# Combination of OipA, BabA, and SabA as candidate biomarkers for predicting *Helicobacter pylori*-related gastric cancer

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## Supplemental Method

### *Molecular genotyping by sequencing*

The genomic DNA of GC *H. pylori* stains (n=5) were extracted and OipA, BabA and SabA genes were amplified by PCR as templates for cycle sequencing with BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The extension products were analyzed by capillary electrophoresis using an ABI 3730 x1 DNA sequencer (Applied Biosystems, Foster City, CA). The primers used for sequencing are listed in Supplemental Table V. The nucleotide sequences were analyzed by ApE version 2.0 and BLAST system at the NCBI.

**Supplemental Table I.** Proteins upregulated (fold change > 1.5) in *H. pylori* strains from GC patients compared to DU patients

| **Accession number** | **Protein name[a]** | **Mascot score** | **Coverage (%)** | **Matched peptides** | **117/115 ratio[b]** |
|---|---|---|---|---|---|
| 118596700 | **Adherence-associated lipoprotein A (AlpA)** | 74.44 | 10.64 | 6 | 4.403 |
| 188144165 | Hypothetical protein HPSH_05890 | 69.15 | 2.78 | 1 | 3.902 |
| 325997176 | Acylamide aminohydrolase | 52.47 | 10.70 | 4 | 2.905 |
| 107837644 | Outer membrane protein (HorJ) | 45.84 | 8.47 | 3 | 2.766 |
| 317182137 | Thioredoxin | 437.38 | 37.74 | 4 | 2.656 |
| 147667712 | **Cag pathogenicity island protein 1 (Cag1)** | 89.65 | 21.74 | 2 | 2.525 |
| 207108417 | Hypothetical protein HpylH_02130 | 50.22 | 8.43 | 2 | 2.399 |
| 15611194 | Bacterioferritin comigratory protein | 50.24 | 7.24 | 1 | 2.379 |
| 207110092 | Flagellar assembly protein (FliW) | 45.18 | 26.92 | 1 | 2.284 |
| 317014107 | **N-methylhydantoinase (SerC)** | 374.00 | 15.95 | 17 | 2.208 |
| 166865295 | Cytotoxin associated protain A | 121.76 | 25.00 | 3 | 2.165 |
| 297379894 | Hydantoin utilization protein B (HyuB) | 459.10 | 18.95 | 20 | 2.149 |
| 315586631 | **Hydantoin utilization protein A (HyuA)** | 880.87 | 18.37 | 13 | 2.123 |
| 207093328 | Hypothetical protein HpylHP_11291 | 139.19 | 25.39 | 5 | 2.047 |
| 317010246 | **Ubiquinol cytochrome c oxidoreductase, cytochrome c1 (PetC)** | 82.32 | 17.89 | 5 | 2.042 |
| 315113175 | Chain A, solution structure of Slyd | 154.74 | 16.56 | 3 | 2.021 |
| 15645213 | **2-oxoglutarate:acceptor oxidoreductase (OorD)** | 80.50 | 13.27 | 1 | 2.008 |
| 317181408 | Hypothetical protein HPF57_0118 | 73.89 | 7.04 | 4 | 1.983 |
