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Association of 4p14 *TLR* locus with antibodies to *Helicobacter pylori*

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Abstract

A genome-wide association study among Europeans related polymorphisms of the *TLR* locus at 4p14 and the *FCGR2A* locus at 1q23.3 to *Helicobacter pylori* serologic status. We replicated associations of 4p14 but not 1q23.3 with anti-*H. pylori* antibodies in 1,402 Finnish males. Importantly, our analysis clarified that the phenotype affected by 4p14 is quantitative level of these antibodies rather than association with seropositivity *per se*. Additionally, we annotated variants at 4p14 as expression quantitative trait loci associated with *TLR6/10* and *FAM114A1.* Our findings suggest that 4p14 polymorphisms are linked to host immune response to *H. pylori* infection but not to its acquisition.

Introduction

Chronic *Helicobacter pylori* infection is causally associated with gastritis, gastroduodenal ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[1] A genome-wide association study (GWAS) of anti-*H. pylori* serologic status among Europeans identified inverse associations with single nucleotide polymorphism (SNPs) in the toll-like receptor (*TLR*) locus at 4p14 and the Fcγ receptor 2a (*FCGR2A*) locus at 1q23.3.[2] Comparing anti-*H. pylori* immunoglobulin G (IgG) antibody levels in the highest quartile vs. lower levels, the 4p14 associations were strongly significant (top-ranked SNP, rs10004195; *P*=1.4e-18) and the 1q23.3 associations were borderline (top-ranked SNP, rs368433; *P*=2.1e-8). In contrast, there were no genome-wide significant associations with anti-*H. pylori* antibody levels in a GWAS among Mexican-Americans.[3] To extend the previous findings among Caucasians, we evaluated associations of anti-*H. pylori* IgG with 4p14 and 1q23.3 loci in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC).

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Results and discussion

Among ATBC participants, rs10004195-A at 4p14 was inversely associated with anti-*H. pylori* antibody levels in the highest 25%. The per-allele odds ratio (OR) was 0.61 (95% confidence interval (CI)=0.47–0.79; *P*=2.2e-4), consistent with the previous report. In contrast, seropositivity (73% of participants) was not associated with rs10004195 (OR=1.00; 95% CI=0.79–1.27; *P*=9.9e-1). Indeed, the minor allele frequency (MAF) of rs10004195 among seronegative individuals (MAF=0.15) was intermediate between the subjects with the highest 25% of antibodies (MAF=0.11) and all other seropositives (MAF=0.17) (Supplementary Table 1).

We also found associations of 4p14 variants with continuous anti-*H. pylori* antibody levels. Notably, the statistical significance as well as magnitude of effects was accentuated when we restricted our analysis to seropositive individuals (Figure 1). For example, the per-allele beta coefficients of rs10004195-A were −0.15 (standard error (SE)=0.05; *P*=3.4e-3; P_{FDR} = 4.0e-2) and −0.20 (SE=0.04; *P*=3.8e-7; P_{FDR} = 4.8e-6) among all and seropositive participants, respectively. The strongest signal was observed at rs6835514 (MAF=0.17; beta=−0.23; SE=0.04; *P*=1.6e-9; P_{FDR} = 1.9e-6), which was in moderate linkage disequilibrium (LD) with rs10004195 (r^2=0.62). Fifty-one nearby SNPs within moderate to high LD (r^2 >0.6) of rs6835514 had p-values ranging from 4.7e-7 (P_{FDR} = 5.6e-6) to 3.0e-9 (P_{FDR} = 1.9e-6) (Supplementary Table 2). In analyses controlling for rs6835514, the effect of rs10004195 did not remain significant (beta=−0.03; SE=0.06; *P*=5.5e-1), indicating that the two SNP associations with IgG levels are not independent.

