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A Meta-Analysis of Genome-Wide Association Scans Identifies IL18RAP, PTPN2, TAGAP, and PUS10 As Shared Risk Loci for Crohn’s Disease and Celiac Disease

Eleonora A. M. Festen¹,²,³, Philippe Goyette³,⁴, Todd Green⁴,⁵, Gabrielle Boucher³, Claudine Beauchamp³, Gosia Trynka⁶, Patrick C. Dubois⁵, Caroline Lagacé³, Pieter C. F. Stokkers⁶, Daan W. Hommes⁷, Donatella Barisani⁸, Orazio Palmieri⁹, Vito Annesi⁹, David A. van Heel⁵, Rinse K. Weersma¹,¹¹*, Mark J. Daly⁴,¹⁰*, Ciska Wijmenga¹⁹, John D. Rioux¹³*,

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Abstract

Crohn’s disease (CD) and celiac disease (CeId) are chronic intestinal inflammatory diseases, involving genetic and environmental factors in their pathogenesis. The two diseases can co-occur within families, and studies suggest that CeId patients have a higher risk to develop CD than the general population. These observations suggest that CD and CeId may share common genetic risk loci. Two such shared loci, IL18RAP and PTPN2, have already been identified independently in these two diseases. The aim of our study was to explicitly identify shared risk loci for these diseases by combining results from genome-wide association study (GWAS) datasets of CD and CeId. Specifically, GWAS results from CeId (768 cases, 1,422 controls) and CD (3,230 cases, 4,829 controls) were combined in a meta-analysis. Nine independent regions had nominal association p-value <1.0×10⁻⁴ in this meta-analysis and showed evidence of association to the individual diseases in the original scans (p-value <1.0×10⁻⁵ in CeId and <1.0×10⁻³ in CD). These include the two previously reported shared loci, IL18RAP and PTPN2, with p-values of 3.37×10⁻⁵ and 6.39×10⁻⁹, respectively, in the meta-analysis. The other seven had not been reported as shared loci and thus were tested in additional CeId (3,149 cases and 4,714 controls) and CD (1,835 cases and 1,669 controls) cohorts. Two of these loci, TAGAP and PUS10, showed significant evidence of replication (Bonferroni corrected p-values <.00071) in the combined CeId and CD replication cohorts and were firmly established as shared risk loci of genome-wide significance, with overall combined p-values of 1.55×10⁻¹⁰ and 1.38×10⁻¹⁰ respectively. Through a meta-analysis of GWAS data from CD and CeId, we have identified four shared risk loci: PTPN2, IL18RAP, TAGAP, and PUS10. The combined analysis of the two datasets provided the power, lacking in the individual GWAS for single diseases, to detect shared loci with a relatively small effect.


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¹ These authors also contributed equally to this work.

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Introduction

Crohn’s disease (CD) and celiac disease (CelD) are both chronic inflammatory diseases of the digestive tract. Both of these diseases are complex genetic traits with multiple genetic and non-genetic risk factors. Recent genome-wide association (GWA) studies have identified some of the genetic risk factors for these diseases. Interestingly, in addition to some similarities in phenotype, these studies have shown that CeID and CD share some genetic risk factors. Specifically, by comparing the results of independent GWA studies of CD and CeID, two genetic risk loci were found in common: the PTPN2 locus and the IL18RAP locus. Therefore, in order to directly test for additional shared genetic risk factors, we combined the GWA results from two large studies of CeID and CD, essentially creating a combined phenotype with anyone with CD or CeID being coded as affected. Association results were then replicated in additional cohorts of CeID and CD. It is expected that shared risk loci should show association in this analysis, whereas the signal of risk loci specific to either of the two diseases should be diluted. With this method of meta-analysis, we identified next to PTPN2 and IL18RAP two loci harbouring TAGAP and PUS10 as shared risk loci for Crohn’s disease and celiac disease at genome-wide significance.

