliac-type HLA really had excessive small-bowel mucosal inflammation. Generally duodenal mucosal lymphocytosis has also been associated with consumption of non-steroidal anti-inflammatory drugs [27,28]. We were not able to reliably trace the regular or occasional use of these drugs from our study subjects, however the study groups had equal history of musculo-skeletal

Table 1

Demographic data and disease history in persistently gliadin antibody (AGA)- and coeliac-type HLA-positive study subjects and in AGA-negative controls. All were also tissue transglutaminase antibody-negative and did not have previously diagnosed coeliac disease (CD).

	Persistently AGA-positive and HLA-positive, n = 49	Persistently AGA-negative, n = 52
Female, n (%)	22 (45)	23 (44)
Median age (range), years	69 (57–80)	69 (57–81)
Family history of coeliac disease, n (%)	3 (6)	3 (6)
Associated immunological disorders, n (%)		
Autoimmune thyroid disorder	2 (4)	2 (4)
Rheumatoid arthritis, juvenile rheumatoid arthritis	3 (6)	2 (4)
Sjögren's syndrome	1 (2)	1 (2)
Psoriasis	1 (2)	3 (6)
Sarcoidosis	2 (4)	0 (0)
Primary osteopenia or osteoporosis, n (%)	2 (4)	3 (6)
Low energy fractures, n (%)	11 (22)	11 (21)
Malignancy, any site, n (%)	5 (10)	8 (15)
History of gastrointestinal diseases or symptoms, n (%) ^a		
Lactose intolerance	3 (6)	7 (13)
Dyspepsia	5 (10)	5 (10)
Irritable bowel syndrome	1 (2)	0 (0)
Constipation	6 (12)	5 (10)
Diarrhoea	5 (10)	2 (4)
Flatulence [*]	9 (18)	1 (2)

^a Data based on interview.

^{*} $p=0.006$.

Table 2
Mean and 95% confidence intervals of Gastrointestinal Symptoms Rating Scale (GSRS) in persistently AGA- and coeliac-type HLA-positive and AGA-negative subjects without tissue transglutaminase antibodies and previous coeliac disease.

GSRS ^a	Persistently AGA+ HLA+ (n = 49)	Persistently AGA- (n = 52)	p-Value
Total score	1.98 (1.76–2.20)	1.70 (1.55–1.84)	0.035
Diarrhoea	1.72 (1.46–1.99)	1.49 (1.29–1.69)	0.159
Indigestion	2.39 (2.11–2.66)	2.16 (1.95–2.37)	0.196
Constipation	1.99 (1.62–2.35)	1.65 (1.39–1.90)	0.137
Abdominal pain	1.76 (1.56–1.96)	1.55 (1.37–1.73)	0.127
Reflux	1.88 (1.57–2.19)	1.41 (1.23–1.60)	0.011

^a Higher score indicates more symptoms.

Table 3
Coeliac disease-related small-bowel mucosal biopsy results in 20 persistently gliadin antibody-positive subjects having coeliac-type HLA.

No.	Sex	Age	HLA	Small bowel biopsy				Gastrointestinal symptoms	Other disorders ^a
				Atrophy	CD3+ IELs	$\gamma\delta$ + IELs	IgA-deposit		
1	m	57	DQ8	+	–	–	+	No	No
2	m	57	DQ8	–	+	+	+	Constipation	No
3	f	80	DQ8, DQB1*02	–	+	+	+	No	Juvenile rheumatoid arthritis
4	f	61	DQ8	–	+	+	–	Flatulence	No
5	m	69	DQ8	–	+	+	–	No	No
6	m	67	DQB1*02	–	+	+	–	No	No
7	f	58	DQ8	–	+	–	–	Constipation, diarrhoea, flatulence	No
8	f	57	DQB1*02	–	+	–	–	Diarrhoea	Osteoporosis
9	m	69	DQ8	–	+	–	–	No	No
10	m	68	DQ8	–	+	–	–	No	No
11	f	58	DQB1*02	–	–	+	+	No	Low-energy fracture
12	m	68	DQ2	–	–	–	+	No	Low-energy fracture
13	f	60	DQ2	–	–	+	–	Flatulence	No
14	m	79	DQ8	–	–	+	–	Constipation, diarrhoea, flatulence	No
15	m	69	DQ2	–	–	+	–	Lactose intolerance	No
16	m	69	DQ8	–	–	+	–	No	Rheumatoid arthritis
17	f	71	DQ8	–	–	+	–	Constipation	No
18	f	69	DQ2, DQ8	–	–	+	–	Lactose intolerance	No
19	f	69	DQ8	–	–	+	–	Dyspepsia	No
20	m	68	DQB1*02	–	–	+	–	No	No

f = Female, m = male, + = positive result, – = normal value, and IEL = intraepithelial lymphocytes.

