Independent and cumulative coeliac disease-susceptibility loci are associated with distinct disease phenotypes

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Abstract

The phenotype of coeliac disease varies considerably for incompletely understood reasons. We investigated whether established coeliac disease susceptibility variants (SNPs) are individually or cumulatively associated with distinct phenotypes. We also tested whether a polygenic risk score (PRS) based on genome-wide associated (GWA) data could explain the phenotypic variation. The phenotypic association of 39 non-HLA coeliac disease SNPs was tested in 625 thoroughly phenotyped coeliac disease patients and 1817 controls. To assess their cumulative effects a weighted genetic risk score (wGRS39) was built, and stratified by tertiles. In our PRS model in cases, we took the summary statistics from the largest GWA study in coeliac disease and tested their association at eight P value thresholds (PT) with phenotypes. Altogether ten SNPs were associated with distinct phenotypes after correction for multiple testing (PEMP2 ≤ 0.05). The TLR7/TLR8 locus was associated with disease onset before and the SH2B3/ATXN2, ITGA4/UBE2E3 and IL2/IL21 loci after 7 years of age. The latter three loci were associated with a more severe small bowel mucosal damage and SH2B3/ATXN2 with type 1 diabetes. Patients at the highest wGRS39 tertiles had OR > 1.62 for having coeliac disease-related symptoms during childhood, a more severe small bowel mucosal damage, malabsorption and anaemia. PRS was associated only with dermatitis herpetiformis (PT = 0.2, PEMP2 = 0.02). Independent coeliac disease-susceptibility loci are associated with distinct phenotypes, suggesting that genetic factors play a role in determining the disease presentation. Moreover, the increased number of coeliac disease susceptibility SNPs might predispose to a more severe disease course.

Introduction

Food antigens do not generally cause a systemic immune response in healthy individuals, but rather lead to the induction of oral tolerance. However, in approximately 1–2% of individuals, the ingestion of dietary gluten results in the development of coeliac disease, an immune-mediated
enteropathy [1]. In some patients, coeliac disease develops already in early childhood, while in others, the tolerance to gluten is lost at a considerably older age. A hallmark of the disease is gluten-dependent small bowel mucosal damage that ranges from minor inflammatory changes to total villous atrophy with crypt hyperplasia [2]. The enteropathy is often coupled with malabsorption and gastrointestinal symptoms, such as diarrhoea, but the disease also presents with diverse extraintestinal manifestations affecting various organs including skin and musculoskeletal system [2, 3]. This multifaceted clinical picture is further diversified by several associated conditions, such as type 1 diabetes and autoimmune thyroidal disease [2].

The predisposition for coeliac disease runs in families, and relatives of coeliac patients are at an increased risk [4]. The disease susceptibility is largely conferred by human leucocyte antigen (HLA) haplotypes encoding for DQ2 or DQ8 heterodimers, which are necessary but not sufficient for disease development [2]. Genome-wide association (GWA) and follow-up studies have identified 94 SNPs in 43 non-HLA risk loci that by themselves modify the disease risk modestly [5–8]. However, combining their independent cumulative effects into a genetic risk score (GRS) improves the prediction of the disease risk [9]. Moreover, by combining the loci of small effect, including those that do not achieve genome wide significance into a polygenic risk score (PRS), it has been possible to examine the influence of several thousands of risk alleles to coeliac disease susceptibility [7, 10].

Currently, there’s only limited information about the connection of the non-HLA risk SNPs with different coeliac disease phenotypes. Therefore, the aim of this study was to investigate whether previously identified coeliac disease-susceptibility SNPs are associated with distinct disease phenotypes and to gain insight into possible biological pathways and processes underlying the identified genotype–phenotype associations. Moreover, the objective was to study whether GRS or PRS give clues to the factors contributing to the clinical heterogeneity of the disease.

**Materials and methods**

**Study population**

Altogether 1048 biopsy-proven coeliac disease patients were recruited by a nationwide newspaper advertisements and the assistance of the Finnish Coeliac Society in Tampere University and Tampere University Hospital 2005–2010. The study protocol was approved by the Regional Ethics Committee of Tampere University Hospital and all study subjects or young children’s legal guardians gave written informed consent. All participants were interviewed either by a physician or by a study nurse with expertise in coeliac disease. The structured interviews included questions on coeliac disease diagnosis, symptoms at the time of diagnosis and in childhood, and associated medical conditions. All relevant medical information was confirmed from the patient records. Whole blood samples were drawn for genetic analysis. As the presence of relatives can lead to genetic bias (inflation of type 1 error), the current study considered only one coeliac case per family resulting in 625 cases grouped into relevant phenotypes (Table 1). The median age of the patients was 41 ranging from 0.5 to 79 years. The individual was selected randomly among the family members with full genotype data available. The healthy controls ($n = 1817$) with information on