Author Summary
Celiac disease and Crohn’s disease are both chronic inflammatory diseases of the digestive tract. Both of these diseases are complex genetic traits with multiple genetic and non-genetic risk factors. Recent genome-wide association (GWA) studies have identified some of the genetic risk factors for these diseases. Interestingly, in addition to some similarities in phenotype, these studies have shown that CeID and CD share some genetic risk factors. Specifically, by comparing the results of independent GWA studies of CD and CeID, two genetic risk loci were found in common: the PTPN2 locus and the IL18RAP locus. Therefore, in order to directly test for additional shared genetic risk factors, we combined the GWA results from two large studies of CeID and CD, essentially creating a combined phenotype with anyone with CD or CeID being coded as affected. Association results were then replicated in additional cohorts of CeID and CD. It is expected that shared risk loci should show association in this analysis, whereas the signal of risk loci specific to either of the two diseases should be diluted. With this method of meta-analysis, we identified next to PTPN2 and IL18RAP two loci harbouring TAGAP and PUS10 as shared risk loci for Crohn’s disease and celiac disease at genome-wide significance.

Results

Meta-analysis
We have performed a meta-analysis of 471,504 SNPs from genome-wide datasets of CD (3230 cases, 4829 controls) and CeID (768 cases, 1422 controls) in order to identify shared risk loci between these 2 diseases.

A quantile-quantile (Q-Q) plot of the association p-values for single-SNP Z scores from the meta-analysis was performed (Figure S1) and shows an excess of significant associations above what would be expected by chance. We observe a low inflation factor of 1.08, which is expected given the inflation observed in each of the original studies: 1.05 for CelD and 1.16 for CD. A Manhattan plot of the current study (Figure S2) highlights many strong association signals, several of which correspond to previously reported CD and CelD loci; however, most of these show strong association in only one of the 2 diseases and have thus not been followed up due to the design of the current study. In addition, given the design of the original CeID GWAS, which included only individuals that were positive for the risk-associated allele HLA-DQ2, association to the major histocompatibility complex (MHC) region in CeID was of no relevance since it was artificially inflated. Therefore the MHC region (Chr6:22700000..35000000 from the NCBI B36 genome build) was removed from our analysis.

The meta-analysis of the CD and CeID datasets identified 25 SNPs, from 10 independent regions, that met our criteria for association (association with CeID at p-value < 1 × 10^{-2} and with CD at p-value < 1 × 10^{-3} in the original scans, as well as a nominal association p-value more significant than 1.0 × 10^{-7} in the meta-analysis) (Table 1 and Table S2). This is more than expected by chance, as we would expect no more than 3 independent regions to meet our criteria, which encouraged us to explore these specific loci further.

The strongest association signal identified in our scan was to the well accepted CD associated risk locus CARD15 (p-values of 3.42 × 10^{-52}, 3.77 × 10^{-5} and 1.30 × 10^{-21} in the CD, CeID and scan datasets respectively). Given the strength and the width of the association signal peak at this locus in the CD dataset, our chances of detecting a false positive shared signal at this locus in the scan were artificially increased. Because of this, the CARD15 locus was
Discussion

By performing a meta-analysis of GWAS data from CD and CelD as a single disease phenotype, we have identified four risk loci shared by these 2 diseases: **PTPN2**, *IL18RAP*, *TAGAP* and *PUS10*. This meta-analysis approach provided the power, lacking in individual disease-specific GWAS datasets, to identify shared risk loci with small effects in each single disease. This approach is a powerful and versatile way of identifying shared risk loci. In fact, two of the shared loci described here, *TAGAP* and *PUS10*, would not have reached genome-wide significance without the power gained from the combined samples (scan and replication) of these 2 diseases. As the GWAS for the individual diseases increase in power, we can expect the power of the current approach to also increase enabling us to identify further shared loci.

The **TAGAP** locus identified in the current study as a shared risk factor for CD and CelD is located on chromosome 6q25.3, within a 200-kb block of linkage disequilibrium (LD). This TAGAP locus was previously identified as a CelD risk locus [9] but not found in previous studies of CD. **TAGAP** is the best candidate of four genes in this region of strong LD [9]. **TAGAP** is a member of the Rho-GTPase protein family, which release GTP from GTP-bound Rho, thereby acting as a molecular switch. The gene is expressed in activated T cells and appears to be important for modulating cytoskeletal changes [14]. Little is known about the

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**Table 1.** Results from the meta-analysis, replication, and combined analysis.

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The meta-analysis was performed using a directional non-weighed Z-score method as explained in the methods section. Combined analyses were performed using a directional weighed Z-score method within diseases and a directional non-weighed Z-score method between diseases. Results for the combined analysis are given only for the SNPs that pass the replication thresholds (directionality in each disease and p-value<0.0071 for the combined replication data).