| 15611698 | Hypothetical protein jhp0631 | 148.11 | 25.90 | 6 | 1.921 |
| 15612304 | **50S ribosomal protein L3 (RplC)** | 138.07 | 26.18 | 6 | 1.905 |
| 15611609 | Hypothetical protein jhp0542 | 52.97 | 7.35 | 4 | 1.898 |
| 315586681 | **Outer inflammatory protein (OipA)** | 40.07 | 5.92 | 2 | 1.890 |
| 188144314 | Conserved hypothetical secreted protein | 357.59 | 39.01 | 10 | 1.873 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 317182005 | Acetyl coenzyme A acetyltransferase | 136.26 | 20.20 | 6 | 1.850 |
| 107836198 | Hypothetical protein HPAG1_1536 | 323.62 | 33.60 | 10 | 1.849 |
| 317182811 | Hypothetical protein HPF57_1521 | 303.84 | 33.60 | 10 | 1.849 |
| 25808535 | **Inorganic pyrophosphatase (Ppa)** | 45.53 | 10.40 | 3 | 1.847 |
| 207092998 | **Carbonic anhydrase (CA)** | 25.02 | 4.96 | 2 | 1.846 |
| 15646105 | Unnamed protein product | 84.14 | 26.40 | 5 | 1.846 |
| 297379391 | Response regulator (OmpR) | 76.91 | 20.00 | 5 | 1.845 |
| 188143179 | Hypothetical protein HPSH_00680 | 69.04 | 50.00 | 2 | 1.844 |
| 317182706 | Putative outer membrane protein | 58.51 | 4.04 | 2 | 1.839 |
| 317181400 | Hypothetical protein HPF57_0110 | 88.91 | 41.27 | 3 | 1.817 |
| 317015027 | Hypothetical protein HPGAM_08495 | 280.74 | 30.83 | 9 | 1.798 |
| 95117797 | Outer membrane protein (Omp22) | 550.30 | 35.20 | 7 | 1.768 |
| 15645472 | Unnamed protein product | 31.50 | 3.00 | 2 | 1.765 |
| 317012238 | Translation initiation factor (IF-2) | 117.26 | 10.36 | 10 | 1.740 |
| 207109410 | **Cytochrome c oxidase cbb3-type subunit II (FixO)** | 79.90 | 22.66 | 4 | 1.736 |
| 46251334 | Outer membrane protein | 339.21 | 33.33 | 5 | 1.730 |
| 127663933 | **Cag pathogenicity island protein 25 (CagC)** | 42.07 | 21.74 | 3 | 1.727 |
| 107837310 | Hypothetical protein HPAG1_1112 | 192.41 | 19.67 | 3 | 1.717 |
| 107836832 | **Aspartate ammonia-lyase (AspA)** | 156.98 | 13.89 | 8 | 1.712 |
| 217033713 | Hypothetical protein HP9810_5g55 | 36.36 | 5.73 | 2 | 1.704 |
| 207109532 | **Replicative DNA helicase (DnaB)** | 38.18 | 5.59 | 1 | 1.695 |
| 325996403 | **ATP synthase F0F1 subunit B (AtpF)** | 56.13 | 17.91 | 3 | 1.690 |
| 107837708 | **Iron(III) ABC transporter, periplasmic iron-binding protein (CeuE)** | 178.01 | 19.70 | 8 | 1.683 |
| 307637404 | Hypothetical protein hp908_0728 | 122.25 | 14.38 | 2 | 1.683 |
| 207108785 | **50S ribosomal protein L13 (RplM)** | 53.39 | 23.53 | 5 | 1.683 |
| 11321265 | **Gamma-glutamyltranspeptidase (Ggt)** | 84.29 | 9.17 | 6 | 1.681 |