| Table 1 Demographic data, clinical characteristics and selected coeliac disease-associated findings in 625 coeliac disease patients at diagnosis |
|---|
| n | % |
| Female | 489 | 78 |
| Childhood diagnosis ≤7 years old | 44 | 7 |
| Coeliac disease-related symptoms during childhood | 273 | 44 |
| Gastrointestinal symptoms | 526 | 84 |
| Malabsorption$^b$ | 267 | 43 |
| Anaemia | 157 | 25 |
| Extraintestinal manifestations$^c$ | 263 | 42 |
| Dermatitis herpetiformis | 69 | 11 |
| Neurological disorders | 38 | 6 |
| Fractures | 70 | 11 |
| Asymptomatic | 53 | 8 |
| Coeliac disease-associated conditions$^d$ | 279 | 94 |
| Autoimmune thyroidal diseases | 100 | 16 |
| Type 1 diabetes | 18 | 3 |
| Small bowel mucosal damage$^e$ | 361 | 66 |
| Total or subtotal villous atrophy | 185 | 34 |
| Partial villous atrophy | 527 | 84 |
| HLA risk$^f$ | 98 | 16 |
| Intermediate/low | 157 | 25 |
| High | 157 | 25 |
| Coeliac disease autoantibodies$^g$ | 38 | 6 |
| Positive | 279 | 94 |
| Negative | 19 | 6 |

$^a$Diarrhoea, abdominal pain, flatulence, heartburn, nausea, vomiting
$^b$Anaemia, vitamin and micronutrients deficiencies
$^c$Dermatitis herpetiformis, rash, neurological and psychiatric disorders, fracture, arthralgia, tiredness, fatigue, osteoporosis, osteomalacia, osteopenia, aphthae, alopecia, growth retardation, elevated liver enzymes, dental defects, infertility
$^d$Small bowel mucosal morphology available from 546 patients
$^e$High risk (DQ2.5/DQ2.5; DQ2.5/DQ2.2), intermediate risk (DQ2.5/X, DQ2.2/D2.2, DQ2.2/X, DQ8/DQ8, DQ8/X), low risk (DQ7/X, DQ7/DQ7)
$^f$Autoantibodies at diagnosis available from 298 patients
gender and HLA-type were obtained from the FINRISK and Health 2000 population cohorts [11]. Ethical committee’s approvals were available from the National Public Health Institute’s ethical committee and the Ethical committee in epidemiology and public health at the hospital district of Helsinki and Uusimaa.

Genotyping and quality control

HLA-DQ typing was performed with the TaqMan chemistry, SSP DQB1 and DRB1 low-resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden), or DELFIA® Coeliac Disease Hybridization Assay Kit (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) [12, 13]. For patients and controls missing HLA-typing results the missing GWAS SNPs were further imputed in full HLA haplotypes with HIBAG R package [14].

All participants had been genotyped for a SNP set on an Illumina 610-Quad BeadChip array (Illumina Inc., San Diego, CA, USA) [5]. The SNPs with established risk for coeliac disease were selected. We included the 39 non-HLA coeliac disease SNPs identified in the earlier GWAS [5] and directly genotyped in our samples. As most of the SNPs identified in Trynka et al. study [6] were either not available at our array or had low genotyping frequency, they were imputed (further details under the ‘imputation’ section).

Genotypes were stored in BC Genome v.4.0 (BC Platforms, Espoo, Finland) and quality checks performed [15]. The genotyped and imputed SNPs passed the quality control (QC) filters for missing genotype rate < 5%, missing genotype rates differences between the cases and controls (<3%), and minor allele frequency (MAF > 1%) [16]. All markers were (P > 1×10^{-6}) in HWE in the controls [6, 16]. Allelic associations with phenotypes were tested in a case–control analysis and within cases, further detailed under the ‘statistical analysis’ section.

Imputation

The genotypes of 94 coeliac disease risk variants [5, 6] were selected to be phased and imputed by using a Finnish population-specific panel of 3775 high-coverage (25–30x) whole-genome sequences (SISu v3) as here described: dx.doi.org/10.17504/protocols.io.nmndc5e. SISu v3 panel was generated at the Broad Institute of MIT and Harvard and at the McDonnell Genome Institute at Washington University; and jointly processed at the Broad Institute.

Genotyping data produced with our chip platform were lifted over to genome build version 38 (GRCh38/hg38) following the protocol described here: https://doi.org/10.17504/protocols.io.nqtdddwn. In sample-wise quality control (QC), samples with sex discrepancies, high genotype missingness (>5%), excess heterozygosity (±4SD) and non-Finnish ancestry were removed. In variant-wise QC, variants with high missingness (>2%), deviation from Hardy–Weinberg equilibrium (HWE) (P < 1e-6) and minor allele count <3 were removed. Pre-phasing (default parameters, except the number of conditioning haplotypes was set to 20,000) and phasing of genotyped data were performed with Eagle 2.3.5 (https://data.broadinstitute.org/alkesgroup/Eagle/)

