Sequence variants in ARHGAP15, COLQ and FAM155A associate with diverticular disease and diverticulitis

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Diverticular disease is characterized by pouches (that is, diverticulae) due to weakness in the bowel wall, which can become infected and inflamed causing diverticulitis, with potentially severe complications. Here, we test 32.4 million sequence variants identified through whole-genome sequencing (WGS) of 15,220 Icelanders for association with diverticular disease (5,426 cases) and its more severe form diverticulitis (2,764 cases). Subsequently, 16 sequence variants are followed up in a diverticular disease sample from Denmark (5,970 cases, 3,020 controls). In the combined Icelandic and Danish data sets we observe significant association of intronic variants in ARHGAP15 (Rho GTPase-activating protein 15; rs4662344-T: \( P = 1.9 \times 10^{-18} \), odds ratio (OR) = 1.23) and COLQ (collagen-like tail subunit of asymmetric acetylcholinesterase; rs7609897-T: \( P = 1.5 \times 10^{-10} \), OR = 0.87) with diverticular disease and in FAM155A (family with sequence similarity 155A; rs6715365-A: \( P = 3.0 \times 10^{-11} \), OR = 0.82) with diverticulitis. These are the first loci shown to associate with diverticular disease in a genome-wide study.

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Divertericul disease is thought to be due to complex interactions between diet, lifestyle, colonic motility, structural changes in the gut, enteric neuropathy and smoking. The intestinal microflora and low-grade inflammation may contribute to diverticular disease and acute diverticulitis. Divertericae are commonly found during routine colonoscopy with increased prevalence from age 50–59 years (32.6%) to age ≥80 years (71.4%) up to 20% will experience complications of the disease but only 1–4% of individuals with diverticula develop acute diverticulitis, with a recurrence risk of 20% within 5 years.

Relative risk of siblings of diverticular disease cases is 2.9 (ref. 8) and the heritability in twin studies estimated to be 40–50% (refs 8,9). This indicates that there is a strong genetic component to the risk. No sequence variants associating with risk of diverticular disease have been found and no genome-wide association studies (GWAS) have been published.

We performed GWAS to search for sequence variants that affect the risk of diverticular disease and diverticulitis in Iceland, with a follow-up in Danish samples of diverticular disease. We identified association of three intronic variants in the genes ARHGAP15 (Rho GTPase-activating protein 15) and COLQ (collagen-like tail subunit of asymmetric acetylcholinesterase) with diverticular disease and in FAM155A (family with sequence similarity 155A) with diverticulitis. These are the first sequence variants found to show genome-wide significant association with diverticular disease.

Results

Association of three loci with diverticular disease. We imputed 32.4 million sequence variants identified through WGS of 15,220 Icelanders into 151,677 chip typed Icelanders and their first- and second-degree relatives and performed two GWAS to search for sequence variants that affect the risk of diverticular disease (5,426 cases) and diverticulitis (2,764 cases) using the same 245,951 controls (Supplementary Table 1). We applied weighted thresholds for genome-wide significance that depend on the functional class of each variant, based on its prior probability of affecting gene function (Supplementary Table 2).

We chose 16 variants for follow-up in a Danish diverticular disease sample set that were within two orders of magnitude from genome-wide significance threshold in a variant class for either diverticular disease or diverticulitis in Iceland (Table 1 and Supplementary Table 3a–c). We do not have information on diverticulitis in the Danish cohort, but chose to follow-up the Icelandic diverticulitis findings based on the assumption that the Danish cohort includes diverticulitis, although the proportion is unknown. With these data sets we identified three loci that are of genome-wide significance in the combined analysis of the Icelandic and Danish samples; intronic variants at the ARHGAP15 and COLQ loci associate significantly with diverticular disease and at FAM155A locus with diverticulitis ($P < 2.3 \times 10^{-9}$, the threshold for intronic variants within a DNAse hypersensitivity site) (Table 1 and Supplementary Table 3). No significant heterogeneity was observed between the study groups and the three singe-nucleotide polymorphisms (SNPs) are markedly significant in the Danish follow-up.