^a None had family history of coeliac disease.

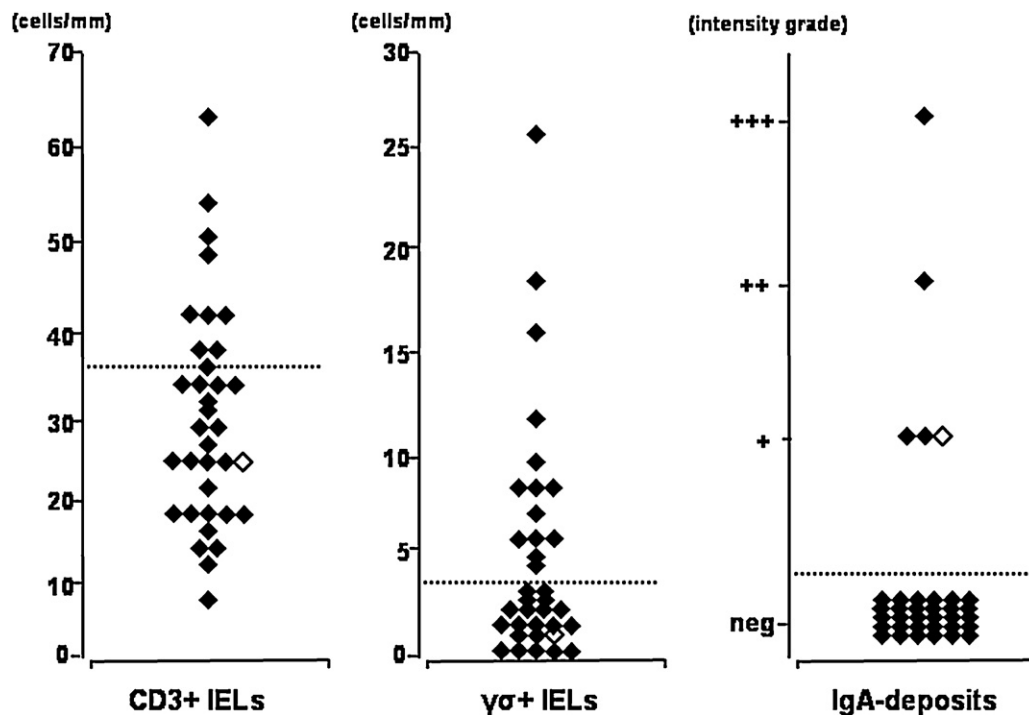


Fig. 2. Densities of CD3+ and $\gamma\delta$ + intraepithelial lymphocytes and intensity of tissue transglutaminase-specific IgA deposits. Dotted line indicates the limit of normal value. Open square indicates a subject with overt villous atrophy on biopsy.

and other chronic diseases (except gastrointestinal complaints), and thus it is unlikely that the consumption of non-steroidal anti-inflammatory drugs would explain the results in persistently AGA-positive subjects. Small-bowel mucosal biopsies were taken from the distal duodenum, as previously recommended. However, there is recent evidence that in some tTGA-positive individuals the villous atrophy can be found only from the duodenal bulb and the more distal duodenum is normal [30]. Whether such phenomenon could be also found in AGA-positive cases is not known.

In our series, most of the AGA-positive subjects had IgA-class antibodies, which is in contrast to earlier data reporting on isolated IgG-AGA positivity in non-coeliac population [12]. IgG-class AGA seems to be the first antibody to appear in genetically predisposed children and, maybe due to changes in immunological reactivity and the epitope spreading, the more coeliac-specific antibodies, IgA-class AGA and IgA-class tTGA appear later [25]. One can speculate that IgG class AGA disappears by time, but at the moment, this is only speculation. It has been also earlier suggested that IgA-class AGA positivity increases with age [31]. Studies on the mechanisms behind such immunological phenomena are required.