Furthermore, we explored putative functional effects of these 52 4p14 SNPs based on publically available data. Except for rs4833095 (Asn248Ser) and synonymous rs5743614 in *TLR1*, all other variants were located in intronic or non-coding regions. However, many of these remaining SNPs fall within proximal or distal regulatory elements (Supplementary Table 3). Using the Roadmap ChromHMM track, we found 7 promoter SNPs and 8 enhancer SNPs in CD19-positive primary blood cells. In ENCODE cell lines, 17 SNPs were mapped to DNaseI hypersensitive regions and 42 SNPs altered binding motifs of at least one transcription factor. GTEx expression quantitative trait loci (eQTL) data on whole-blood samples identified multiple SNPs significantly correlated (*P*<0.01) with mRNA transcript levels of *TLR6/10* or *FAM114A1* but not with *TLR1* (Supplementary Table 3). The low IgG allele (G) of rs6835514 was inversely associated with mRNA levels of *TLR6* (beta=−0.11; *P*=3.2e-3) and positively associated with *FAM114A1* expression (beta=0.31; *P*=3.7e-5) (Figure 2). Of particular interest, rs10034903, which was mapped to an active promoter of *TLR10* as well as a transcription factor binding site, appeared to be a significant eQTL for both *TLR10* and *FAM114A1* (Figure 2). Similar to rs6835514, the low IgG allele (G) of rs10034903 was associated with decreased mRNA expression of *TLR10* (beta=−0.13; *P*=6.5e-3) and increased mRNA expression of *FAM114A1* (beta=0.27; *P*=8.0e-4).

Although roles in pathogen recognition and innate immunity have been well established,[4] little is known about TLR1/6/10 with respect to *H. pylori* response.[5, 6] In a recent report, heterodimeric TLR2/TLR10 was suggested to mediate *H. pylori* lipopolysaccharide recognition in activating the NF-kB signaling pathway.[7] Additionally, a growing number of

studies suggest genetic polymorphisms in *TLR* genes are associated with infectious disease susceptibility[8]. For example, rs5743618 (Ser602Ile of *TLR1*; r^2=0.80 among CEU) in high LD with rs6835514 (r^2=0.80 among CEU) has been associated with susceptibility to tuberculosis[9], *Chlamydia trachomtis* infection[10] and leprosy[11]; while rs4833095 (Asn248Ser of *TLR1*; r^2=0.85 with rs6835514 among CEU) has been associated with *Atopobium vaginae* infection[12] and placental malaria[13].

Intriguingly, 4p14 has also been implicated as a susceptibility locus for IgE-mediated allergic sensitization and for hay fever-related asthma; minor alleles were associated with decreased levels of IgE[14] and decreased risk of asthma[15] in Caucasians, in parallel with our finding of an inverse association with anti-*H. pylori* antibody levels. However, three *TLR* SNPs included in the current report were inconsistently associated with active *H. pylori* infection determined by ^{13}C-urea breath test in a Chinese population.[16] Further studies are warranted of these genetic polymorphisms in relation to target gene regulation and disease consequences. Fine mapping studies are also needed to pinpoint the functionally relevant causal variants.

As for the borderline association reported for 1q23.3, we did not find qualitative or quantitative associations with anti-*H. pylori* antibodies. In particular, rs368433 was not associated with the highest quartile of antibody levels (OR=1.05; 95% CI=0.72–1.52; *P*=8.1e-1) nor with seropositivity overall (OR=0.84; 95% CI=0.58–1.23; *P*=3.8e-1) (Supplementary Table 1). Moreover, there were no significant associations with continuous IgG levels among either all participants or seropositives only (Supplementary Figure 1). Based on our observed 0.06 MAF of rs368433, estimated power to detect the previously reported 0.73 OR at a 5% significance level was 71% among all individuals and 80% among seropositives.

In conclusion, we confirmed the association of the 4p14 locus with anti-*H. pylori* antibodies among Caucasians, and clarified the phenotype affected by these polymorphisms. Our findings suggest that the 4p14 locus may modulate intensity of host immune response rather than acquisition of *H. pylori* infection *per se*. The clinical significance of higher levels of antibody to *H. pylori* remains to be determined; conflicting associations with either increased[17, 18] or decreased[19, 20] gastric cancer risk have been reported. Antigen specificity of the 4p14 locus associations should also be examined in future studies. These findings await extension to other ethnic/racial groups with differences in exposure patterns, bacterial strain pathogenicity, host genetic characteristics and population burdens of *H. pylori*-associated diseases.