Imputation was carried out with Beagle 4.1 (version 08Jun17.d8b, https://faculty.washington.edu/browning/beagle/b4_1.html) as described in the following protocol: dx.doi.org/10.17504/protocols.io.nmndc5e. Variant callset was produced by following GATK best-practices. Genotype-, sample- and variant-wise QC was applied in an iterative manner by using the Hail framework v0.1 [https://github.com/hail-is/hail/]

Functional identification of enriched pathways related to the phenotype-associated SNPs

Given that coeliac disease associated SNPs might not be causal variants but situated in their close proximity e.g. in high linkage disequilibrium (LD) with them, FUMA (Functional Mapping and Annotation of GWAS) platform [17] was used for the functional annotation of the 39 genotyped SNPs. Publicly available GWAS summary statistic results in which our cohort has been included [5] and a pre-defined list of associated SNPs with phenotypes in this study were provided. All proxies in LD (r^2 ≥ 0.8) with the phenotype-associated SNPs were identified using HaploReg v.4.1 [18]. The RegulomeDB 2.0 [19] was used to assign all the variants a score ranging from 1a to 7. Scores from 1a to 3a are likely to affect the expression of a gene. The lesser the score the higher the likelihood, thus 1a indicating a maximum and 3a a minimum likelihood to affect gene expression [19]. Variants scoring ≥3a but predicted as likely deleterious [Combined Annotation Dependent Depletion (CADD) score closest or >12.37)] were also included [20]. For those phenotype-associated SNPs scoring >3a, we selected their proxy with the lowest RegulomeDB score [19]. Using publicly available databases to study tissue-specific gene expression, we examined significant eQTLs associations (FDR < 0.05) with the functional variants, located nearby (cis-eQTL) or distal (trans-eQTL) to genes. They were assessed in the whole and peripheral blood using the Blood eQTL [21], BIOS QTL [22] and eQTLGen [23] browsers. Moreover,
as the phenotype of coeliac disease varies considerable and the symptoms may affect the function of several cell types tissues we also used the Genotype-Tissue Expression data (brain, nerve, colon, oesophagus, EBV-transformed lymphocytes, muscle-skeletal, pancreas, skin, small intestine, stomach, cultured fibroblasts, thyroid, whole blood) [24]. Their participation in pathways and biological processes were identified by using KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) databases [25].

**Genetic risk score**

In order to assess the cumulative effects of the genotyped 39 non-HLA SNPs on phenotypes, we created a weighted GRS model (wGRS39), calculated by multiplying each allele by the natural logarithms of the previously reported OR values (β-coefficients) [5], followed by dividing their sum by the total number of alleles [9]. We analysed the wGRS39 model in tertiles, adjusted for sex, calculated according to the distribution of the average weighted risk alleles in the controls as published [26]. Based on these tertiles, all participants were categorised into the low-, medium- and high-risk groups.

**Polygenic risk score**

We derived a PRS using summary statistics from the most recent and largest GWAS in coeliac disease with 24,269 participants [6] as the discovery dataset and tested its representation analysis adjusted by Benjamini & Hochberg correction, adjusting for SNP-gender interaction. Multiple comparisons were tested by using the chi-squared method with one degree of freedom, measured by OR values and 95% confidence intervals. Logistic regression adjusted for SNP-gender interaction. We performed $10^4$ permutation tests, generating an empirical $P_{EMP2}$ for the evaluation of subgroups with a small sample size and adjusting for multiple tests [16]. Associations were statistically significant at $P_{EMP2} \leq 0.05$. The Breslow-Day (BD) test examined the homogeneity of the association’s OR ($P_{BD}$ values $< 0.01$) in each male and gender strata [29].

Phenotype-association testing by using post-imputation genotype probabilities was performed under an additive genetic effect model using the frequentist likelihood score method implemented in SNPTEST v2.5.2 [30]. All the associations were adjusted for gender with the SNPTEST method Newml. For ChrX region we additionally used the ChrX-specific SNPTEST method with stratify-on gender option. Associations that reached our permutation threshold ($P$ value $\leq 0.001$) were considered as statistically significant.

Using the Genetic Power Calculator [31], we computed the statistical power reached by the sample size set for each associated phenotype to detect allele association at permutation ($P \leq 0.001$) and at nominal ($P \leq 0.01$ and $P \leq 0.05$) thresholds (detailed results in Supplementary Table S1). SNPs were assumed to be independent causal variants with no other causal variants in linkage disequilibrium ($D’ = 1$).

The eQTL associations by the selected variants located nearby (cis-eQTL) or distal (trans-eQTL) to genes were significant at a false discovery rate (FDR) $< 0.05$. An overall representation analysis adjusted by Benjamini & Hochberg procedure was performed by using WEB-based GEne ScE T AnaLysis online toolkit [25] to identify significant (FDR $< 0.05$) eQTL gene-sets enriched in KEGG pathways and GO biological processes.