Interestingly, in our study AGA-positive subjects had more gastrointestinal symptoms than AGA-negative subjects irrespective of small-bowel biopsy findings. *H. pylori* gastritis was not more common in AGA-positive individuals than in the Finnish general population at large and thus does not explain the gastrointestinal symptoms in our study population [32]. It has been hypothesized that gluten can induce functional bowel symptoms [33–36]. A previous study of irritable bowel syndrome (IBS) patients has shown that AGA-positive IBS patients with coeliac-type HLA may benefit from a gluten-free diet [34]. IBS patients may also evince mild inflammation in their morphologically normal small-bowel mucosa and a link between potential coeliac disease and IBS has thus been speculated [28,35,37]. An abnormal immunological reaction to gluten and increased gut permeability has been shown in IBS patients and is thought to cause gastrointestinal symptoms [38,39]. AGA-positivity has also been linked to increased gut permeability although results have been controversial [10,40]. Interestingly, AGA- and HLA DQ8-positive transgenic mice have been shown to develop neuromotor, permeability and low-grade inflammatory changes in small intestinal mucosa but no atrophy [41]. As no intervention with a gluten-free diet was carried out in this present study, it is not known whether small-bowel minor mucosal changes or gastrointestinal symptoms were gluten-dependent in our persistently AGA-positive study population with coeliac-type HLA. As coeliac disease is strongly linked to HLA DQ2 and DQ8 haplotypes, it was hypothesized that those persistently AGA-positive subjects who do not have coeliac-type HLA are very unlikely to suffer from coeliac disease, and therefore HLA DQ2 and DQ8 negative, AGA positive subjects were not invited to further investigations. Interestingly, recently published papers suggest that gluten sensitivity might extend also beyond coeliac-type HLA [36,42] warranting more studies in the whole spectrum of gluten sensitivity, including gliadin positive subjects without coeliac-type HLA.

To conclude our findings would indicate that persistent AGA-positivity is seldom indicative of overt coeliac disease but it seems to be related to gastrointestinal symptoms. Long-term follow-up studies are needed to establish whether these persistently AGA-positive subjects with gastrointestinal symptoms are truly gluten-intolerant and would benefit from a gluten-free diet.

Conflict of interest statement

None declared.

List of abbreviations

AGA, gliadin antibodies; BMI, body mass index; CD, coeliac disease; DPG-AGA, deamidated gliadin peptide antibodies; EMA, endomysial antibodies; ELISA, enzyme-linked immunosorbent assay; GSRS, Gastrointestinal Symptom Rating Scale; HLA, human leucocyte antigen; IBS, irritable bowel syndrome; IELs, intraepithelial lymphocytes; tTGA, tissue transglutaminase antibodies; Vh/CrD, villous height and crypt depth ratio.

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Gliadin antibodies in older population and neurological and psychiatric disorders

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Objectives – A variety of neurological and psychiatric disorders have recently been linked to coeliac disease and gluten sensitivity. We here explored whether persistently positive gliadin antibodies (AGA) and coeliac-type HLA increase the risk of gluten sensitivity-related neurological and psychiatric manifestations. The study was carried out in an older population who had consumed gluten for decades but who had no previous coeliac disease diagnosis. **Materials and Methods** – The original study population comprised 4272 randomly selected older individuals, of whom 2089 had AGA and transglutaminase 2 antibodies (antiTG2) measured twice within a 3-year interval. Forty-nine persistently AGA-positive but antiTG2-negative subjects with coeliac-type HLA and 52 randomly selected persistently AGA- and antiTG2-negative age- and sex-matched controls were clinically examined for neurological disorders. The Psychological General Well-Being (PGWB) questionnaire, the SF-36 health survey questionnaire and the Depression Scale (DEPS) were employed to evaluate psychological well-being. The medical files of all the study subjects were analysed for previous illnesses. **Results** – Persistently AGA-positive but antiTG2-negative older subjects carrying coeliac disease-type HLA did not evince significantly more neurological symptoms or diseases than AGA-negative control subjects ($P = 0.682$, $P = 0.233$). There were no statistically significant differences between AGA-positive and AGA-negative groups in psychological well-being and quality of life when measured by PGWB ($P = 0.426$), SF-36 questionnaires ($P = 0.120$) and DEPS ($P = 0.683$). **Conclusions** – At population level, persistent AGA positivity did not indicate gluten sensitivity-related neurological and psychiatric disorders.

Introduction

Coeliac disease is an autoimmune disorder where ingestion of dietary wheat-, rye- and barley-derived gluten initiates and drives chronic inflammation in the small-bowel mucosa, leading eventually to villous atrophy and enteropathy in genetically predisposed individuals. Gluten-induced immune responses may extend beyond the intestine and the disease may also manifest itself with various non-specific and extra-intestinal symptoms (1). A wide range of neurological disorders, including cerebellar ataxia, peripheral

neuropathy, epilepsy and cognitive impairment, have been reported in patients with coeliac disease (1–4). Likewise, psychiatric conditions, including anxiety, depression and other mood alterations, have also been associated with the disease (5–8). In many cases, coeliac disease presents without gastrointestinal symptoms and malabsorption and may therefore go undiagnosed for many years. The untreated condition may eventually lead to chronic ill-health and a diminished sense of psychological well-being (9, 10).