| | | | | | |
|---|---|---|---|---|---|
| 207109162 | **DNA-directed RNA polymerase subunit omega (RpoZ)** | 42.47 | 32.88 | 3 | 1.676 |
| 188144468 | Hypothetical protein HPSH_07460 | 249.33 | 16.19 | 3 | 1.675 |
| 15611420 | Copper-associated protein | 70.71 | 16.67 | 1 | 1.675 |
| 317010684 | **3-dehydroquinate dehydratase (AroD)** | 62.57 | 10.78 | 1 | 1.674 |
| 110377354 | **Heat shock protein 60 (GroEL)** | 3425.88 | 77.11 | 42 | 1.665 |
| 208435298 | ATP/GTP binding protein | 138.71 | 4.35 | 2 | 1.659 |
| 317177289 | Outer membrane protein (HofC) | 357.43 | 14.77 | 8 | 1.657 |
| 207092727 | ATP-binding protein (Mpr) | 38.35 | 21.32 | 3 | 1.655 |
| 15644733 | Unnamed protein product | 416.82 | 18.76 | 10 | 1.653 |
| 107837702 | Translation elongation factor (EF-Ts) | 231.96 | 28.17 | 12 | 1.650 |
| 188143969 | **Acetate kinase (AckA)** | 36.68 | 5.24 | 4 | 1.646 |
| 712831 | Heat shock protein | 2499.12 | 76.15 | 40 | 1.635 |
| 188144216 | **Aspartate-semialdehyde dehydrogenase (Asd)** | 66.17 | 7.23 | 3 | 1.630 |
| 217034805 | Hypothetical protein HP9810_866g2 | 360.08 | 40.13 | 7 | 1.622 |
| 317013097 | **30S ribosomal protein S17 (RpsQ)** | 68.83 | 24.42 | 4 | 1.621 |
| 15612352 | Rod shape-determining protein (MreB) | 129.06 | 11.82 | 3 | 1.615 |
| 317181264 | Hypothetical protein HPF32_1468 | 188.68 | 25.23 | 11 | 1.612 |
| 317011348 | **Signal recognition particle protein (Ffh)** | 83.67 | 6.92 | 3 | 1.608 |
| 317008739 | Urease accessory protein (UreH) | 42.01 | 7.92 | 2 | 1.603 |
| 15644849 | Unnamed protein product | 73.05 | 15.95 | 6 | 1.603 |
| 207109368 | Biopolymer transport protein | 42.87 | 8.18 | 1 | 1.597 |
| 315587202 | **30S ribosomal protein S3 (RpsC)** | 148.34 | 33.76 | 13 | 1.593 |
| 207108543 | **Pyridoxal phosphate biosynthetic protein (PdxJ)** | 55.12 | 8.85 | 3 | 1.590 |
| 107836475 | ATP-dependent nuclease | 28.68 | 8.14 | 4 | 1.588 |
| 294662397 | Chain B, crystal structure of Ycei (Hp1286) | 250.52 | 36.59 | 9 | 1.587 |
| 308062217 | **Blood group antigen binding adhesin (BabA)** | 414.23 | 9.62 | 7 | 1.586 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 210135477 | **30S ribosomal protein S10 (RpsJ)** | 59.24 | 13.46 | 2 | 1.583 |
| 15611308 | Outer membrane protein | 37.53 | 11.25 | 7 | 1.582 |
| 317013104 | **50S ribosomal protein L23 (RplW)** | 253.69 | 49.46 | 5 | 1.573 |
| 217034319 | Hypothetical protein HP9810_491g12 | 154.44 | 29.52 | 6 | 1.570 |
| 107836496 | **50S ribosomal protein L21 (RplU)** | 128.73 | 20.19 | 3 | 1.556 |
| 15645018 | Unnamed protein product | 158.50 | 29.52 | 6 | 1.556 |
| 15611904 | Hydrogenase expression/formation protein | 88.21 | 10.33 | 5 | 1.555 |
| 315586121 | Conserved hypothetical protein | 87.74 | 13.14 | 4 | 1.552 |
| 15611134 | Urease accessory protein | 129.96 | 23.62 | 5 | 1.550 |
| 207093235 | **30S ribosomal protein S7 (RpsG)** | 217.42 | 32.03 | 6 | 1.550 |
| 148717246 | **Sialic acid-binding adhesin (SabA)** | 35.62 | 2.15 | 2 | 1.538 |
| 188143129 | **30S ribosomal protein S9 (RpsI)** | 239.38 | 37.98 | 6 | 1.537 |
| 15645683 | Unnamed protein product | 56.31 | 8.86 | 7 | 1.536 |
| 188143477 | **Serine protease DO (HtrA)** | 544.56 | 30.04 | 14 | 1.535 |
| 317010118 | Ferrodoxin-like protein | 38.57 | 4.37 | 2 | 1.535 |
| 217034426 | Hypothetical protein HP9810_11g8 | 87.29 | 9.25 | 6 | 1.533 |
| 107837687 | **Ubiquinol cytochrome c oxidoreductase, 2Fe-2S subunit (PetA)** | 69.89 | 10.18 | 2 | 1.533 |
| 15644814 | Unnamed protein product | 92.97 | 17.98 | 5 | 1.530 |
| 189011844 | Heat shock protein | 241.29 | 53.93 | 6 | 1.521 |
| 15645986 | **(3R)-hydroxymyristoyl-ACP dehydratase (FabZ)** | 55.73 | 16.35 | 3 | 1.519 |
| 317014518 | **F0F1 ATP synthase subunit beta (AtpD)** | 574.90 | 22.60 | 10 | 1.516 |
| 207108692 | **2-dehydro-3-deoxyphosphooctonate aldolase (KdsA)** | 87.11 | 9.55 | 2 | 1.515 |
| 298736833 | Bifunctional methionine sulfoxide reductase A/B | 152.90 | 18.82 | 9 | 1.504 |
| 15646161 | **30S ribosomal protein S2 (RpsB)** | 109.01 | 18.18 | 7 | 1.500 |