Potential causal genes at the diverticular disease loci. The strongest diverticular disease association in Iceland was with 45 correlated ($r^2 > 0.97$) sequence variants (minor allele frequency (MAF) = 17.6–17.8%) in 88 kb region spanning introns 9 and 10 of ARHGAP15 (Rho GTPase-activating protein 15) (Fig. 1a). The variants are represented by rs4662344-T that associates at genome-wide significance in Iceland (chr2:143,591,289, odds ratio (OR) = 1.23, $P = 4.9 \times 10^{-13}$) (Table 1). In Iceland rs4662344-T confers similar risk of diverticulitis (OR = 1.26, $P = 4.5 \times 10^{-9}$) and uncomplicated diverticulitis disease (OR = 1.20, $P = 2.6 \times 10^{-9}$) ($P_{het} = 0.36$). No association signal remains at the locus after conditional analysis using rs4662344-T as a covariate (Fig. 2a), indicating that one of the 45 intronic variants is likely to mediate the signal at this locus. The association replicates in the Danish samples (OR = 1.22 and $P = 7.3 \times 10^{-7}$) for a combined P value of $1.9 \times 10^{-18}$ and OR of 1.23 for the Icelandic and Danish samples. None of the three missense variants in ARHGAP15 (15 exons 475 amino acids) associate with diverticular disease (P > 0.44). No other gene is within 100 kb of any of the SNPs in linkage disequilibrium (LD) ($R^2 > 0.8$) with rs4662344-T.

rs4662344-T did not associate with expression of ARHGAP15 or any other gene in the region ($±500$ kb) in any of the tissues in GTExV6 database (including whole blood and small intestine, the most relevant tissues) nor in RNA sequencing data at deCODE from whole blood ($n = 2,246$) and adipose tissue ($n = 708$) (Supplementary Fig. 1, shown for each transcript and each exon). ARHGAP15 encodes Rho GTPase-activating protein 15, a member of the Rac-specific GTPase-activating protein (GAP). Rac is a small GTPase, important for cell proliferation, apoptosis, attachment and motility. ARHGAP15 activation of Rac affects the actin cytoskeleton and cell morphogenesis and overexpression of ARHGAP15 causes increase in actin stress fibres and cell contraction. Neutrophils of mice that are knockout for ArhGAP15 show increased migration, phagocytosis, reactive oxygen species (ROS) production and bacterial killing, and reduced inflammation. We therefore tested the effect of rs4662344-T on ROS production by neutrophils stimulated with E. coli, phorbol 12-myristate 13-acetate or N-formyl-Met-Leu-Phe, but found no effect of rs4662344-T on ROS production for any of the stimulants (Supplementary Fig. 2).

The second strongest signal that associates with diverticular disease in the Icelandic samples is captured by a single intronic SNP in COLQ, rs7609897-T (chr3:15,461,174, MAF = 24.7%) (Fig. 2b), that associates with diverticular disease (OR = 0.85, $P = 1.6 \times 10^{-9}$) in Iceland; this association replicates nominally in the Danish samples with a consistent direction of the effect (OR = 0.91, $P = 0.010$) for a combined OR of 0.87 and $P$ value of $1.5 \times 10^{-10}$ for the Icelandic and Danish samples. In the Icelandic and in 1,000G European data sets, rs7609897-T is weakly correlated with other markers ($r^2 < 0.26$ and $r^2 < 0.48$, respectively). We performed conditional analysis to look for additional signals at the locus (Supplementary Table 5). We found one rare missense variant rs146687198-G (p.Gly246Ala, MAF = 0.22%) in COLQ with large effect on diverticular disease in Iceland (OR = 2.06, 95% confidence interval (CI): 1.4, 3.0, $P = 3.5 \times 10^{-4}$). This rare missense variant is not correlated with the intronic rs7609897-T ($r^2 < 0.001$). Follow-up genotyping in the Danish samples showed a weaker and not significant effect (OR = 1.15, 95% CI: 0.63, 2.10, $P = 0.65$, MAF = 0.26%). However, the effect is consistent in the two populations ($P_{het} = 0.11$, for Iceland and Danish samples). Although the association of this rare missense variant with diverticular disease is not of genome-wide significance, the prior probability established by the association of rs7609897-T suggests that the association of rs146687198-G may be real and points to COLQ as the causative gene at this locus.

COLQ has 18 exons that span a 71 kb region (Supplementary Table 5). COLQ is expressed in most tissues (GTExV6) but did not associate with mRNA expression of COLQ or any of the 11 genes within 500 kb of rs7609897-T in any of the tissues in the GTExV6 database or in the deCODE in blood or adipocyte RNA sequencing data (Supplementary Fig. 3).
COLQ encodes a subunit of a collagen-like molecule (ColQ), associated with acetylcholinesterase (AChE), whose catalytic subunits are anchored in the basal lamina of neuromuscular junction through ColQ. Homozygote mutations (or compound heterozygotes) in COLQ can reduce AChE availability resulting in muscle weakness and congenital myasthenic syndromes\(^{17}\).