Recent evidence shows that gluten-related problems are not restricted to classical coeliac

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disease enteropathy. The term gluten sensitivity has been launched to take into account the various extraintestinal gluten-dependent manifestations, even in patients with an apparently normal intestinal mucosa (1). As a marker of gluten sensitivity, increased levels of antibodies against wheat gluten, gliadin have been found in a variety of neurological disorders of unknown aetiology, cerebellar ataxia and peripheral neuropathy being the most common problems (1). Furthermore, increased immune sensitivity to dietary gluten proteins has been reported in psychiatric conditions such as depression, schizophrenia and bipolar disorder (11–13). Many of these gliadin antibody (AGA)-positive subjects remain negative for coeliac disease-specific transglutaminase 2 antibodies (antiTG2). However, in gluten ataxia, even though AGA-positive and antiTG2-negative subjects often have normal small-bowel mucosal villous morphology, the majority have coeliac disease-type HLA DQ2 or DQ8, antibodies against neuronal transglutaminase (transglutaminase 6) and signs of minor small-bowel mucosal inflammation indicative of early stage coeliac disease (1). This suggests that these gluten ataxia cases belong to the same gluten-induced disease spectrum as coeliac disease patients showing classical enteropathy. As the above-mentioned neurological and psychiatric gluten-sensitive disorders are potentially treatable with a gluten-free diet (1, 14–16), active case-finding by coeliac serology has been suggested. So far, however, association between AGA and neurological and psychiatric disorders has been studied in specialized centres in selected patient series, which may overestimate the association between AGA and various neurological and psychiatric conditions. The clinical relevance of AGA positivity at population level thus needs to be elucidated.

We have recently shown AGA positivity to be common (14%) in the older Finnish population, and the positivity was persistent in 81% (11, 17). Even though this was rarely indicative of overt coeliac disease, it was often associated with minor small-bowel mucosal inflammation in subjects carrying coeliac-type HLA (17). The hypothesis in this study was that as gluten sensitivity is markedly genetically predisposed and takes years to develop, we could detect gluten sensitivity-related neurological and psychiatric manifestations in older subjects with persistently positive AGA and coeliac-type HLA, as they will have consumed gluten for decades. We now sought to establish whether persistent AGA positivity without positive antiTG2 is related to neurological problems, decreased self-perceived psychological

well-being and depression in this older cohort. To find previously undiagnosed and subclinical conditions, we personally thoroughly examined all study subjects. Randomly selected persistently AGA- and antiTG2-negative subjects of the same age and gender were invited as controls. This study enlightens further and more precisely the issues of our previous study based on medical files (11).

Materials and methods

Patients, controls and study design

The Good Ageing in the Lahti Region (GOAL) survey on ageing and well-being (18) provided a basis for the current case–control study. The original study population comprised 4272 randomly selected individuals born in the years 1946–50, 1936–40 and 1926–30, representing the general population in the respective age groups (Fig. 1). Of those approached, 2815 agreed to participate and filled in questionnaires on their health condition, present and past diseases and special diets. Sera were collected for the analysis of IgA- and IgG-class AGA and IgA-class antiTG2 in 2002 (11), and 3 years later, all eligible subjects ($n = 2216$) were asked to undergo a new serological test. Serum samples were available in both