Logistic regression was used to test the associations of the wGRS39 tertiles with the coeliac phenotypes after comparing each group (medium [2nd tertile] and high [3rd tertile] wGRS39 risk categories) to a reference (low wGRS39 risk category, 1st tertile). The associations were quantified by ORs with 95% CIs, significant at $P$ value $< 0.05$. In our case only model, we tested the associations between PRS and phenotypes using linear logistic regression, adjusting for age and sex. Multiple comparisons were addressed by applying $10^4$ permutations to identify the best fit PRS ($P_{EMP2} \leq 0.05$). Results are presented as change in variance ($R^2$), with both unadjusted $P$ values and $P_{EMP2}$ values.

All data analysis, conversion of required input formats in the imputation analysis and graphs were performed using PLINK 1.90 (https://www.cog-genomics.org/plink/1.9), PLINK 2.0 (https://www.cog-genomics.org/plink/2.0/), PRSice v2 and RStudio (version 1.1.463-20 2009–2019 RStudio Inc., Boston, USA).
Table 2  Associations of genotyped coeliac disease risk variants to different phenotypes in the Finnish case–control material

| Phenotypes                                      | N   | Marker  | Gene        | Chr | A1/A2 | MAF all | MAF cases | MAF controls | OR (95% CI)          | P<EMP2 |
|------------------------------------------------|-----|---------|-------------|-----|-------|---------|-----------|--------------|----------------------|--------|
| **Gender, age**                                 |     |         |             |     |       |         |           |              |                      |        |
| Female, CeD diagnosed ≤ 7 years old            | 30  | rs597985| TLR7/TLR8   | X   | G/A   | 0.25    | 0.03      | 0.24         | 0.11 (0.03–0.47)      | 0.015* |
| Age                                             |     |         |             |     |       |         |           |              |                      |        |
| CeD diagnosed >7 years old                      | 573 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.47      | 0.41         | 1.28 (1.12–1.46)      | 0.015  |
| Age                                             |     |         |             |     |       |         |           |              |                      |        |
| CeD related symptoms in childhood               | 273 | m3098911| CCR1/CCR3   | 3   | T/C   | 0.14    | 0.21      | 0.14         | 1.58 (1.26–1.99)      | 0.003  |
| Gastrointestinal symptoms                       |     |         |             |     |       |         |           |              |                      |        |
| All                                             | 267 | m7810546| IL12A      | 3   | G/A   | 0.11    | 0.15      | 0.1          | 1.57 (1.21–2.04)      | 0.024  |
| Anaemia                                         |     |         |             |     |       |         |           |              |                      |        |
| Extraintestinal manifestations                  | 157 | m2327832| OLIG3/TNFAIP3 | 6   | C/T   | 0.19    | 0.26      | 0.19         | 1.53 (1.24–1.89)      | 0.002  |
| All                                             | 267 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.48      | 0.41         | 1.36 (1.13–1.63)      | 0.042  |
| Neurological disorders                          | 38  | m3098911| CCR1/CCR3   | 3   | T/C   | 0.14    | 0.28      | 0.14         | 2.54 (1.41–3.91)      | 0.034  |
| Fractures                                       | 70  | m1055659| MYNN       | 3   | G/A   | 0.27    | 0.41      | 0.26         | 1.84 (1.34–2.50)      | 0.021  |
| CeD-associated conditions                       |     |         |             |     |       |         |           |              |                      |        |
| All                                             | 123 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.52      | 0.41         | 1.61 (1.24–2.09)      | 0.012  |
| AITD                                            | 100 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.54      | 0.41         | 1.68 (1.26–2.24)      | 0.012  |
| T1D                                             | 18  | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.69      | 0.41         | 3.32 (1.63–6.67)      | 0.027  |
| Small bowel mucosal damage                      |     |         |             |     |       |         |           |              |                      |        |
| TVA/SVA                                         | 361 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.48      | 0.41         | 1.35 (1.15–1.58)      | 0.012  |
| PVA                                            | 185 | m3098911| CCR1/CCR3   | 3   | T/C   | 0.14    | 0.21      | 0.14         | 1.59 (1.21–2.08)      | 0.030  |
| Intermediate HLA riska                          | 517 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.47      | 0.4          | 1.35 (1.16–1.58)      | 0.006  |
| Intermediate HLA riska                          | 517 | m653178 | SH2B3/ATXN2 | 12  | C/T   | 0.42    | 0.47      | 0.4          | 1.35 (1.16–1.58)      | 0.006  |
| CeD-antibodies                                  |     |         |             |     |       |         |           |              |                      |        |
| Negative CeD-antibodies                         | 19  | m3098911| CCR1/CCR3   | 3   | T/C   | 0.14    | 0.34      | 0.14         | 3.19 (1.62–6.28)      | 0.020  |

A total of 1817 controls were used in all the analysis. Associations that resisted the 10,000 permutation corrections for multiple testing (P<EMP2 ≤ 0.05) are reported in the table along with the respective odds ratios (ORs), 95% confidence intervals (95% CI) and corrected P values (P<EMP2).