**[a] Proteins that were categorized by KEGG pathway analysis are shown in bold.**

**[b] The 117/115 iTRAQ ratios represent the results for three independent repeats.**

## Supplemental Table II Gender effect on the odds ratio of an increased number of recognized antigens

| Recognized antigens | | Number positive (%) | | | OR (95% CI), *p* value[b] | |
|---|---|---|---|---|---|---|
| **Male** | **Antigens** | **GC (n=48)** | **DU (n=56)** | **non-GC[a] (n=69)** | **GC *vs.* DU** | **GC *vs.* non-GC** |
| | None | 5 (10.4) | 34 (60.7) | 43 (62.3) | 0.08 (0.03- 0.22), <0.0001 | 0.07 (0.02-0.20), <0.0001 |
| | Any one antigen | 14 (29.2) | 15 (26.8) | 18 (26.1) | 1.13 (0.48-2.66), 0.79 | 1.17 (0.51-2.65), 0.71 |
| | Two of the four antigens | 12 (25.0) | 6 (10.7) | 6 (8.7) | 2.78 (0.95-8.09), 0.05 | 3.5 (1.21-10.12), 0.02 |
| | Three of the four antigens | 10 (20.8) | 1 (1.8) | 2 (2.9) | 14.47 (1.78-117.82), 0.0016 | 8.82 (1.84-42.35), 0.0022 |
| | All four antigens | 7 (14.6) | 0 (0) | 0 (0) | ND[c] | ND |
| **Female** | **Antigens** | **GC (n=28)** | **DU (n=24)** | **non-GC (n=31)** | **GC *vs.* DU** | **GC *vs.* non-GC** |
| | None | 5 (17.9) | 17 (70.8) | 22 (71.0) | 0.09 (0.02-0.33), 0.0001 | 0.09 (0.03-0.31), <0.0001 |
| | Any one antigen | 5 (17.9) | 3 (12.5) | 4 (12.9) | 1.52 (0.32-7.16), 0.71 | 1.47 (0.35-6.12), 0.72 |
| | Two of the four antigens | 5 (17.9) | 2 (8.3) | 3 (9.7) | 2.39 (0.42-13.64), 0.43 | 2.03 (0.44-9.41), 0.46 |
| | Three of the four antigens | 11 (39.3) | 2 (8.3) | 2 (6.5) | 7.12 (1.39-36.48), 0.01 | 9.38 (1.85-47.47), 0.0024 |
| | All four antigens | 2 (7.1) | 0 (0) | 0 (0) | ND | ND |

[a] The non-GC group consisted of normal subjects, gastritis patients with or without *H. pylori* infection, and DU patients.

[b] *p* value calculated using the Chi-squared test.

[c] ND, not determined.

## Supplemental Table III. Age effect on the odds ratio of an increased number of recognized antigens

| Recognized antigens | | Number positive (%) | | | OR (95% CI), *p* value[b] | |
|---|---|---|---|---|---|---|
| **<50** | **Antigens** | **GC (n=11)** | **DU (n=31)** | **non-GC[a] (n=48)** | **GC *vs.* DU** | **GC *vs.* non-GC** |
| | None | 1 (9.1) | 17 (54.8) | 29 (60.4) | 0.08 (0.01-0.72), 0.01 | 0.07 (0.01-0.55), 0.0021 |
| | Any one antigen | 4 (36.4) | 9 (29.0) | 13 (27.1) | 1.40 (0.33-5.97), 0.71 | 1.54 (0.39-6.14), 0.71 |
| | Two of the four antigens | 2 (18.2) | 3 (9.7) | 3 (6.3) | 2.07 (0.30-14.44), 0.59 | 3.33 (0.49-22.90), 0.23 |
| | Three of the four antigens | 3 (27.3) | 2 (6.5) | 3 (6.3) | 5.44 (0.77-38.33), 0.10 | 5.63 (0.96-32.97), 0.07 |
| | All four antigens | 1 (9.1) | 0 (0.0) | 0 (0) | ND[c] | ND |
| **≥50** | | **GC (n=65)** | **DU (n=49)** | **non-GC (n=52)** | **GC *vs.* DU** | **GC *vs.* non-GC** |
| | None | 9 (13.8) | 34 (69.4) | 36 (69.2) | 0.07 (0.03-0.18), <0.0001 | 0.07 (0.03-0.18), <0.0001 |
| | Any one antigen | 15 (23.1) | 9 (18.4) | 9 (17.3) | 1.33 (0.53-3.36), 0.54 | 1.43 (0.57-3.60), 0.44 |
| | Two of the four antigens | 15 (23.1) | 5 (10.2) | 6 (11.5) | 2.64 (0.89-7.85), 0.07 | 2.3 (0.82-6.43), 0.11 |
| | Three of the four antigens | 18 (27.7) | 1 (2.0) | 1 (1.9) | 18.38 (2.36-143.30), 0.0003 | 19.53 (2.51-152.08), 0.0002 |
| | All four antigens | 8 (12.3) | 0 (0) | 0 (0) | ND | ND |

[a] The non-GC group consisted of normal subjects, gastritis patients with or without *H. pylori* infection, and DU patients.

[b] *p* value calculated using the Chi-squared test.

