SHORT COMMUNICATION

Association of 4p14 TLR locus with antibodies to Helicobacter pylori

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A genome-wide association study among Europeans related polymorphisms of the Toll-like receptor (TLR) locus at 4p14 and the Fcγ receptor 2a locus at 4q21.3 to Helicobacter pylori serologic status. We replicated associations of 4p14 but not 1q23.3 with anti-H. pylori antibodies in 1402 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. In addition, we annotated variants at 4p14 as expression quantitative trait loci (eQTL) 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.

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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 of anti-H. pylori serologic status among Europeans identified inverse associations with single-nucleotide polymorphisms (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 genome-wide association study 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).

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 antibody levels (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 (s.e. = 0.05; P = 3.4e–3; P_{FDR} = 4.0e–2) and −0.20 (s.e. = 0.04; P = 3.8e–7; P_{FDR} = 4.8e–4) among all and seropositive participants, respectively. The strongest signal was observed at rs6835514 (MAF = 0.17; beta = −0.23; s.e. = 0.04; P = 1.6e–9; P_{FDR} = 1.9e–6), which was in moderate linkage disequilibrium (LD) with rs10004195 (r² = 0.62). Fifty-one nearby SNPs within moderate to high LD (r² > 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 rs10004195, the effect of rs6835514 did not remain significant (beta = −0.03; s.e. = 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 rs5473614 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 seven promoter SNPs and eight enhancer SNPs in CD19-positive primary blood cells. In ENCODE cell lines, 17 SNPs were mapped to DNase I hypersensitive regions and 42 SNPs altered binding motifs of at least one transcription factor. Genotype-Tissue Expression (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 = 5.6e–7).

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Figure 1. 4p14 locus (−log10 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 infection10 and leprosy; 11 whereas rs4833095 (Asn248-H. pylori Ser of TLR1) was in high LD with rs6835514 (beta = 0.85 among CEU) has been observed in 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 increased17,18 or decreased19,20 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. This study included 1402 participants who had both genotyping and H. pylori serology data available.

Antibodies to H. pylori were measured by enzyme-linked immunosorbent22–23 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 s.d. 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, R2.

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, 1380 SNPs at 4p14 and 1127 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 with seronegatives. Finally, 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 genotyping 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 1380 SNPs, ignoring the high correlation among the tested SNPs. Analyses were conducted using SAS v9.3 (SAS Institute Inc., Cary, NC, USA) and R v3.1.3 (https://www.r-project.org/). Statistical power was estimated with CaTS (http://csg.sph.umich.edu/abecasis/CaTS/index.html).

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.

Informed consents were obtained from all participants. The study was approved by institutional review boards of the National Public Health Institute of Finland and the US National Cancer Institute.

| 4p14 | CC | CC | CC |
|------|----|----|----|
| AA   | 91 | 88 | 88 |
| AG   | 66 | 63 | 63 |
| GG   | 10 | 16 | 16 |

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 DNase I 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 sites (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.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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