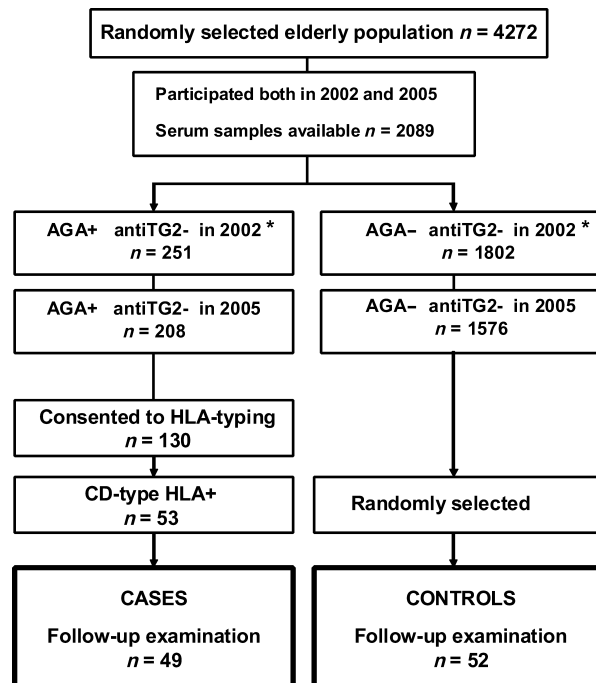


Figure 1. Flowchart of the study. AGA, antigliadin antibodies; antiTG2, antibodies against transglutaminase-2; CD, coeliac disease; *no previous CD diagnosis.

2002 and 2005 for 2089 cases (Fig. 1). Altogether 203 persistently (both in 2002 and 2005) AGA-positive but antiTG2-negative subjects without known coeliac disease were offered HLA testing, and 53 of the 130 tested had coeliac-type HLA. Eventually, 49 of those persistently AGA-positive and genetically predisposed individuals consented to undergo a general and thorough neurological examination (study group, cases; median age 69 years, range 57–80 years; 45% women); furthermore, 36 of them were willing to undergo endoscopy and small-bowel biopsy. Equal numbers of randomly selected persistently AGA- and antiTG2-negative subjects of the same age and gender were invited to attend a clinical examination (control group; median age 69 years, range 57–81 years; 44% women). All cases and controls were examined and interviewed in similar manner by the same investigator; however, for ethical reasons, HLA-typing and endoscopy were not performed on control subjects (17).

For further comparisons, data on clinical histories of 77 persistently AGA-positive subjects without coeliac-type HLA (median age 67 years, range 56–80 years; 48% women) were extracted from the medical records of Päijät-Häme Central Hospital.

Neurological examination

The occurrence of neurological symptoms and diseases was recorded and the medical files of the subjects were analysed. On examination, we focused especially on finding previously undiagnosed and subclinical disorders. Gait, balance, muscle strength, deep tendon reflexes, vibration, pin-prick and light touch senses and the functions of cranial nerves were evaluated. Cognition was assessed by clinical interview and a combination of the Mini Mental State Examination (MMSE) and the clock-drawing test (19). All evaluations were made by the same, non-blinded investigator (AR), but in borderline cases another (blinded) investigator (LL) co-evaluated the subject. If any relevant clinical symptoms or signs were detected, further studies such as electroneuromyography (ENMG), skin biopsy, lumbar puncture, radiological scans, neuropsychological and laboratory tests were performed according to clinical needs and good clinical practice. When neuropathy was suspected, ENMG using a structured study protocol and a three mm punch skin biopsy 5–10 cm above the lateral malleolus to detect small-fibre neuropathy (20) was carried out. In cases where neuropathy was diagnosed, full blood count, liver and thyroid function tests, blood glucose, serum creatinine, vitamin-B12, -B6, -B1, folic acid levels,

serum and urine protein fractions, antineuronal and antiganglioside antibodies were measured and if possible, cerebrospinal fluid analysed for cells, glucose, protein, albumin, immunoglobulins, IgG index, antineuronal antibodies and antibodies against *Borrelia burgdorferie*. In addition, when ataxia was detected, vitamin-E, gene tests for MIRAS/POLG1, Friedrich's ataxia and FragileX were performed.

Psychiatric conditions and psychological well-being

Present and past histories of psychological conditions were inquired into during the clinical examination. Psychological well-being and quality of life were evaluated applying questionnaires widely used in coeliac disease and of proven validity and reliability (10, 21, 22). The Psychological General Well-Being (PGWB) questionnaire contained 22 items comprising six subdimensions: anxiety, depression, well-being, self-control, general health and vitality, scoring being based on a six-grade Likert scale, higher scores indicating better psychological well-being (21). The SF-36 health survey questionnaire, containing eight subdimensions (mental health, physical functioning, role limitations because of physical problems, bodily pain, general health, vitality, social functioning and role limitations because of emotional problems), was used to assess health-related quality of life. The raw scores on all 36 items were rescored from 0 to 100, higher scores indicating better health and quality of life (10).

The Depression Scale (DEPS) has been widely applied as a self-rating depression questionnaire and is suitable in recognizing depression in primary care patients (23). The DEPS includes ten items covering the core symptoms of depression with four alternatives in scoring: 0 = not at all, 1 = a little, 2 = quite a lot and 3 = extremely. A higher score indicates more severe symptoms (24) and the cut-off level for depressive symptoms is nine (23).