The third locus harbours sequence variants in the first intron of FAM155A, marked by rs67153654-A, showing a suggestive association with diverticulitis in Iceland (Fig. 1b and Fig. 2c)\(^{7}\). Although diagnosis of diverticulitis is not exclusive to diverticular disease\(^{11,12}\), our results suggest that FAM155A may be a candidate gene for diverticulitis. The 13 variants at the other loci selected for validation in the Icelandic and Danish samples lack evidence for association with either diverticular disease or diverticulitis (0.05/184 loci). (Supplementary Table 7).

The 16 missense variants found in FAM155A associates with diverticular disease or diverticulitis (Supplementary Table 5). FAM155A is mainly expressed in the hypothalamus and pituitary gland\(^{18,19}\), with low expression in the colon and blood (GTEX V6)\(^{16}\). We found no effect of rs67153654-A on the expression of FAM155A or nearby genes in GTEX V6 or in blood or adipocytes using RNA sequencing (Supplementary Fig. 4). Little is known about the function of FAM155A but close SNPs (\(r^2\leq0.01\) with rs67153654-A) have been associated with increased fat mass in children\(^{20}\) and anorexia nervosa\(^{21}\). We tested the association of diverticulitis versus uncomplicated diverticular disease for variants at the ARHGAP15, COLQ and FAM155A loci (Supplementary Table 4). Only the FAM155A variant is significantly less frequent in diverticulitis (OR = 0.84, 95% CI: 0.74, 0.94, \(P = 3.8 \times 10^{-3}\)).

The 13 variants at the other loci selected for validation in the Danish samples lack evidence for association with either diverticular disease or diverticulitis (Supplementary Table 3a–c and Supplementary Note).

Inflammation contributes to the development and recurrence of diverticulitis\(^{22}\). Therefore, we tested the effects of the diverticulitis disease variants at the ARHGAP15, COLQ and FAM155A loci on other inflammatory diseases of intestine and colon, namely ulcerative colitis (UC) and Crohn’s disease (CD) (inflammatory bowel disease (IBD)) and found no association with a \(P < 1 \times 10^{-3}\). Furthermore, none of the 184 IBD/UC/CD variants (from the GWAS catalogue\(^{23–29}\)) associate with diverticulitis or diverticular disease (0.05/184 = \(P < 2.7 \times 10^{-4}\)) in Iceland. Neither did polygenic risk scores (PRS) for IBD, UC and CD capture risk of diverticular disease or diverticulitis (Supplementary Table 6).

Few sequence variants have previously been reported to associate with diverticular disease, diverticulitis or diverticular disease-related diseases in small candidate gene studies\(^{30–33}\). We show no evidence for association of these variants with the disease in the Icelandic data (Supplementary Table 7).

### Table 1 | Icelandic GWAS results, follow-up in a Danish diverticular disease sample set and association in the Icelandic and Danish sample sets combined.

| Nearest gene | SNP | Amin/Amaj | Icelandic diverticulitis disease\(^{7}\) | Danish diverticulitis disease* | Combined Icelandic and Danish diverticulitis disease sample sets |
|--------------|-----|-----------|-----------------------------------|-------------------------------|-------------------------------------------------------------|
|              |     |           | 4,262 cases; 245,951 controls     | 5,970 cases; 3,020 controls   | 11,396 cases; 248,971 controls                                |
|              |     |           | MAF % cases/controls OR (95% CI) P value | MAF % cases/controls OR (95% CI) P value | MAF % cases/controls OR (95% CI) P value P\(_{\text{het}}\) |
| ARHGAP15     | rs4662344 | T/C     | 20.9/17.7 1.23 (1.16, 1.31) 4.9 \times 10^{-12} | 21.7/18.5 1.22 (1.13, 1.32) 7.0 \times 10^{-7} | 12.3 (17, 129) 1.9 \times 10^{-18} 0.86 |
|              | rs7609897 | T/G     | 22.1/24.7 0.85 (0.80, 0.89) 1.6 \times 10^{-9} | 21.6/23.3 0.91 (0.84, 0.98) 1.0 \times 10^{-2} | 0.87 (0.83, 0.91) 1.5 \times 10^{-8} 0.17 |
| FAM155A      | rs67153654 | A/T    | 17.0/18.6 0.89 (0.84, 0.94) 8.7 \times 10^{-5} | 17.4/20.1 0.84 (0.78, 0.91) 2.2 \times 10^{-5} | 0.87 (0.83, 0.91) 3.0 \times 10^{-11} 0.43 |

