

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

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## Abstract

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

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

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

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

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

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**KEYWORDS**

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

**INTRODUCTION**

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

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

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

**METHODS****Patients and study design**

The study was conducted in Tampere University and Tampere University Hospital in the period 2017–2021. It continued an earlier family screening study carried out in the same centre in the period 2006–2010.<sup>6</sup> The first study comprised altogether 3115 non-coeliac relatives of previously diagnosed coeliac disease index patients from 706 families (Figure 1). The main intention was to invite FDRs, but more distant relatives were also approved. All participants were tested for serum IgA-class endomysium (EmA) and tissue transglutaminase antibodies (TGA) and coeliac disease-associated HLA. Corresponding IgG-class antibody tests were used in cases of selective IgA deficiency. Altogether 148 of the screened relatives had positive EmA – the main screening outcome and definition for seropositivity – and were referred to local healthcare facilities for

**Key summary****Established knowledge on this subject:**

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

**New findings of this study:**

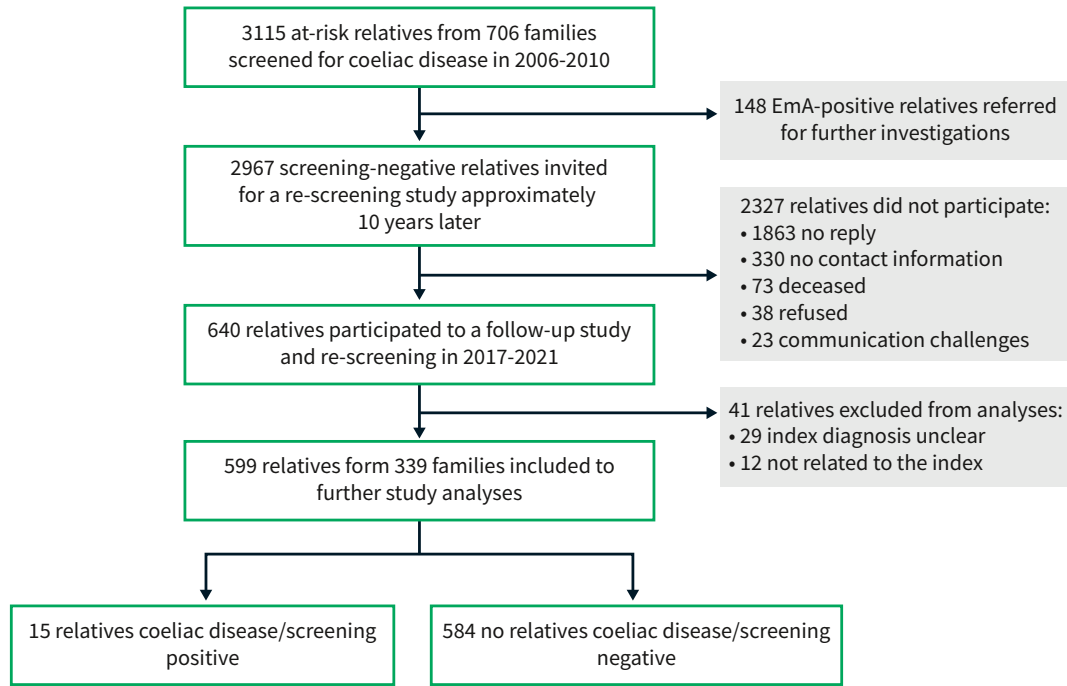
- The incidence rate for later coeliac disease diagnosis or new seropositivity in the once tested relatives was 221/100,000 person-years after 10 years of follow-up.
- The figure increased to 336/100,000 among those carrying at-risk HLA genetics.
- More detailed HLA-typing could help to target a subgroup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

further diagnostic investigations (Figure 1). The remaining 2967 relatives were informed that one-time negative testing does not exclude coeliac disease for a lifetime and that they should contact their local healthcare facilities in case of symptoms or signs suggestive of the disease.