Serology and HLA-typing

Serum IgA- and IgG-class AGA were measured by enzyme-linked immunosorbent assay (ELISA) (25); the results were obtained from the standard curve established according to dilutions of positive reference serum and converted to concentrations of arbitrary ELISA units per millilitre (EU/ml). The limits of positivity were set at 0.20 and 20 EU/ml, respectively. Serum IgA-class antibodies against TG2 and IgA- and IgG-class antibodies against TG6 were detected by ELISA using

human recombinant TG2 (Celikey, Phadia, GmbH, Freiburg, Germany) and TG6 (E003 and E004 Neuronal transglutaminase ELISA kits; Zedira, Darmstadt, Germany) as antigens according to the manufacturer's instructions. Concentrations over 5.0 U/ml, over 21 U/ml and over 38 U/ml were considered positive for IgA-class antiTG2, IgA- and IgG-class antiTG6 antibodies, respectively, as recommended by the manufacturers.

The study subjects were genotyped for HLA-DQB1*02, DQB1*0302 and DQA1*05 alleles using the DELFIA Coeliac Disease Hybridization Assay (Perkin-Elmer Life and Analytical Sciences, Turku, Finland). The genotypes DQB1*02 and DQA1*05 correspond to the serological HLA type DQ2 and DQB1*0302 to HLA-DQ8. These genotypes together or DQB1*02 alone are found in 96–100% of patients with coeliac disease and in this study were considered to constitute coeliac-type HLA (17).

Upper gastrointestinal endoscopy and small-bowel mucosal biopsies

Four small-bowel mucosal biopsy samples were taken by upper gastrointestinal endoscopy from the distal part of the duodenum. The biopsies were processed and evaluated as described in our previous studies (17, 26). Only one of 36 persistently AGA-positive but antiTG2-negative subjects was found to have villous atrophy with crypt hyperplasia indicative of overt coeliac disease. In addition, 19 (54%) had signs of minor coeliac disease-type small-bowel mucosal inflammation (increased density of CD3⁺ or $\gamma\delta$ ⁺ IELs or the presence of small-bowel mucosal TG2-targeted IgA deposits) (17). In this study, these biopsy findings were compared to the occurrence of neurological and psychological conditions.

Statistics

Quantitative data were expressed as medians or means and 95% confidence intervals (CI). Statistical differences between study groups were evaluated using Pearson's chi-square test, Fisher's exact test, *t*-test or Mann–Whitney *U*-test, as appropriate. Values of *P* < 0.05 were considered significant. The statistics were calculated with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

The study was accepted by the research ethics committee of Päijät-Häme Central Hospital. All participants gave written informed consent.

Results

Based on the interview and follow-up examination persistently AGA-positive but antiTG2-negative older subjects carrying coeliac disease-type HLA did not have more neurological diseases or symptoms than AGA-negative control subjects (Table 1). Four AGA-positive and three AGA-negative subjects suffered from polyneuropathy (Table 2). Only one AGA-positive subject, a 79-year-old man, had ataxia. He had suffered an ischaemic stroke 6 years previously with residual mild aphasia and mild right-sided hemiparesis. In brain magnetic resonance imaging, profound cerebral and cerebellar atrophy was found along with cerebral ischaemic white matter lesions. His erythrocyte folic acid concentrations were low, but he refused duodenal biopsy and a treatment trial with a gluten-free diet. Furthermore, in the examination, mild cognitive problems were suspected in three AGA-positive and two AGA-negative subjects, but none evidenced dementia in further neuropsychological evaluations. In the AGA-positive group, neurological findings were not related to small-bowel mucosal coeliac disease-type morphological or inflammatory changes (data not shown).

Altogether 12 (25%) of the 48 persistently AGA-positive but antiTG2-negative subjects with coeliac-type HLA had antibodies against TG6; seven were IgA-class, three IgG-class antiTG6-positive and two were positive in both IgA and IgG classes. Two of the antiTG6-positive cases had polyneuropathy and small-fibre neuropathy (Table 2), while the rest evinced no neurological dysfunction.