N sample size, Chr chromosome, A1/A2 Minor Allele_Major Allele, MAF Minor allele frequency, AITD autoimmune thyroidal disease, TVA/SVA total or subtotal villous atrophy, PVA partial villous atrophy, CeD coeliac disease.

*Cases and controls categorised as low HLA risk were dropped out from the analysis, and HLA intermediate risk participants included. Logistic regression adjusted the effect of gender on SNPs. The Breslow–Day (BD) test was used examine the homogeneity of the ORs in each gender strata and a *P<BD value < 0.02 was considered statistically significant. All markers were in Hardy–Weinberg equilibrium in the controls (P > 1 x 10^-6).
Results

Genetic associations with coeliac disease phenotypes

Of the previously identified 94 coeliac disease-associated variants, 39 were typed to sufficient extent and thus were selected for the study. When comparing coeliac disease patients to controls, ten SNPs were associated with specific phenotypes (Table 2, and Supplementary Table S2). Rs5979785 in the TLR7–TLR8 region was associated with coeliac disease diagnosis ≤7 years of age and stratifying for gender revealed that this association only occurs in girls (Breslow–Day test, \( P < 0.001 \)). This SNP was associated with the age of coeliac disease diagnosis in girls (OR = 0.11; 95% CI = 0.03–0.46) also in the case only analysis comparing coeliac disease patients with the given phenotype to those without (detailed results in Supplementary Table S3). As regards coeliac disease diagnosis above 7 years of age in the case–control analysis, rs653178 (at SH2B3/ATXN2), rs13010713 (at ITGA4/UBE2E3), rs13151961 (at IL2/IL21), rs11712165 (at CD80) and rs10936599 (at MYNN) showed an association. Except for rs10936599, the same variants were also associated with an intermediate HLA risk. Moreover, rs653178, rs13010713 and rs13151961 showed an association with the presence of gastrointestinal symptoms and with the presence of more severe mucosal damage (TVA/SVA) at diagnosis. In addition, rs653178 was also associated with extraintestinal manifestation as well as coeliac disease associated conditions in general and more specifically withAITD and T1D.

In order to address the association of all the 94 coeliac disease associated SNPs, we carried out imputation. Out of the 2442 subjects in our study, 299 individuals (285 cases and 14 controls) were excluded due to heterozygosity and missingness. Of the 94 coeliac disease-associated SNPs, we carried out imputation. Out of the 148 extracted proxies to the 2442 subjects in our study, 299 individuals (285 cases and 13098911) had strong regulatory function on transcription with a RegulomeDB score ≤ 3a, indicating that the variant likely lies within a potential functional region. Of these rs10936599 was enriched with proxies with the lowest scores (1a and 1f). Out of the 148 extracted proxies to the other phenotype-associated SNPs (rs653178, rs13010713, rs17810546 and rs2298428), 42 had a putative functional role in gene regulation (RegulomeDB score ≤ 3a) (Supplementary Table S5). Thereafter we searched for eQTL effects for both the queried SNPs or the proxies that were likely to regulate gene expression or to be deleterious. We identified several significant highly tissue-specific eQTL effects (FDR < 0.05) (Supplementary Table S6). The eQTL genes connected to each of the phenotypes by the associated SNPs or their proxies were then subjected to KEGG pathways and GO terms analysis.

The analysis of the cis-eQTL genes of individual phenotypes, revealed significant enrichment only in the case of coeliac disease symptoms in childhood, neurological disorders, PVA and negative coeliac disease antibodies (Supplementary Table S7). The four cis-eQTL genes (CCR5, CCR3, CCR2, CXCR6) were enriched (FDR ≤ 0.002) in chemokine signalling and cytokine-cytokine receptor interaction KEGG pathways. These genes are likely to be involved in modulation of chemokine and cellular defence responses, cell chemotaxis second messenger-mediated signalling and divalent inorganic cation homeostasis based on the GO associated terms (FDR ≤ 0.0001). Coeliac disease diagnosis above 7 years of age, gastrointestinal symptoms, coeliac disease-associated conditions, EI manifestations and intermediate HLA risk were the phenotypes connected to a set of trans-eQTL genes. Among KEGG pathways, they were most significantly enriched (FDR ≤ 0.01) in the B cell receptor signalling, Pertussis, NOD-like receptor signalling and in MicroRNAs in cancer. They were enriched (FDR ≤ 2.2E-09) for biological processes GO
Independent and cumulative coeliac disease-susceptibility loci are associated with distinct disease phenotypes. Variants with imputation data only are highlighted in bold.