[c] ND, not determined.

**Supplemental Table IV.** Clinical features of the gastric cancer patients

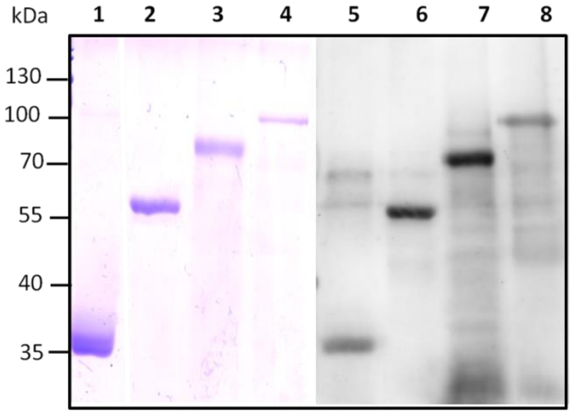
| **Feature** | | **No. (%)** |
|---|---|---|
| **Total** | | **76 (100)** |
| **Gender** | | |
| | **Male** | **48 (63)** |
| | **Female** | **28 (37)** |
| **Age (yr)** | | |
| | **Average** | **64.2** |
| | **Range** | **23-87** |
| | **SD** | **13.3** |
| **Staging** | | |
| | **EGC**[a] | **23 (30)** |
| | **AGC**[b] | **53 (70)** |
| **Histological type** | | |
| | **Diffuse** | **27 (36)** |
| | **Non-diffuse** | **49 (64)** |
| **Tumor location** | | |
| | **Antrum** | **43 (57)** |
| | **Non-antrum** | **33 (43)** |

[a] Early gastric cancer with cancer cell invasion confined to the mucosa or submucosa.

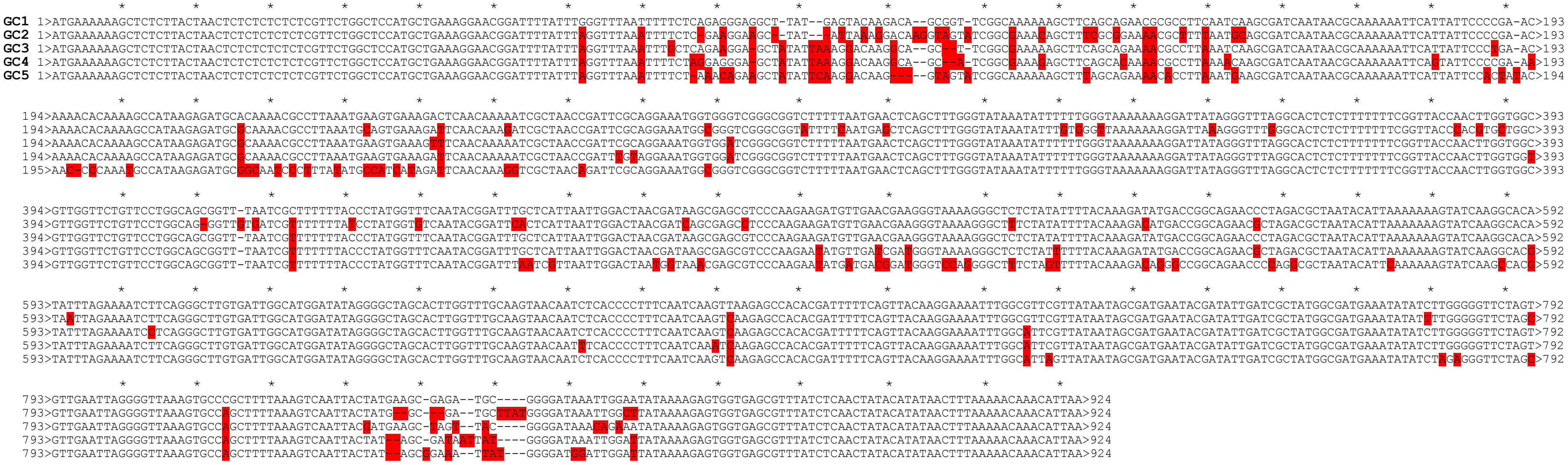
[b] Advanced gastric cancer with cancer cell invasion beyond the muscularis propria.