| Nearest gene | SNP | Amin/Amaj | Icelandic diverticulitis disease\(^{7}\) | Danish diverticulitis disease* | Combined Icelandic and Danish diverticulitis disease sample sets |
|--------------|-----|-----------|-----------------------------------|-------------------------------|-------------------------------------------------------------|
|              |     |           | 2,662 cases; 245,951 controls     | 5,970 cases; 3,020 controls   | 8,734 cases; 248,971 controls                                |
|              |     |           | MAF % cases/controls OR (95% CI) P value | MAF % cases/controls OR (95% CI) P value | MAF % cases/controls OR (95% CI) P value P\(_{\text{het}}\) |
| ARHGAP15     | rs4662344 | T/C     | 21.3/17.7 1.26 (1.16, 1.36) 4.5 \times 10^{-5} | 21.7/18.5 1.22 (1.13, 1.32) 7.0 \times 10^{-7} | 1.24 (1.17, 1.31) 1.8 \times 10^{-14} 0.62 |
|              | rs7609897 | T/G     | 21.0/24.7 0.8 (0.74, 0.86) 1.9 \times 10^{-9} | 21.6/23.3 0.91 (0.84, 0.98) 1.0 \times 10^{-2} | 0.85 (0.80, 0.89) 1.0 \times 10^{-9} 0.02 |
| FAM155A      | rs67153654 | A/T    | 15.6/18.6 0.8 (0.74, 0.87) 2.3 \times 10^{-7} | 17.4/20.1 0.84 (0.78, 0.91) 2.2 \times 10^{-5} | 0.82 (0.78, 0.87) 3.0 \times 10^{-11} 0.43 |

| Nearest gene | SNP | Amin/Amaj | Icelandic uncomplicated diverticulitis disease | Danish diverticulitis disease* | Combined uncomplicated diverticulitis disease and Danish diverticulitis disease sample sets |
|--------------|-----|-----------|-----------------------------------------------|-------------------------------|------------------------------------------------------------------------------------------|
|              |     |           | 2,662 cases; 245,951 controls                 | 5,970 cases; 3,020 controls   | 8,632 cases; 248,971 controls                                                            |
|              |     |           | MAF % cases/controls OR (95% CI) P value       | MAF % cases/controls OR (95% CI) P value | MAF % cases/controls OR (95% CI) P value P\(_{\text{het}}\)                               |
| ARHGAP15     | rs4662344 | T/C     | 20.4/17.7 1.2 (1.11, 1.30) 2.6 \times 10^{-5} | 21.7/18.5 1.22 (1.13, 1.32) 7.0 \times 10^{-7} | 1.21 (1.15, 1.28) 8.6 \times 10^{-12} 0.77 |
|              | rs7609897 | T/G     | 23.7/24.7 0.91 (0.84, 0.98) 6.1 \times 10^{-9} | 21.6/23.3 0.91 (0.84, 0.98) 7.0 \times 10^{-7} | 0.91 (0.86, 0.95) 1.7 \times 10^{-4} 1.0 |
| FAM155A      | rs67153654 | A/T    | 18.8/17.8 0.99 (0.9, 1.06) 0.72              | 17.4/20.1 0.84 (0.78, 0.91) 2.2 \times 10^{-5} | 0.91 (0.87, 0.97) 1.4 \times 10^{-3} 4.9 \times 10^{-3} |

Amaj, major allele; Amin, minor allele; 95% CI, 95% confidence interval; MAF, minor allele frequency; OR, odds ratio of the minor allele; P\(_{\text{het}}\), P value for the heterogeneity between cohorts; SNP, single-nucleotide variant polymorphism.

MAF is calculated on the chip-typed samples, excluding familialy imputed genotypes. The three variants rs4662344, rs7609897 and rs67153654 are annotated as intronic variant within a DNase hypersensitivity site, giving the class-specific Bonferroni threshold for genome-wide significance as \(P < 2.3 \times 10^{-9}\) (ref.12).