### Table 3: Associations of imputed 88 coeliac disease risk variants to different phenotypes in the Finnish case-control material

| Phenotypes                        | Marker      | Gene        | Chr  | A1/A2 | MAF all | MAF cases | MAF controls | OR (95% CI) | Beta   | P_adj value |
|-----------------------------------|-------------|-------------|------|-------|---------|-----------|--------------|-------------|--------|-------------|
| **Gender, CeD diagnosed ≤ 7 years old** | rs5979785  | TLR7/TLR8   | X    | TC    | 0.25    | 0.06      | 0.25         | 0.19 (0.06–0.62) | −5.17  | 3.8E−04     |
| Female, CeD diagnosed > 7 years old | rs653178    | SHEB3/ATXN2 | 12   | TC    | 0.42    | 0.49      | 0.40         | 1.43 (1.21–1.70) | 0.35   | 4.7E−05     |
|                                   | rs3184504   | SHEB3/ATXN2 | 12   | CT/T  | 0.41    | 0.48      | 0.39         | 1.40 (1.18–1.66) | 0.33   | 1.5E−04     |
|                                   | rs76830465  | IL12A       | 3    | AC    | 0.09    | 0.13      | 0.08         | 0.62 (0.47–0.80) | −0.49  | 6.1E−04     |
|                                   | rs13010713  | ITGA4/UBE2E3| 2    | CT    | 0.50    | 0.44      | 0.51         | 0.76 (0.64–0.90) | −0.28  | 0.001       |
| **CeD related symptoms in childhood** | rs7999785   | TLR7/TLR8   | X    | TC    | 0.24    | 0.15      | 0.25         | 0.52 (0.38–0.71) | −0.87  | 3.2E−04     |
|                                   | rs7616215   | CCR1        | 3    | TC    | 0.38    | 0.47      | 0.37         | 1.47 (1.17–1.83) | 0.37   | 0.001       |
| **Gastrointestinal symptoms**     | rs76830465  | IL12A       | 3    | AC    | 0.09    | 0.13      | 0.08         | 0.58 (0.45–0.76) | −0.55  | 1.5E−04     |
|                                   | rs653178    | SHEB3/ATXN2 | 12   | TC    | 0.41    | 0.48      | 0.40         | 1.36 (1.14–1.62) | 0.30   | 7.4E−04     |
| **Malabsorption**                 |             |             |      |       |         |           |              |             |        |             |
| *All*                             | rs76830465  | IL12A       | 3    | AC    | 0.09    | 0.16      | 0.08         | 0.48 (0.34–0.67) | −0.75  | 4.7E−05     |
|                                   | rs17810546  | IL12A       | 3    | GA    | 0.10    | 0.16      | 0.10         | 0.57 (0.41–0.79) | −0.56  | 0.001       |
| **Anemia**                        | rs17206432  | OLIG3/TNFAP5| 6    | GA    | 0.19    | 0.29      | 0.18         | 0.56 (0.41–0.78) | −0.58  | 8.0E−04     |
| **Extraintestinal manifestations**|             |             |      |       |         |           |              |             |        |             |
| *All*                             | rs653178    | SHEB3/ATXN2 | 12   | TC    | 0.41    | 0.53      | 0.40         | 1.64 (1.29–2.10) | 0.49   | 8.0E−05     |
|                                   | rs3184504   | SHEB3/ATXN2 | 12   | CT/T  | 0.40    | 0.51      | 0.39         | 1.59 (1.24–2.03) | 0.45   | 2.7E−04     |
| **Neurological disorders**        | rs243323    | SOCS1       | 16   | GA    | 0.30    | 0.09      | 0.30         | 4.36 (1.56–12.21) | 1.45   | 0.001       |
| *All*                             | rs653178    | SHEB3/ATXN2 | 12   | TC    | 0.40    | 0.79      | 0.79         | 5.86 (2.18–15.72) | 1.74   | 8.3E−05     |
| **CeD-associated conditions**     | rs3184504   | SHEB3/ATXN2 | 12   | CT/T  | 0.40    | 0.79      | 0.40         | 5.65 (2.10–15.16) | 1.72   | 1.1E−04     |
| **Small bowel mucosal damage**    | rs13010713  | ITGA4/UBE2E3| 2    | CT/T  | 0.50    | 0.42      | 0.51         | 0.72 (0.58–0.88) | −0.34  | 0.001       |
| *TVS*                             | rs12351961  | IL2/IL21    | 4    | GA    | 0.11    | 0.04      | 0.12         | 2.84 (1.45–5.59) | 1.04   | 4.2E−04     |
| *PVA*                             | rs1233208   | IL2/IL21    | 4    | GA    | 0.11    | 0.04      | 0.12         | 2.84 (1.45–5.59) | 1.04   | 4.1E−04     |
| **Intermediate HLA risk**          | rs653178    | SHEB3/ATXN2 | 12   | TC    | 0.41    | 0.50      | 0.40         | 1.46 (1.22–1.75) | 0.38   | 3.8E−05     |
| *All*                             | rs3184504   | SHEB3/ATXN2 | 12   | CT/T  | 0.40    | 0.48      | 0.39         | 1.41 (1.18–1.69) | 0.34   | 2.2E−04     |
|                                   | rs76830465  | IL12A       | 3    | AC    | 0.09    | 0.14      | 0.08         | 0.55 (0.42–0.73) | −0.60  | 3.6E−05     |
| *CeD-antibodies*                  | rs7890171   | IL13R1, IL18RAP| 2  | GA    | 0.50    | 0.56      | 0.49         | 0.75 (0.63–0.90) | −0.30  | 0.001       |
| *Positive CeD-autoantibodies*      | rs676830465 | IL12A       | 3    | AC    | 0.09    | 0.14      | 0.08         | 0.56 (0.39–0.78) | −0.61  | 0.001       |

A total of 1749 controls were used in all the analysis.