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

**Clinical data and diagnostic findings**

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



**FIGURE 1** Flowchart of the study

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

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

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

### Serological testing and genetics

EmA were measured by indirect immunofluorescence using human umbilical cord as an antigen and considering titres 1:  $\geq 5$  positive. A

commercial EliA test (Celikey, Phadia) was used to test TGA, applying a cut-off of  $> 7$  U/L for seropositivity. At the initial screening in 2006–2010, TGA was tested with commercial ELISA test (QUANTA Lite h-tTG IgA, INOVA Diagnostics), applying a cut-off of  $> 20$  U/L for seropositivity. The corresponding IgG class serological tests were used if selective IgA deficiency was suspected based on abnormal EmA staining pattern and low TGA value. For the purposes of the study, a positive screening result was defined as positivity for both antibodies (EmA, TGA) and presence of coeliac disease-related HLA.<sup>17</sup>

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

### Ethics

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

## Statistics

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

## RESULTS

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

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

The clinically diagnosed ( $n = 8$ ) and screening-positive ( $n = 7$ ) relatives did not differ significantly on any study parameters (Table 2). Three of the screening-positive subjects were from the

same family and the remaining 12 from separate families. Coeliac disease was confirmed endoscopically in all clinically diagnosed patients except one elderly subject who was diagnosed based on TGA level  $>10\times$  upper limit of normal. The biopsy has also been taken from four of the now detected screening-positive subjects and three of them are still considering undergoing endoscopy. Three of the four subjects had subtotal villous atrophy consistent for coeliac disease, whereas one (EmA 1:100, TGA Celikey® 26.0 IU/L) subject was reported to have normal mucosal morphology. One half of the HLA haplotype was uncertain in one clinically diagnosed subject, although she was found to be carrying HLA DQ2.5.

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

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

## DISCUSSION

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

**TABLE 1** Characteristics of the relatives who had coeliac disease excluded in the first screening but either had a later coeliac disease diagnosis or new screening positivity<sup>a</sup> or remained seronegative

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

<sup>a</sup>Positive endomysial and transglutaminase antibodies and HLA DQ2/8.

<sup>b</sup>Time from the first screening to the present study or new coeliac disease diagnosis.

<sup>c</sup>Data missing from 86 subjects.

<sup>d</sup>DQ2.5/2.5 and DQ2.5/2.2.

<sup>e</sup>DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

<sup>f</sup>DQ2/8 negative.

<sup>g</sup>Subject has ≥2 previously diagnosed first-/second-degree relatives.

<sup>h</sup>Comparison between first-degree relatives.

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

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

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

<sup>a</sup>Positive endomysial and transglutaminase antibodies and HLA DQ2/8.

<sup>b</sup>Time from the first screening to the present study or new coeliac disease diagnosis.

<sup>c</sup>Data missing from 86 subjects.

<sup>d</sup>DQ2.5/2.5 and DQ2.5/2.2.

<sup>e</sup>DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

<sup>f</sup>DQ2/8 negative.

<sup>g</sup>Subject has ≥2 previously diagnosed first-/second-degree relatives.

<sup>h</sup>Comparison between first-degree relatives.

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

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

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

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

Abbreviation: CI, confidence interval.

<sup>a</sup>DQ2.5/2.5 and DQ2.5/2.2.

<sup>b</sup>DQ2.5 heterozygotes or DQ2.2 and/or DQ8 positive.

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

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

By applying a more detailed risk stratification,<sup>6</sup> we observed a strong positive predictive role of high-risk HLA DQ2.5/2.5 or 2.5/2.2 compared to intermediate-risk HLA in re-testing. Further supporting such a targeted approach, individuals with the high-risk haplotypes may be at increased risk for coeliac disease-associated complications.<sup>28</sup> Here it is important to note that no detailed HLA determination is currently available for use in clinical practice and determination of high-risk HLA alone would miss the majority of affected cases carrying the more common intermediate-risk

haplotype. In the future, more precise genetic risk scores also including non-HLA alleles may further help by targeting the serological surveillance of at-risk relatives.<sup>19</sup> It must, however, be kept in mind that awareness of the hereditary susceptibility for coeliac disease may also cause increased anxiety and influence individual health care behaviour.<sup>29</sup> More evidence on the cost-effectiveness of genetic testing is also needed.

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

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

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

## Strengths and limitations

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



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

## CONCLUSIONS

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

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## ETHICS APPROVAL

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

## AUTHOR CONTRIBUTIONS

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

## DATA AVAILABILITY STATEMENT

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

## INFORMED CONSENT

Written informed consent was obtained from each participant.

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## SUPPORTING INFORMATION

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