### Notes

- **IL18RAP** and **PTPN2** were not followed up because they are known shared risk loci for CD and CelD.
- **SNPs** that were imputed in the CelD replication datasets.

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not moved forward to replication. An evaluation of the association signal in the *in silico* CelD GWAS replication datasets confirmed that this locus did not show replication in CelD. Several of the SNPs meeting our criteria for association mapped to the known shared risk loci *IL18RAP* and *PTPN2*. Identifying these shared risk loci in the initial phase of our analysis provides proof of the effectiveness of our method. Interestingly, these two loci either reach or are very near genome-wide significance in the current meta-analysis (p-value = 8.37×10<sup>-8</sup> for **IL18RAP** and of 6.39×10<sup>-9</sup> for **PTPN2**), validating their previously identified role in both CD and CelD. The remaining 12 SNPs were located in seven independent regions and for each of these loci we selected the most associated SNP for testing for evidence of replication.

### Replication phase

All SNPs selected for follow-up were genotyped in additional replication cohorts of CelD patients (n = 3149) and healthy controls (n = 4714) and of CD patients (n = 1941) and healthy controls (n = 1669). Given that these putative shared risk loci were selected through the combined analysis of our CD and CelD scan cohorts, a positive threshold for replication was therefore set at a corrected p-value of 0.0071 (Bonferroni corrected p-value of 0.05 for 7 independent tests) in the combined analysis of CD and CelD replication cohorts.

Only two of the 7 loci tested, *PUS10* (pseudouridylate synthase 10; RefSeq NM_144709.2) and **TAGAP** (T-cell activation GTPase activating protein; RefSeq NM_054114), showed significant replication (p-values of 6.03×10<sup>-7</sup> and 3.03×10<sup>-6</sup> respectively) with matching direction of association between the scan and replication datasets, as well as replication p-values more significant than 0.05 in both CD and CelD replication cohorts independently (Table 1). While neither *PUS10* nor **TAGAP** were identified as loci of genome-wide significance in the combined dataset from each disease (p-values = 1.34×10<sup>-6</sup> and 7.00×10<sup>-7</sup> in CelD and p-values = 6.16×10<sup>-8</sup> and 2.13×10<sup>-6</sup> in CD respectively), both reach genome-wide significance in a combined analysis of CD and CelD cohorts (p-values of 1.38×10<sup>-11</sup> and 1.55×10<sup>-10</sup> respectively). Based on the results calculated from the replication datasets, we also observe that the effects at these 2 loci are similar in size and direction for both CD and CelD (Table 1).

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exact role of *TAGAP* in immune function, but it has been found to be co-regulated with *IL2* and is expected to play a role in T-cell activation [14].

The current study also identifies a shared risk locus between CD and CelD in the *PUS10* gene region, a locus previously described as a risk locus for CD [15]. This locus was recently identified as a risk locus in both ulcerative colitis (UC) and CelD, indicating that this locus may be a shared risk locus for these three diseases [7,16]. This latter finding further validates the approach use in this study to identify risk factors that are shared across diseases. Interestingly, the UC study identified three independent signals in this region which seem to be shared differently across these three diseases: one signal seems to be shared only between CD and UC, a second only between CelD and UC, while the third, identified in the current study, seems shared between all three diseases. Further analysis of this locus will be necessary in order to clarify the role of these different alleles in disease risk.

In this study we aimed to find shared genetic risk factors for CelD and CD by meta-analysis of GWAS data of both diseases, defining a single phenotype for these analyses. Using readily available data, we were able to reliably establish four shared loci: *PTPN2*, *IL1R1RAP*, *TAGAP* and *PUS10*. For many diseases with overlapping phenotypic characteristics, GWAS data is available and joint analysis of GWAS datasets of these related diseases could lead to the identification of many new shared susceptibility loci.

**Materials and Methods**

**Subjects**

For the CD aspect of the meta-analysis, we used the previously published data (available at http://www.broadinstitute.org/~jcbarret/ibd-meta/) from the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) meta-analysis of 3230 CD cases and 4829 healthy controls taken from three independent CD GWAS (Table S1) [15]. A more in depth description of these cohorts and their origin can be obtained from the original publication of this meta-analysis.