*Note that the Danish diverticular disease results are the same in all three parts of the table, since diverticulosis diagnosis was not available."
Figure 1 | Manhattan plot for genome-wide association results. The P values (− log_{10}) are plotted against their respective positions on each chromosome. P value thresholds for the different annotation classes are indicated with gray lines. For intronic/intergenic variants outside DNase hypersensitivity site: P = 1.1 × 10^{-9}, intronic/intergenic variants within DNase hypersensitivity site P = 2.3 × 10^{-9}, low-impact variants: P = 5.3 × 10^{-9}, medium-impact variants: P = 7.4 × 10^{-8} and high-impact variants: P = 3.7 × 10^{-7}. The plots were created using qqman: an R package for visualizing GWAS results using Q-Q and Manhattan plots54. (a) GWAS results for diverticular disease (cases n = 5,292; controls n = 245,951). ARHGAP15 P = 5.1 × 10^{-12}, COLQ P = 2.8 × 10^{-9} and FAM155A P = 8.0 × 10^{-5}. (b) GWAS results for diverticulitis (cases n = 2,764 cases; controls n = 245,951) (excluding diverticular disease). ARHGAP15 P = 6.0 × 10^{-9}, COLQ P = 3.0 × 10^{-9} and FAM155A P = 1.7 × 10^{-7}. The three variants rs4662344, rs7609897 and rs67153654 are annotated as intronic variant within a DNase hypersensitivity site, giving the class-specific Bonferroni threshold for genome-wide significance as P < 2.3 × 10^{-9} (ref. 12).

Discussion

We have found common sequence variants in introns of the ARHGAP15, COLQ and FAM155A that associate with risk of diverticular disease or diverticulitis. These sequence variants do not overlap with known GWAS signals in other diseases or traits, including established risk loci for immune-mediated and inflammatory diseases34. Diverticulitis occurs when the mucosa of diverticula becomes inflamed. Often the flat colon mucosa between the orifices of the diverticula is inflamed, with changes indistinguishable from those of UC or CD35. We found no genetic overlap between diverticular disease and UC and CD. We found no association of the diverticular disease variants reported here with these diseases and well-established risk variants, and PRS for IBD, UC and CD do not associate with diverticular disease or diverticulitis. This indicates that the pathogenic mechanisms differ from those of autoimmune diseases of the colon and intestine. This is further supported by the complete lack of association with the HLA region that associates strongly with IBD and UC25.

The stronger association of the FAM155A variants with diverticulitis than diverticular disease in general may reflect effects on disease progression, such as inflammation or infection. Various inflammatory components have been suggested as biomarkers of diverticular disease and diverticulitis, including C-reactive protein, white blood cell count, erythrocyte sedimentation rate and faecal calprotectin36. Still we found that the FAM155A variants have no effect on C-reactive protein levels, white blood cell count or neutrophil count, erythrocyte sedimentation rate (P < 10^{-3}) (Supplementary Methods), neither do the ARHGAP15 and COLQ variants. Despite the role of Rho GTPase-activating protein 15, encoded by ARHGAP15, on phagocyte function and inflammation the ARHGAP15 variant associating with diverticular disease does not affect ROS production by neutrophils. Whether the diverticulosis disease variants mediate their effects by modulating inflammation is thus unclear. None of the diverticular disease variants affect the expression of ARHGAP15, COLQ or FAM155A or of nearby genes, neither in deCODE’s RNAseq data on blood and adipocytes nor in data from the various tissues of the GTEx database. Thus, the mechanism by which they affect the risk of diverticular disease remains to be elucidated.

Smoking is a risk factor for symptomatic diverticular disease in both men and women increasing risk of developing complicated diverticular disease1 and hospital admission for acute colonic diverticulitis37. We found that heavy smokers (N = 26,113, >10 pack-years)38,39 have higher risk of developing diverticular disease than never smokers (N = 22,815) (Supplementary Methods), with relative risk = 1.35; 95% CI: 1.21–1.51, P = 1.11 × 10^{-7} (adjusted for sex and age). However, smoking showed no interaction with the effect of any of the three diverticular disease variants.