Table 1 Neurological disorders in persistently gliadin antibody (AGA)-positive subjects with coeliac-type HLA and in persistently AGA-negative controls revealed in interview and follow-up examination. All were also transglutaminase 2-antibody-negative and did not have diagnosed coeliac disease

	Persistently AGA-positive and HLA-positive <i>n</i> = 49	Persistently AGA-negative <i>n</i> = 52	<i>P</i> -value
Neurological symptoms, <i>n</i> (%)	18 (37)	17 (33)	0.682
Neurological disease, <i>n</i> (%)	8 (16)	14 (27)	0.233
Polyneuropathy	4 (8)	3 (6)	0.710
Small-fibre neuropathy	3 (6)	1 (2)	0.353
Ataxia	1 (2)	0 (0)	0.485
Nerve root lesion	1 (2)	5 (10)	0.363
Migraine	2 (4)	1 (2)	0.610
Essential tremor	1 (2)	2 (4)	1.000
Ischaemic stroke	3 (6)	7 (13)	0.320
Epilepsy	0 (0)	1 (2)	1.000
Intracranial haemorrhage	1 (2)	0 (0)	0.485

Table 2 Clinical details of cases evincing polyneuropathy

Sex age	AntiTG6 +/-	Small-bowel mucosal biopsy	Type of neuropathy	Clinical findings	Medical history and laboratory findings
Persistently AGA-positive with coeliac-type HLA					
F 78	IgA+	nd	Axonal polyneuropathy and small-fibre neuropathy	Absent achilles tendon reflexes, mild balance problem	High vitamin-B6
M 80	IgG+	Normal	Axonal polyneuropathy and small-fibre neuropathy	Ostostatic hypotension, absent achilles tendon reflexes, reduced sensation of touch	Antiganglioside antibodies positive, prostate cancer
F 80	–	Minor mucosal inflammation	Axonal polyneuropathy	Absent achilles tendon reflexes, mild balance problem, reduced sensation of touch, mild muscle weakness	Deficiency of vitamin-D and folic acid
M 79	–	Normal	Axonal polyneuropathy and small-fibre neuropathy	Absent achilles tendon reflexes, mild balance problem	Deficiency of vitamin-E and vitamin-B12, DM II, prostate cancer
Persistently AGA-negative					
M 79	nd	nd	Polyneuropathy*	Mild balance problem, reduced sensation of vibration and touch, absent achilles tendon reflexes	Prostate cancer, polymyalgia rheumatica
M 69	nd	nd	Axonal polyneuropathy and small-fibre neuropathy	Mildly unsteady gait, mildly reduced sensation of vibration	
M 59	nd	nd	Axonal polyneuropathy	Mild balance problem, reduced sensation of vibration, absent achilles tendon reflexes	Primary hyperparathyreosis, DM II

Nd, not done; antiTG6, transglutaminase 6 antibodies; DM II, diabetes mellitus type-II.

*Diagnosed 2 years before entering the study; skin biopsy not performed.

Only one subject in the AGA-negative control group reported depression in her past history; no other previously diagnosed psychiatric disorders were reported in the study or control groups. There were no statistically significant differences between AGA-positive and AGA-negative subjects in psychological well-being or quality of life as measured by PGWB and SF-36 questionnaires (Table 3). Furthermore, persistently AGA-positive subjects with coeliac-type HLA did not suffer from depression more often than persistently AGA-negative controls as assessed by DEPS score (mean 4.6 [95% CI 3.4–5.8] vs 4.3 [3.4–5.2], $P = 0.683$). There were no differences in DEPS scores between the persistently AGA-positive subjects who had coeliac-type small-bowel mucosal minor changes, those who had entirely normal small-bowel mucosa or those who did not undergo small-bowel biopsy ($P = 0.898$).

According to the hospital records, persistently AGA-positive subjects without coeliac-type HLA did not have more often neurological diseases (18 of 77, 23%) than persistently AGA-positive cases with coeliac-type HLA (16%, Table 1) or persistently AGA-negative cases (27%) (difference between the groups, $P = 0.430$). Three AGA-positive subjects who did not have coeliac-type HLA suffered from polyneuropathy; none had

Table 3 Mean and 95% confidence intervals of Psychological General Well-Being (PGWB) and SF-36 scores in persistently gliadin antibody (AGA)-positive subjects with coeliac-type HLA and AGA-negative subjects. All were also transglutaminase 2-antibody-negative and did not have diagnosed coeliac disease