Two independent cohorts were used for the CD replication phase (Table S1). The first consisted of 1217 Dutch CD cases from three Dutch university medical centers: the Academic Medical Centre Amsterdam (n = 661), the University Medical Centre Groningen (n = 322) and the University Medical Centre Leiden (n = 234); the 804 Dutch controls used for this replication cohort were obtained from cohorts of healthy partners of IBD patients from the UMC Leiden (n = 151) and the UMC Groningen (n = 120) and from healthy blood donors recruited through the Sanquin Blood bank by the UMC Utrecht and the VUMC Amsterdam (n = 533) [17,18]. The second replication cohort consisted of an Italian IBD case – control cohort (724 CD patients and 892 controls) collected at the S. Giovanni Rotondo "CSS" (SGRC) Hospital in Italy. This cohort has previously been used and characterized in several association reports [19,20].

All patients and controls were of European Caucasian descent. The diagnosis of CD required objective evidence of inflammation from radiologic, endoscopic, and/or histopathologic evaluation. All affected subjects fulfilled clinical criteria for CD. Recruitment of study subjects was approved by local and national institutional review boards, and informed consent was obtained from all participants. Some controls were shared between the WTCCC component of the CD meta-analysis and the two UK CelD cohorts (one used in the original scan and one used as replication). This was taken into account as explained in the meta-analysis description.

**Imputation of GWAS data**

Imputation of the CelD datasets used in the initial and the replication phases of this study were performed with BEAGLE using HapMap phase II and HapMap phase III as reference datasets [21]. A minimum quality score for statistical certainty of the imputation of 0.98 was adhered to.

Imputation of the CD dataset for the initial CD-CelD meta-analysis was performed with the programs MACH and IMPUTE, using HapMap phase II as a reference dataset, as previously described [15,22,23].

**Meta-analysis**

For the original CD dataset, association tests were described previously [15]. Briefly, results for each SNP from three independent GWAS were summarized as Z-scores and combined in a weighted fashion into a single test statistic; imputation uncertainty was taken into account into Z-score and weight calculation using empirical variance calculated from allele dosage. For the original CelD GWAS scan, best guess imputed genotype frequency data was obtained, and association P-values were calculated using chi-square tests (1 df) of SNP allele counts.

The initial meta-analysis was performed using the statistical program R (http://www.r-project.org/). For both the CD and the CelD dataset the p-values signifying the evidence for association were converted to directional Z-scores, and an overall Z-score and two-tailed p-value for the average of the individuals was subsequently calculated. Given the fact that some controls were shared between one component of the CD meta-analysis and the CelD scan, we expect a correlation of 0.187 between CD and CelD Z-scores. We took this correlation into account in the variance term of the overall Z-score [24].

Unweighed Z-scores were used when combining the data from CD and CelD in the initial meta-analysis, since the CD cohort was...
substantially larger than the CelD cohort and weighing would lead to an overrepresentation of the CD signal in the meta-analysis. A locus was selected for replication when SNPs met the following criteria: a p-value for the locus of <1×10^{-5} in the meta-analysis, in combination with a p-value of <1×10^{-2} in the CelD dataset and a p-value of <1×10^{-3} in the CD dataset. Different thresholds for CD and CelD for inclusion in the replication phase were used in order to reflect the difference in power between the scans for the two phenotypes. For each of the loci that met these criteria, the most strongly associated SNP was analyzed in the CD and CelD replication cohorts.

In order to evaluate the expected number of SNPs that would pass our thresholds (p-value <1×10^{-2}, <1×10^{-3} and <1×10^{-5} for CelD, CD and meta-analysis, respectively) by chance, we first evaluated the probability of a particular SNP to reach those thresholds under the null hypothesis of no association and the expected correlation between the two datasets. This probability can be evaluated from the distribution of two correlated normal variables (correlation of 0.186), combined as described for the meta-analysis. We evaluated this probability to be approximately 6.0×10^{-6}. If the 468,378 SNPs tested in the scan were independent, we would then expect less than 3 (468,378×6.0×10^{-6}) independent SNPs to be selected by chance. Under a binomial model, we evaluated the probability that 9 or more independent SNPs passes our thresholds to be lower than 0.0025. Those are obviously upper bounds, as we know correlation exists among the SNPs tested.