Materials and methods

Our study included participants from the ATBC, a randomized, double-blind, placebo-controlled trial conducted 1985–1993 in 29,133 Finnish male smokers aged 50 to 69 years.[21] Participants completed questionnaires at enrollment and serum samples were collected and stored at −70°C for future analyses. The current study included 1,402 participants who had both genotyping and *H. pylori* serology data available.

Antibodies to *H. pylori* were measured by enzyme-linked immunosorbent[22, 23, 24, 25] and multiplex bead-based assays[26, 27], as described previously. In order to combine measurements based on different technologies, we standardized levels using laboratory-specific means and standard deviations. Detailed information about genotyping and quality control was published previously[28, 29]. Briefly, a genome-wide scan was performed with Illumina HumanHap550/610 arrays. Imputation was performed using the hidden-Markov model algorithm implemented in MACH, based on HapMap CEU reference panel build 36, R22.

We defined candidate regions as ±200 kb from the previously reported top SNPs,[2] rs10004195 at 4p14 (chr4:38584724–38984724, Hg19) and rs368433 at 1q23.3 (chr1:161284210–161684210, Hg19). We additionally included SNPs located in flanking regions of rs6835514 (chr4:38694380–39094380, Hg19), the most significant 4p14 SNP in our linear regression analysis. After quality control, 1,380 SNPs at 4p14 and 1,127 SNPs at 1q23.3 were available from genotyping or imputed data. Average call rate for the genotyped SNPs was 1 and average quality score for imputed SNPs (Rsq) was 0.92.

To refine the phenotype affected by gene polymorphisms, we tested several definitions of *H. pylori* serologic status. First, we used the same definition as the previous report in Caucasians, which compared individuals with IgG levels in the highest 25% to individuals in the other 75%. Second, to determine whether the loci are associated with *H. pylori* acquisition, we compared seropositives to seronegatives. Lastly, we analyzed IgG levels as a continuous variable. We assumed an additive genetic model with number of minor alleles as a predictor, using 10-year age groups and genotying principal components as covariables. We used logistic regression for dichotomous outcome variables and linear regression for the continuous outcome variable. Adjustment for multiple comparisons at 4p14 was performed by the false discovery rate (FDR) based on 1,380 SNPs, ignoring the high correlation among the tested SNPs. Analyses were conducted using SAS v9.3 (SAS Institute Inc., Cary, NC) and R v3.1.3. Statistical power was estimated with CaTS (http://csg.sph.umich.edu/abecasis/CaTS/index.html).

We focused functional annotation on the 52 SNPs located in moderate to high (r^2>0.6) LD with rs6835514. The UCSC Genome browser (http://genome.ucsc.edu) was used to confirm genomic regions and to screen NIH Epigenomics Roadmap (http://www.roadmapepigenomics.org/) and ENCODE (http://genome.ucsc.edu/ENCODE/) tracks. HaploReg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) and RegulomeDB (http://regulomedb.org/) were also used to confirm SNP functions and to compile summary results.

To map promoter, enhancer, polycomb-repressed or heterochromatin regions, we used the chromatin state segmentation by Hidden Markov Model (ChromHMM) track from Roadmap reported for CD19 primary cells (presumably, circulating B-lymphocytes). DNase cluster assigned by DNase I hypersensitive assay results from 125 cell types, transcription factor binding sites defined by chromatin immunoprecipitation sequencing for 161 factors, and transcription levels determined by RNA-seq in GM12878 were tracked using ENCODE.

To identify putative target genes regulated by SNPs, we compiled eQTL results assessed in whole blood samples (n=168) from Genotype-Tissue Expression (http://www.gtexportal.org/home/). Linear regression was conducted for the 52 SNPs on log and quantile normalized RNA-seq levels of four genes to which any of these SNPs were mapped, including *TLR1* (ENSG00000174125.3), *TRL6* (ENSG00000174130.8), *TLR10* (ENSG00000174123.6), and *FAM114A1* (ENSG00000197712.7). Covariables included three genotyping principle components, 15 peer factors and sex. Based on the number of genes tested, our Bonferroni-corrected significance threshold was P=0.012 (0.05/4 genes).