**Supplemental Table V.** Primer sequences used for constructs, RT-PCR, and genotyping

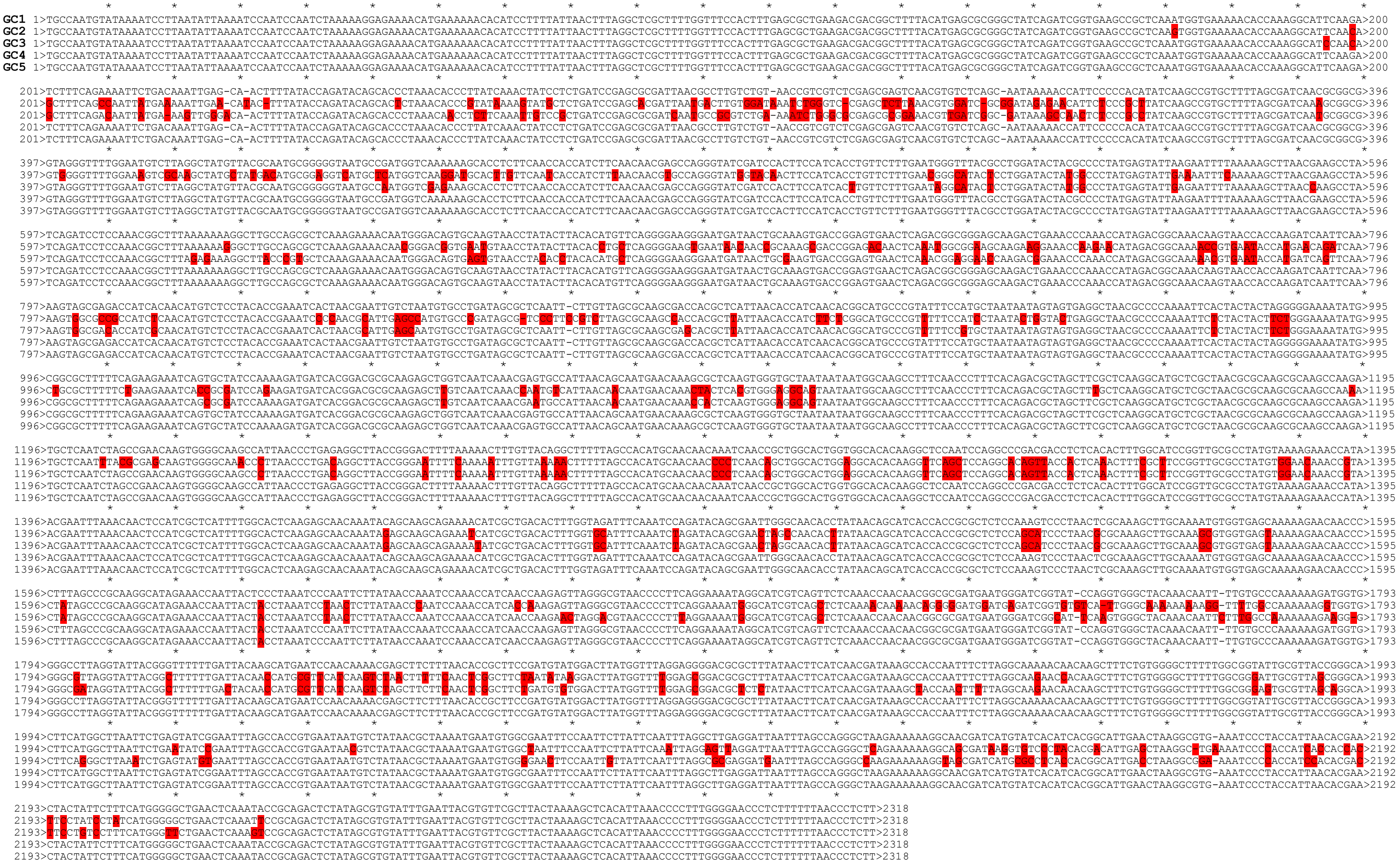
| Gene | Primer sequence (F : forward, R : reverse ) |
|---|---|
| AlpA | F : 5’-GGATCCGAATCCTGCGCTAGCATAAGTTA-3’ |
|  | R : 5’-CTCGAGCTCGAGGAATGAATACCCATAAGA-3’ |
| OipA | F : 5’-GGATCCGAATCCCACGCTGAAAGG-3’ |
|  | R : 5’-CTCGAGCTCGAGATAAACGCTCAC-3’ |
|  | F1: 5’-CATGCTGAAAGGAACGGATTTTAT-3’ |
|  | R1: 5’-ATAAACGCTCACCACTCTTTTATA-3’ |
| BabA | F : 5’-GGATCCGAATCCGAAGACGACGGCTTTTACATGAGC-3’ |
|  | R : 5’-CTCGAGCTCGAGAAATACACGCTATAGAGCCTGCGG-3’ |
|  | F1: 5’-GAAGACGACGGCTTTTACATGAGC-3’ |
|  | R1: 5’-AAATACACGCTATAGAGCCTGCGG-3’ |
|  | F2: 5’-CGGGACGGTGAATGTAACCT-3’ |
|  | R2: 5’-ATTTTCCTGAAGGGGTTACG-3’ |
| SabA | F : 5’-GGATCCGAATCCGAAGACAACGGCTTTTTTGT-3’ |
|  | R : 5’-CTCGAGCTCGAGTTAATAAGCAAACACATAATTGAG-3’ |
|  | F1: 5’-GAAGACAACGGCTTTTTTGT-3’ |
|  | R1: 5’-TTAATAAGCAAACACATAATTGAG-3’ |
|  | F2: 5’-TGCTCAGGAATTGAAAAC TG-3’ |
|  | R2: 5’-CCGTTCAAAGCGCCGTTATTGTT-3’ |
| UreB | F : 5’-ATGAAAAAGATTAGCAGAAAAGAATAT-3’ |
|  | R : 5’-CTAGAAAATGCTAAAGAGTTGCGCCA-3’ |