Replication phase

For replication in CD, SNPs selected for testing were designed into multiplex assays, and genotyped using primer extension chemistry and mass spectrometric analysis (iPLEX assay, Sequenom, San Diego, California, USA) on the Sequenom MassArray. This was performed at the Laboratory for Genetics and Genomic Medicine of Inflammation (www.inflammgen.org) of the Université de Montréal. Quality control was performed, excluding samples showing >10% missing data, as well as SNPs with >10% missing data or significantly out of Hardy-Weinberg equilibrium (p-value <0.001). The overall genotyping call rate in the CD replication dataset following quality control analyses was >99%. The CD replication datasets from the two groups (Dutch and Italian) were combined and analyzed using a weighted and directional Z-score approach.

For replication in CelD, genotype frequencies and association data for five replication SNPs were obtained from genome-wide genotyping datasets on Illumina Human 670Quad or 610Quad Genotyping BeadChips (Illumina, Inc., San Diego, CA, USA). Each GWAS dataset was analyzed using PLINK 1.05 and association p-values were calculated using chi-square tests (1 df) of SNP allele counts [25]. Two of the replication SNPs were not included on the Illumina Human 670Quad or 610Quad Genotyping BeadChips. For these SNPs best guess genotype frequency data was obtained by imputation as described above, and association p-values were calculated using chi-square tests (1 df) of SNP allele counts. The CelD replication datasets from the three groups (UK, the Netherlands and Italy) were combined and analyzed using a weighted and directional Z-score approach.

Since selection of specific SNPs for replication was based on their association p-values in the combined CD-CelD dataset, a significant threshold for replication was set at a Bonferroni corrected p-value of 0.05 for 7 independent tests (p-value more significant than 0.0071) in the combined CD-CelD replication dataset. As for the initial meta-analysis, the data from the replication in the CD and CelD cohorts were combined through an unweighted Z-score approach. In addition, for a SNP to be replicated, both effect and direction of association trend needed to match between scan and replication within each disease.

For each SNP showing positive replication, an overall disease-specific association p-value, combining the scan and replication data, was also calculated using a weighted meta-analysis approach. Finally, an overall CD-Celiac meta-analysis of the scan and replication phases of this study was obtained, by combining these within-CD and within-CellD datasets in an unweighted meta-analysis. Given the fact that some controls were shared between the within-CD and the within-CelD, we expect a correlation of 0.149 between CD and CelD Z-scores. As for the initial scan, we took this correlation into account in the variance term of the overall Z-score as per Lin and colleagues [24].

Supporting information

Figure S1 Q-Q plot of meta-analysis scan. A meta-analysis scan of 471,504 SNPs from genomewide datasets of CD and CelD was performed using a directional non-weighed Z-score method (as explained in the methods section). The Q-Q plot was generated from the p-values for single-SNP Z scores. Given the strength of association signal for the MHC region (chr6:22,700,000-35,000,000) in CelD, this region overwhelmed the Q-Q plot and was therefore removed from the dataset for purpose of clarity; removing the MHC region had little impact on the observed inflation. Found at: doi:10.1371/journal.pgen.1001283.s001 (0.06 MB DOC)

Figure S2 Manhattan plot of meta-analysis scan. A meta-analysis scan of 471,504 SNPs from genomewide datasets of CD and CelD was performed using a directional non-weighed Z-score method (as explained in the methods section). The Manhattan plot was generated from the p-values for single-SNP Z scores. Given the strength of association signal for the MHC region (chr6:22,700,000-35,000,000) in CelD, this region overwhelmed the Manhattan plot and was therefore removed from the dataset for purpose of clarity. Found at: doi:10.1371/journal.pgen.1001283.s002 (0.10 MB DOC)

Table S1 Subjects included in study. Found at: doi:10.1371/journal.pgen.1001283.s003 (0.14 MB DOC)

Table S2 Complete list of associated markers from meta-analysis. Found at: doi:10.1371/journal.pgen.1001283.s004 (0.01 MB XLSX)

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Author Contributions
Conceived and designed the experiments: EAMF PG TG MJD CW JDR. Performed the experiments: CB PCD CL DAvH. Analyzed the data: EAMF PG TG GB CB GT PCD CL DAvH MJD CW JDR. Contributed reagents/materials/analysis tools: PCFS DWH DB OP VA DAvH RKW CW JDR. Wrote the paper: EAMF PG TG GB RKW MJD CW JDR.

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