This first genome-wide association scan may pave the way for studies on the mechanism underlying the development of diverticular disease and diverticulitis.
Genotyping and association. Genotyping and imputation methods and the association analysis in the Icelandic samples was performed as follows: In brief, we sequenced the whole genome of 15,220 Icelanders using Illumina sequencers to a mean depth of at least $10 \times$ (mean 30, median $\times 32$), using three different library preparation methods from Illumina: (a) the standard TruSeq DNA library preparation method; Illumina GAIIx and/or HiSeq 2000 sequencers; (b) the TruSeq DNA PCR-free library preparation method; Illumina HiSeq 2500 sequencers; and (c) the TruSeq Nano DNA library preparation method. Illumina HiSeq X sequencers (see Supplementary Methods for a detailed description of the sequencing methods). Genotypes of SNPs and indels were called using joint calling with the Genome Analysis Toolkit HaplotypeCaller (GATK version 3.3.0) using information about haplotype sharing genotype calls were improved by taking advantage of chip-typing and long-range phasing of all the sequenced individuals. In total, 32,463,443 genetic variants were called (info > 0.8 and MAF > 0.01%). SNPs and indels that met the quality criteria were imputed into the 151,677 chip-typed Icelanders with the help of extensive genealogical information and long-range phased haplotypes. The sequence variants were imputed into 294,212 untyped relatives of the chip-typed individuals to further increase the sample size for association analysis and increase the power to detect associations.

We used the variant effect predictor to predict the maximal consequence of each sequence variant on all neighbouring RefSeq genes. There is a substantial variation in the enrichment of phenotype-associating sequence variants based on their annotations. On the basis of these enrichments, it is possible to group sequence variants into categories, in order of decreasing impact on biological function. We used the enrichment of variant classes to correct the threshold for genome-wide significance with a weighted Bonferroni adjustment. With 32,463,443 sequence variants tested, we applied the method of genomic control (Supplementary Table 2) in the case–control analysis, we used logistic regression; disease status was treated as the response and genotype counts were used as covariates. We also included in the model as nuisance variables the following available individual characteristics that correlate with disease status: county of birth, sex, current age or age of death (first- and second-order terms included), availability of blood sample for the individual and an indicator function for the overlap of the timespan of phenotype collection with lifetime of the individuals. To distinguish polygenicity from population stratification in the GWAS results, we applied LD score regression to estimate a correction factor to yield significance thresholds of $P < 2.6 \times 10^{-7}$ for high-impact variants ($N = 8,474$, including stop gained, frameshift, splice acceptor or donor), $P < 5 \times 10^{-8}$ for moderate-impact variants ($N = 149,983$, including missense, splice-region variants, and in-frame INDELs), $P < 6 \times 10^{-9}$ for low-impact variants ($N = 2,283,889$, including synonymous variants $\beta$- and $\gamma$-untranslated region variants), $P < 2.3 \times 10^{-9}$ for other variants overlapping DNase hypersensitivity sites ($N = 3,913,058$) and $P < 7.9 \times 10^{-10}$ for other non-DNase hypersensitivity site variants, intergenic and deep intronic ($N = 26,108,039$) (Supplementary Table 2). For association testing in the case–control analysis, we used logistic regression; disease status was treated as the response and genotype counts were used as covariates. We also included in the model as nuisance variables the following available individual characteristics that correlate with disease status: county of birth, sex, current age or age of death (first- and second-order terms included), availability of blood sample for the individual and an indicator function for the overlap of the timespan of phenotype collection with lifetime of the individuals. We applied LD score regression to estimate a correction factor to distinguish polygenicity from population stratification in the GWAS results. To render the fact of relatedness of the Icelandic individuals included in this study, we applied the method of genomic control where the inflation in the $\chi^2$ values was estimated on the basis of a subset of about 300,000 common variants, and $P$ values were adjusted by dividing the corresponding $\chi^2$ values by this factor. For the divergicentric disease, this factor was 1.18 and for diverticulitis 1.17.

A total of 5,426 individuals with diverticural disease were included in the association analysis; 3,368 of these were genotyped using various Illumina chips and imputed the remaining 1,058 were imputed on the basis of genotypes of first- and second-degree relatives. The same population controls, 245,951 individuals recruited through different deCODE projects, were used for association analysis of the three diverticular disease phenotypes: 124,228 genotyped and 121,723 imputed on the basis of genotypes of first- and second-degree relatives. All individuals with diverticular disease were excluded from the control list.