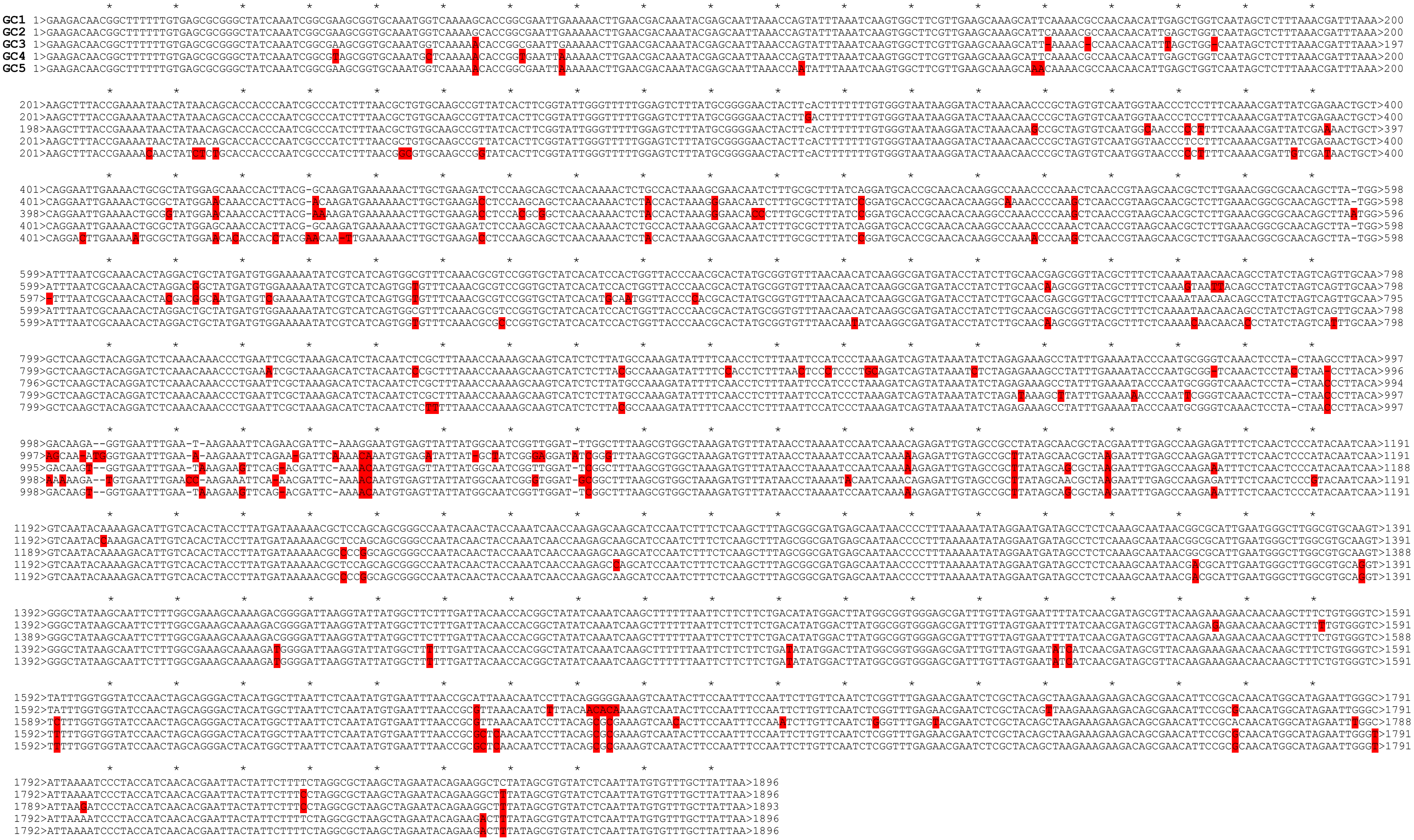
**Supplemental Figure 1.** Purity of the recombinant proteins and reactivity with a GC patient serum. Purified recombinant OipA (lanes 1 and 5), AlpA (lanes 2 and 6), SabA (lanes 3 and 7), and BabA (lanes 4 and 8) were electrophoresed on a 10 % SDS-PAGE and either stained with Coomassie Blue (lanes 1-4) or transferred to a PVDF membrane and immunoblotted with serum from a GC patient serum (lanes 5-8).