* N sample size, *Chr* chromosome, *A1/A2 Minor Allele, Major Allele, MAF minor allele frequency, *TVS/SVA* total or subtotal villous atrophy, *PVA* partial villous atrophy, *CeD* coeliac disease.

*Cases and controls categorised as low HLA risk were dropped out from the analysis, and HLA intermediate risk participants included. Imputation analysis was carried out by using a Finnish population-specific panel of 3775 high-coverage (25–30x) whole-genome sequences (SiSu v3) under the genome build version 38 (GRCh38/hg38). Phenotype-association testing was performed by using post-imputation genotype probabilities by using logistic regression under an additive model with the SNPTEST v2.5 software [30]. A frequentist test with Newm method corrected for gender. The ChrX-specific SNPTEST stratify-on gender method was used for associations in the ChrX region. Associations that reached our 10,000 permutation threshold (*P* value ≤ 0.001) are reported in the table along with their respective odds ratios (ORs), 95% confidence intervals (95% CI), beta coefficient and adjusted *P* values (*P* adj value). All imputed variants had good imputation metrics (INFO score of ≥0.9) MAF > 0.01, and were in Hardy–Weinberg equilibrium in the controls (*P* > 1 × 10^−6).
terms mostly involved in adaptive immune response, T cell activation, response to interferon-gamma, leucocyte cell-cell adhesion, cytokine secretion, regulation of leucocyte activation and response to molecule of bacterial origin (Supplementary Table S7).

**Genetic risk score and polygenic risk score associations with coeliac phenotypes**

In order to study the combined effect of the 39 SNPS on different phenotypes, we applied the wGRS39 tertiles (Supplementary Table S8). Patients at the highest wGRS39 tertiles had significantly higher risk for having coeliac disease-related symptoms during childhood (OR = 1.76, 95% CI = 1.12–2.77), a more severe small bowel mucosal damage (OR = 1.76, 95% CI = 1.08–2.90), malabsorption (OR = 1.62, 95% CI = 1.04–2.54) and anaemia (OR = 1.68, 95% CI = 1.03–2.78) (Table 4, and Supplementary Table S8).

As regards the PRS analysis we found a significant effect of PRS on DH at P values threshold (P_T = 0.2, R^2 = 0.06, P = 0.007, P_{EMP2} = 0.02) (Fig. 1a). Best fit PRS at P_T of 0.001 predicted the presence of fractures but did not persist permutation correction (R^2 = 0.025, P = 0.043, P_{EMP2} = 0.12) (Fig. 1b, and Supplementary Table S9).

**Table 4** Association of weighted genetic risk score (wGRS) (in tertiles) with coeliac phenotypes at disease diagnosis in the Finnish coeliac population

| Coeliac disease-related symptoms during childhood | P value |
|--------------------------------------------------|---------|
| wGRS39 tertiles^a | OR [95% CI] |
| Low | Reference |
| Medium | 1.27 [0.84–1.92] | 0.258 |
| High | 1.76 [1.12–2.77] | **0.014** |

| Total/Subtotal villous atrophy | P value |
|-------------------------------|---------|
| wGRS39 tertiles^a | OR [95% CI] |
| Low | Reference |
| Medium | 1.31 [0.85–2.03] | 0.220 |
| High | 1.76 [1.08–2.90] | **0.024** |

| Malabsorption | P value |
|---------------|---------|
| wGRS39 tertiles^a | OR [95% CI] |
| Low | Reference |
| Medium | 1.13 [0.75–1.71] | 0.563 |
| High | 1.62 [1.04–2.54] | **0.035** |

| Anaemia | P value |
|---------|---------|
| wGRS39 tertiles^a | OR [95% CI] |
| Low | Reference |
| Medium | 0.92 [0.58–1.50] | 0.742 |
| High | 1.68 [1.03–2.78] | **0.041** |

CI confidence interval, OR odds ratio

^aCoeliac phenotypes were categorised into low-, medium- and high-risk wGRS39 tertiles, according to the distribution of the average weighted risk alleles in the controls. Phenotypes grouped by wGRS39 tertiles with fewer than five events in the reference group were not tested.

**Discussion**

In this study, by using genotyped variants in our cohort, we identified a genotype-phenotype association for ten SNPs previously associated with coeliac disease susceptibility. In addition, our results demonstrate that combining 39 coeliac disease-associated genotyped SNPs into wGRS39 was more informative than a PRS to assess the genetic risk for distinct phenotypes of coeliac disease.