Informed consents were obtaind from all participants. The study was approved by IRBs of the National Public Health Institute of Finland and the US National Cancer Institute.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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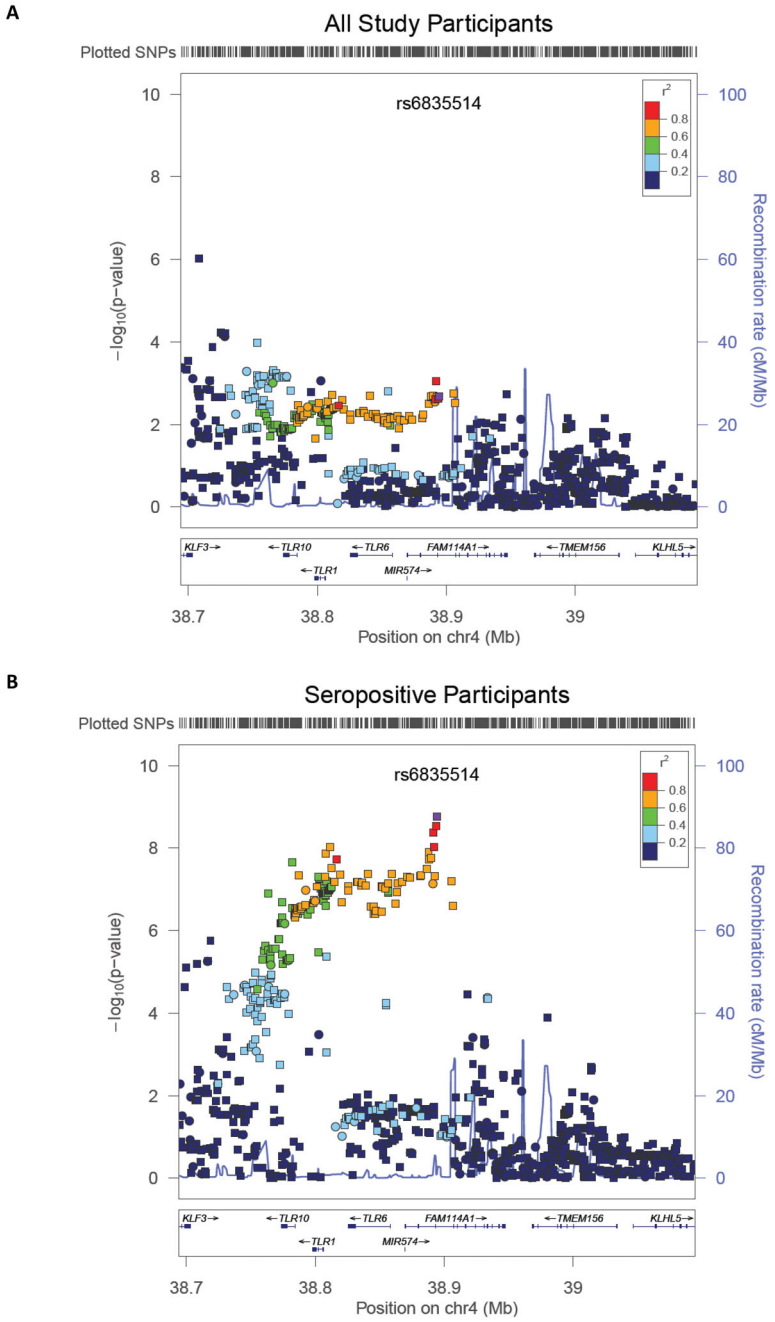

Figure 1.

4p14 locus ($-\log_{10} P$) associations with anti-*H. pylori* antibodies estimated among (A) all and (B) seropositive ATBC participants.

Genomic region was defined as ±200 kb surrounding the index SNP (rs6835514, purple). Circles and squares indicate genotyped and imputed SNPs, respectively. Figure was generated with LocusZoom version 1.1 (http://csg.sph.umich.edu/locuszoom/) using Hg18/ HapMap Phase II CEU as genome build/LD population.