	Persistently AGA-positive and HLA-positive <i>n</i> = 49	Persistently AGA-negative <i>n</i> = 52	<i>P</i> -value
PGWB*			
Anxiety	25.0 (24.0–26.0)	26.0 (24.9–27.0)	0.195
Depression	16.8 (16.3–17.2)	16.8 (16.3–17.4)	0.852
Well-being	17.7 (16.9–18.5)	17.6 (16.6–18.5)	0.848
Self-control	15.6 (15.1–16.1)	15.8 (15.2–16.4)	0.598
General health	13.8 (13.1–14.6)	13.2 (12.3–14.1)	0.323
Vitality	18.9 (18.0–19.7)	19.5 (18.7–20.3)	0.293
Total	107.6 (103.9–111.2)	109.8 (105.7–114.0)	0.426
SF-36*			
Mental health	76.6 (69.0–84.2)	83.5 (79.3–87.7)	0.120
Physical functioning	80.3 (75.2–85.4)	77.2 (71.5–82.9)	0.437
Social functioning	87.5 (82.6–92.4)	91.9 (87.7–96.1)	0.180
Role limitations because of emotional problems	75.0 (65.3–84.7)	83.3 (74.5–92.1)	0.218
Role limitations because of physical problems	70.7 (60.5–81.0)	66.2 (55.0–77.3)	0.557
Vitality	68.3 (62.3–74.3)	72.9 (67.9–77.9)	0.247
General health	60.5 (55.9–65.0)	59.4 (54.5–64.3)	0.759
Bodily pain	78.7 (73.5–83.9)	79.0 (73.3–84.6)	0.953

*Higher score indicates better quality of life.

ataxia. AGA-positive but coeliac-type HLA-negative subjects did not have more often depression (8%) than AGA-negative control group ($P = 0.241$).

Discussion

While in more selected patient materials, AGA has proved a good biomarker for gluten-sensitive neurological and psychiatric conditions (1, 12, 13, 26), at population level persistent AGA positivity could not uncover these disorders in older subjects who have in fact been exposed to gluten for decades. By thorough clinical examination, we found new neurological conditions equally in both AGA-positive and AGA-negative groups, which would indicate that AGA is unspecific for coeliac disease and gluten-related neurological or psychiatric conditions.

Although we could not show persistently AGA-positive subjects to have more neurological disorders than controls, it is interesting that some AGA-positive subjects with neurological disorders yielded laboratory results previously reported to be associated with coeliac disease and its neurological manifestations (Table 2). One neuropathy patient had antiganglioside antibodies (27) and two were antiTG6-positive (28). Altogether three had malabsorption of vitamins and one of these three evinced minor small-bowel mucosal inflammation. Although other conditions such as diabetes or cancer may have caused the neurological disorders in these individuals, we cannot rule out the possibility that gluten sensitivity may have played a role and predisposed the patients to develop neuropathy or ataxia more easily.

While in previous studies, antiTG6 has proved specific to gluten-sensitive neurological manifestations (1, 28), in our series, it was not associated with neurological symptoms or disorders. Methodological differences might partly explain this, because we used a commercial test while others have used in-house assays (28) and it is conceivable that the antigens used in the different tests may vary (29). Similarly, minor variability has previously been reported between commercial antiTG2 ELISA tests from different manufacturers (30). Of note, in coeliac disease, we have learned that antiTG2 positivity often predicts or anticipates the development of small-bowel mucosal atrophy and full-blown coeliac disease (31). It remains to be seen whether the antiTG6-positive subjects found in this study develop gluten-dependent neurological disorders in the follow-up.

Depression and mood alterations have been linked to coeliac disease (5–8), AGA positivity

(11) and small-bowel mucosal inflammation (22). Here, however, we found no difference in the occurrence of depression or in psychological well-being between AGA-positive subjects and AGA-negative controls. Psychiatric disorders associated with AGA might not be coeliac-type HLA-dependent (32). It has recently been shown that gluten sensitivity extends beyond coeliac-type HLA (33, 34), and this should be noted in further studies. Moreover, in this study, we asked participants to contact us and attend for laboratory and clinical examination. This may have left depressed and poorly functioning subjects out of the study, which could explain the difference in results from our previous study based on medical records of the same background population (11).

As far as we know this is the first large study focusing on gluten-sensitive neurologic and psychiatric conditions in a randomly selected adult population. The study population was representative of the general Finnish older population and was not originally selected for screening of coeliac disease. The strength of this study is that the controls were selected from the same background population and they were studied in the same way. As this was a population-based study, intervention by gluten-free diet was not possible.

To conclude, gluten sensitivity-related neurological and psychiatric manifestations do not seem to constitute any major health problem at the older population level. In this context, positive AGA without coeliac disease is not a bad prognostic sign. Further studies are needed to assess the value of AGA analysis in the diagnostic work-up of patients with neuropathy, ataxia or psychiatric problems.

Acknowledgements

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Conflicts of interest

None.

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