According to our results, rs5979785 located in the proximity of the TLR7 and TLR8 genes was associated with a decreased risk of diagnosis before 7 years of age in girls and notably this association was detected also in the case only comparison. Our RegulomeDB analysis did not retrieve rs5979785 as having regulatory function of transcription although it has previously been reported to decrease the TLR8 expression in the blood [5]. TLR7 and TLR8 are both members of the toll-like receptor family and they detect distinct forms of viral nucleic acids and initiate antiviral responses [32]. As distinct viruses, including enterovirus has been implicated as a risk factor for coeliac disease [33, 34], additional studies on this loci in relation with viral infections and early coeliac disease onset are called for.

Our results also revealed an association of rs653178 at SH2B3/ATXN2 with an increased risk to several other phenotypes, including concomitant T1D. Our findings are thus in accordance with previous studies where the 12q24/SH2B3 locus has been associated with both coeliac disease and T1D [8, 35, 36]. In these studies, rs3184504, a functional proxy in LD with rs653178 [37], has been indicated as the true SNP behind the association. Although rs3184504 was not included in our initial set of genotyped SNPs, we found it to be associated with concomitant T1D in the analysis carried out with the imputed genotypes.

In addition to having a likely regulatory function on transcription and an eQTL effect of the expression of SH2B3 along with many other genes in our analysis, rs3184504 SNP has been also associated with a higher expression of SH2B3 in the intestinal mucosal of patients with active coeliac disease [38], islet autoimmunity [39] and also implicated with bacterial infections [40], making the
12q24/SH2B3 locus associations in our cohort interesting for further research.

In pursuit of clarifying the molecular mechanisms exerted by the susceptibility SNPs on the phenotypes, we identified that only three of our phenotype-associated SNPs had significant eQTL effects on regulating gene expression. This would suggest that the SNPs included in our analysis are mostly proxies and either situated in the proximity of the causative variant or further away. In the KEGG pathway and GO biological process analysis the phenotype-associated rs13098911 SNP was identified as the only variant having cis-eQTL effects on a set of genes enriched in chemokine pathway. This finding most likely reflects the location of this variant in the chemokine gene cluster.

The enrichment results of the trans-eQTL genes identified largely identical pathways for most of our phenotypes. All these phenotypes were associated with rs653178 and its proxy rs3184504 revealed considerable effects on the expression of several distal genes. This pleiotropic effect of rs3184504 thus likely explains our finding of the pathway enrichment analysis of the trans-eQTL genes. Thus, further studies to infer the true phenotype-causal variants and the mechanisms that might mediate their effects on phenotypic variation are needed.

Regarding our weighted 39 SNP-based risk score model, the high-risk tertile in wGSR39 was associated with a higher risk of having coeliac disease symptoms in childhood, more severe small bowel mucosal damage, malabsorption and the occurrence of anemia. These phenotypes can be considered as signs of a more severe disease course [2, 41], thus raising the possibility that increased number of coeliac disease susceptibility SNPs might predispose to a more severe disease. In contrast, combining information from thousands of genomic variants into a PRS contributed to explain very little of the variance in phenotypes apart from DH. One major reason for this might be related to the fact that genetic loci not directly associated with disease status in large case–control GWAS may moderate the relationship between the coeliac polygenic burden and phenotypes [7, 10]. Thus, the main genetic contribution to phenotypic variations seems to derive from loci associated with the disease susceptibility.

A major strength of the current study is the clinically well-characterised large cohort of coeliac patients. In addition, a strength is the careful phenotyping of the patients, allowing us to investigate the association of different genotypes with various phenotypes. Moreover, the imputation analysis allowed us to explore the phenotype association of further 49 previously coeliac disease SNPs not typed in our cohort. The use of the most comprehensive available imputation panel in Finnish population increased our opportunities for identifying new coeliac disease SNPs associated with the phenotypes. Our highly conservative procedures in the imputation analysis diminished the risk of false positive associations. It must be noted though that 47.8% of coeliac disease patients dropped out from the analysis. Moreover, for distinct phenotypes, we had a fairly low number of patients which affected the statistical power in some cases. Moreover, we included in our GRS study only the 39 genotyped SNPs from independent coeliac disease risk loci, and not all the 94 previously associated with coeliac disease, and it might be the case that other coeliac susceptibility SNPs have a modulatory role on the phenotypes.

We conclude that independent coeliac disease-susceptibility loci are associated with distinct disease phenotypes, suggesting that distinct SNPs might play a role in modulating the disease presentation in a yet to determined mechanism. Moreover, while PRS seems not to explain the variance in phenotypes, more severe coeliac disease phenotypes could possibly be contributed by higher amount of coeliac disease risk SNPs. Our GRS approach might thus be useful to identify patients at risk of developing a severe disease course unless identified and treated...
early. Further studies with a larger number of SNPs are called for in independent well-characterised patient cohorts to better understand how genetic variants contribute to the different coeliac disease phenotypes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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