Single SNP genotyping in the replication cohort was performed at deCODE genetics with the Centaurus (Nanogen) platform. The rs761545809 indel was typed using a PCR-based method using NED-labelled (yellow fluorescent dye-labelled primer) primers. An internal control was added to the resulting PCR products and the fragments were separated and detected on an Applied Biosystems Model 3730 Sequencer, using in-house Allele Caller Software. Test for association in the Danish replication samples was done using logistic regression implemented in the NEMO Software. The results from the replication were combined with the discovery results using a Mantel–Haenszel statistic.

Expression analysis. RNA sequencing. Preparation of Poly-A cDNA sequencing libraries. Isolated total RNA samples were assessed for quality and quantity using the Total RNA 6000 Nano Chip for the Agilent 2100 Bioanalyzer. We generated cDNA from Illumina’s mRNA using Illumina’s TruSeq RNA Sample Prep Kit. Briefly, using hybridization to Poly-T beads we isolated Poly-A mRNA from total RNA samples (1–4 µg input). The Poly-A mRNA was fragmented at 94°C, and first-strand cDNA prepared using SuperScript II Reverse Transcriptase (Invitrogen) and random hexamers, followed by second-strand cDNA synthesis, end repair, addition of a single A base, adapter ligation, AMPure bead precipitation and PCR amplification. The resulting cDNA was measured using the DNA 1000 Lab Chip on a Bioanalyzer.

Figure 2 | Regional association plot for the three associated loci. P values (− log10) for the marker associations are plotted against the chromosomal location (human genome build 38) at each locus. The colour of the genomic variants reflects the linkage disequilibrium (LD) with the lead SNP in the Icelandic dataset. The blue line indicates recombination rates from the Icelandic recombination map for males and females. Known genes and exons are shown below using data from the UCSC genes track. The plot was created with a stand-alone version of the LocusZoom Software. (a) Locus plot for the marker rs4662344-T (chr2:143,591,289) at the ARHGAP15 locus. P values plotted are for association with diverticulitis in Iceland. (b) Locus plot for the marker rs7609897-T (chr3:15,461,174) in intron of the COLQ gene. P values plotted are for association with diverticulitis in Iceland. (c) Locus plot for the marker rs67153654-A (chr13:107,572,636) at the FAM155A–iT1 locus. P values plotted are for association with diverticulitis in Iceland.
Sequencing. We used Illumina’s iBiot and the TruSeq PE Cluster Kits v2 to cluster the samples onto to flow cells. Then, we performed paired-end sequencing with either HiSeq 2000 Instruments using TruSeq v3 Flow Cells/SBS Kits or GAIIx Instruments using the TruSeq SBS Kits v5 from Illumina. Read lengths were 2 × 125 cycles.

Read alignment. We aligned the RNA sequencing reads to Homo Sapiens (Build 38) with TopHat version 2.0.12 with a set of known transcripts supplied in GTF format (ReFseq hg38; Homo sapiens, NCBI, build 38). TopHat was configured in such a way to first attempt to align reads to the provided transcriptome, followed, for reads that do not map fully to the transcriptome, an attempt to map them onto the genome.

RNA-seq quality control. RNA libraries were excluded if the number of mapped reads were <10× or number of mapped read pairs were <5× or if the mapping rate of the first or second read fell below 80% relative to the mapping of the other read end. Genotype concordance was determined by comparing imputed genotypes to those derived from RNA-seq. Samples surpassing exclusion had median 103 million mapped reads.

RNA transcript expression. Transcript abundance was estimated with kallisti\textsuperscript{1} version 0.43 using Ensembl r87 transcriptome with subset to transcripts annotated as GENECODE Basic or Transcript support level 1. Transcripts with minimum five

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Author contributions
S.S., U.T., D.F.G., T.S., I.J. and K.S. designed the study, coordinated the project and interpreted the results. S.S., B.F., E.S., A.N.-C., J.B., J.A., H.S.I., E.P.-M., H.U., M.M., T.R., T.T., I.J. and T.S. coordinated and managed collection of samples and ascertainment of phenotype data. S.G. and A.S. performed experiments and analysed results. S.S., K.F.A., P.S., G.H.H., S.O., G.Th., G.M. and D.F.G. performed statistical and bioinformatic analysis. S.S., D.F.G., I.J. and K.S. drafted the manuscript. All authors contributed to the final version of manuscript.

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Competing interests: S.S., K.F.A., P.S., S.G., G.H.H., S.O., A.S., T.R., G.Th., G.M., U.T., D.F.G., I.J. and K.S. are employees of deCODE Genetics/Amgen Inc. The remaining authors declares no competing financial interests.

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