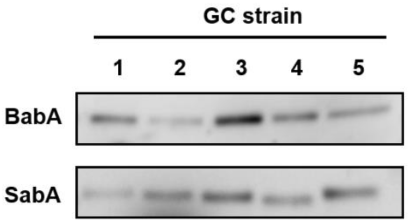
**Supplemental Figure 2.** Genotyping of the OipA gene in the 5 GC strains. The sequence of OipA gene in the clinical GC strains GC1-GC5 are compared. The nucleotides in red indicated difference and the "-" in sequences denoted a gap.



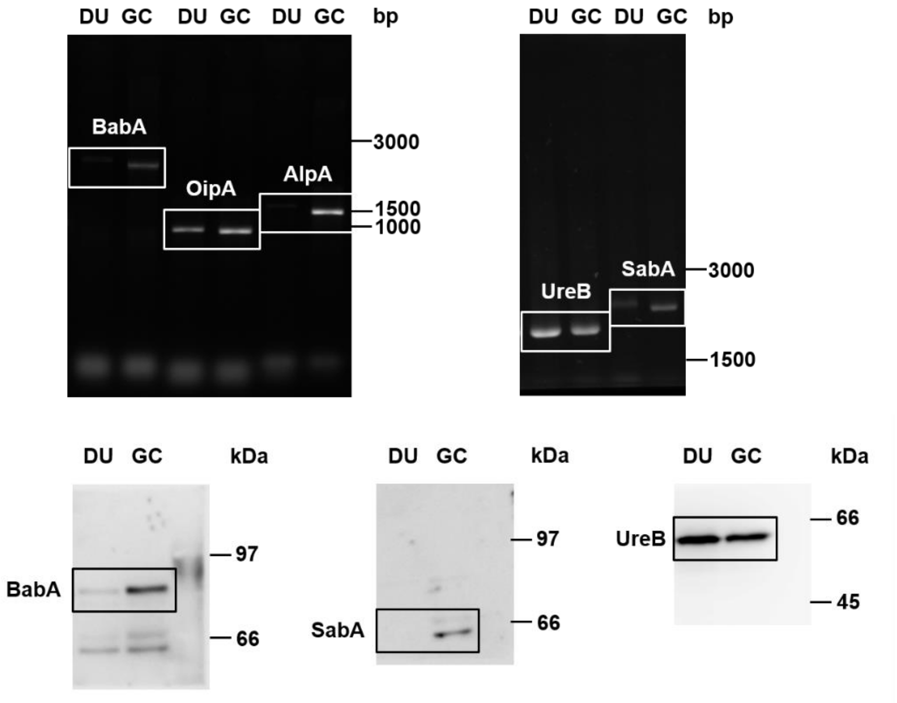
**Supplemental Figure 3.** Genotyping of the BabA gene in the 5 GC strains. The sequence of BabA gene in the clinical GC strains GC1-GC5 are compared. The nucleotides in red indicated difference and the "-" in sequences denoted a gap.



**Supplemental Figure 4.** Genotyping of the SabA gene in the 5 GC strains. The sequence of SabA gene in the clinical GC strains GC1-GC5 are compared. The nucleotides in red indicated difference and the “-“ in sequences denoted a gap.



**Supplemental Figure 5.** BabA and SabA protein expression of the 5 GC strains. Protein extracts from 5 GC-derived *H. pylori* samples were prepared and protein levels of BabA and SabA were examined by Western blotting. The data shown are representative of the results obtained in three independent experiments, all gels and blots were run in the same experimental conditions.



**Supplemental Figure 6.** Uncropped gels and blots of Figure 2B.