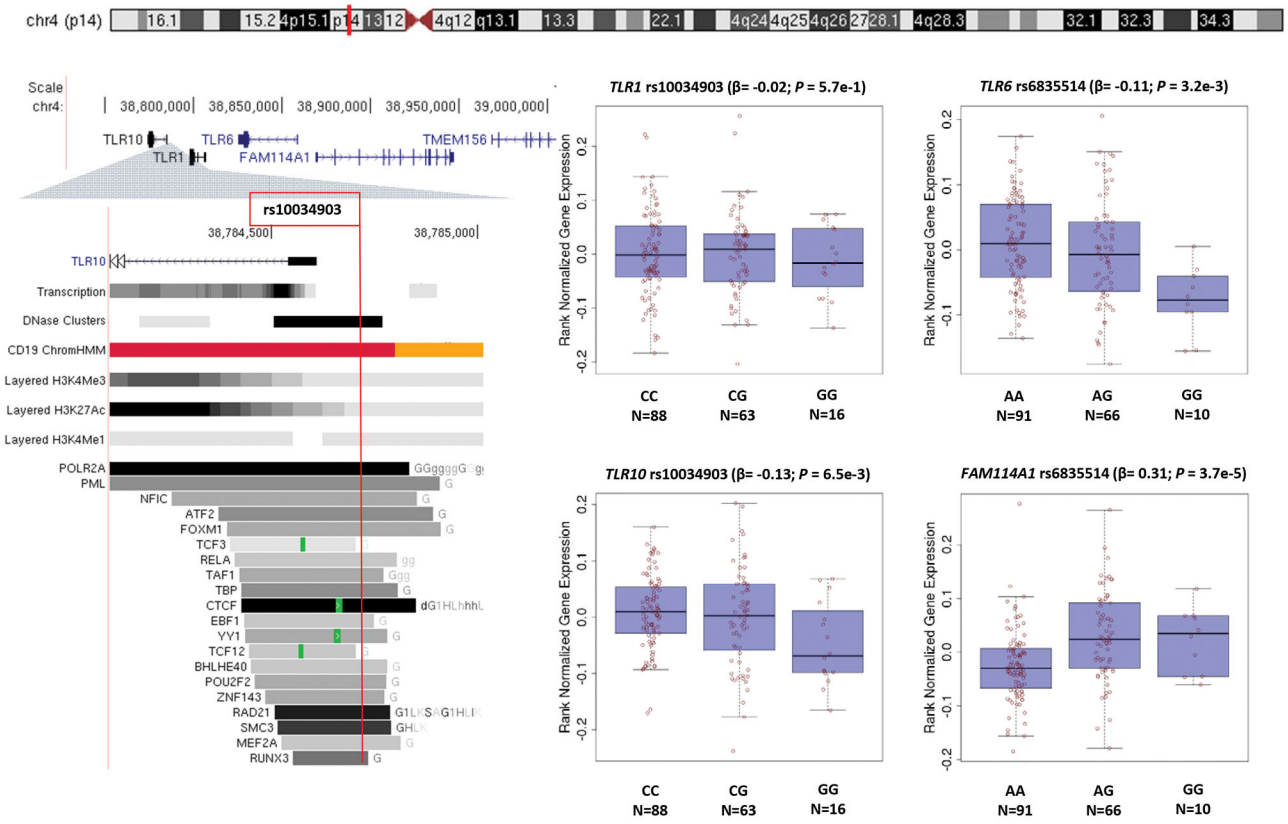

Figure 2.

Selected functional annotations of 4p14 locus SNPs.

NIH Epigenomics Roadmap and ENCODE data were screened using the UCSC Genome Browser to track transcription levels in GM12878 (ENCODE) and regulatory elements, including DNaseI hypersensitivity cluster (open chromatin structure; gray box indicating the extent of the hypersensitive region with shading proportional to the maximum signal strength observed in any cell line) from 125 cell types (ENCODE), Roadmap Chromatin State Segmentation using a Hidden Markov Model (ChromHMM) from CD19 Primary Cells (Promoter [Red] and Enhancer [Orange]), layered core histone marks H3K4Me3, H3K27Ac, and H3K4Me1 in GM12878 (ENCODE), and transcription factor (TF) binding site (gray box with shading proportional to the maximum signal strength; green highlight indicating the highest scoring site of a canonical motif for the corresponding TF) identified by ChIP-seq (ENCODE) experiments. GTEx data on 168 whole-blood samples were analyzed with box plots and regression statistics for expression quantitative trait loci (eQTL). The genomic location of rs10034903 is shown by the red vertical line.