Correlations between Genetic Polymorphisms in Long Non-Coding RNA PRNCR1 and Gastric Cancer Risk in a Korean Population

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Abstract: We evaluated the association between prostate cancer non-coding RNA 1 (PRNCR1) polymorphisms and the risk of developing gastric cancer (GC) and GC subgroups in Korea. A case–control study was conducted with 437 GC patients and 357 healthy controls using a TaqMan genotyping assay. A chi-squared test, binary logistic regression, and genetic models were used to explore the association between five PRNCR1 polymorphisms and GC risk. After adjusting for gender and age, overall analyses using the recessive model indicated that the rs13252298 GG genotype was significantly associated with increased risk of intestinal-type gastric cancer (IGC). In the stratification analyses, the recessive model indicated that the rs1016343 TT genotype was significantly associated with decreased GC risk in individuals aged <60 years showing lymph node metastasis (LNM)-negative results. The rs13252298 GG genotype in the recessive model showed increased GC risk in subjects aged ≥60 years showing LNM-positive results and those aged ≥60 years in tumor stage III. In the dominant model, the rs16901946 combined genotype (AG/GG) was significantly associated with increased GC risk in subjects aged <60 years with tumor stage III. In the recessive model, the rs16901946 GG genotype was associated with decreased risk of GC and IGC in males aged ≥60 years. Thus, genetic variations in PRNCR1 may contribute to susceptibility to GC.

Keywords: PRNCR1; polymorphism; gastric cancer; intestinal type; lymph node metastasis

1. Introduction

Gastric cancer (GC) is one of the most common forms of cancer worldwide. Despite a steady decline in GC incidence and mortality rates over the past few decades, the rates are still high in Asian countries. According to a report by the Korean National Cancer Center, GC is the third most common cancer, with 25,872 new cases and 7138 deaths recorded in Korea in 2016 [1–3].

Approximately 80% of disease-related polymorphisms occur in non-coding regions consisting of introns and intergenic regions [4]. Genome-wide association studies have reported that a large number of polymorphisms are associated with cancer and that these cancer-related polymorphisms
are associated with long non-coding RNAs (lncRNAs) [5–7]. LncRNAs are non-translated RNA molecules over 200 nucleotides in length. Recently, it was reported that lncRNAs are involved in tumorigenesis [8,9]. Moreover, germline variants can affect lncRNA expression, regulating cancer development and progression [10]. Indeed, several studies have demonstrated that lncRNA genetic variants are related to susceptibility of various cancers, including breast cancer [11,12], colon cancer [13], gastric cancer [14,15], lung cancer [16], and prostate cancer [10,17]. Particularly, lncRNA prostate cancer non-coding RNA 1 (PRNCR1), transcribed from a non-coding region of chromosome 8q24, is involved in the carcinogenesis of prostate cancer (PC) through activation of the androgen receptor [18], and lncRNA PRNCR1 polymorphisms have been correlated with various cancers, including GC [14,18–23].

Based on previous findings, we hypothesized that polymorphisms in the lncRNA PRNCR1 might affect genetic susceptibility to GC. Therefore, we conducted a case–control study to elucidate the association between single nucleotide polymorphisms (SNPs) in PRNCR1 and risk of developing GC in a Korean population. We further analyzed the impact of PRNCR1 polymorphisms on GC risk in combination with various characteristics and clinical features, including age, sex, tumor differentiation, histologic type, T classification, lymph node metastasis (LNM), and tumor stage.

2. Results

2.1. Patient Characteristics and Single Nucleotide Polymorphisms (SNP) Selection

The characteristics and clinical features of the 437 patients with GC and the 363 cancer-free controls are presented in Table 1. There was a significant difference in the age and gender distribution between the two groups ($p < 0.001$ and $p < 0.001$, respectively). The mean age was 65.3 ± 11.1 years for GC patients and 58.1 ± 8.9 years for the controls. The proportion of male subjects was significantly higher in the group with GC (69.6%), whereas the number of female subjects was higher in the control group (67.2%). Of the 437 patients with diagnosed GC, more than half were classified as having the intestinal type (55.8%), making it the most common type, followed by the diffuse-type and the mixed-type. The majority of the patients did not show lymph node metastasis (LNM) (60.9%) and were classified as T1 (50.6%) and tumor stage I (58.8%). We selected five PRNCR1 SNPs: rs1016343, rs13252298, rs7841060, rs16901946, and rs1456315, which have been previously associated with cancer.

### Table 1. Characteristics and clinical features of the gastric cancer group and the control group.

| Variables                        | Gastric Cancers N (%) | Controls N (%) | $p$  |
|----------------------------------|-----------------------|----------------|------|
| Age (years) (mean ± SD)          |                       |                |      |
| <60                              | 185 (42.3)            | 186 (52.1)     | 0.007† |
| ≥60                              | 252 (57.7)            | 171 (47.9)     |      |
| Gender (%)                       |                       |                |      |
| Male                             | 304 (69.6)            | 117 (32.8)     | <0.001‡ |
| Female                           | 133 (30.4)            | 240 (67.2)     |      |
| Tumor differentiation            |                       |                |      |
| Differentiated                   | 208 (47.6)            |                |      |
| Undifferentiated                 | 190 (43.5)            |                |      |
| Missing                          | 39 (8.9)              |                |      |
| Histological type (%)            |                       |                |      |
| Intestinal                      | 244 (55.8)            |                |      |
| Diffuse                          | 140 (32.1)            |                |      |
| Mixed                            | 53 (12.1)             |                |      |
| T classification (%)             |                       |                |      |
| T1                               | 221 (50.6)            |                |      |
| T2                               | 59 (13.5)             |                |      |
| T3                               | 16 (3.6)              |                |      |
| T4                               | 141 (32.3)            |                |      |
Table 1. Cont.

| Variables                           | Gastric Cancers | Controls | p   |
|-------------------------------------|-----------------|----------|-----|
|                                     | N (%)           | N (%)    |     |
| Lymph node metastasis (%)           |                 |          |     |
| Negative                            | 266 (60.9)      |          |     |
| Positive                            | 171 (39.1)      |          |     |
| Tumor stage (%)                     |                 |          |     |
| I (A+B)                             | 257 (58.8)      |          |     |
| II (A+B)                            | 50 (11.5)       |          |     |
| III (A+B+C)                         | 130 (29.7)      |          |     |

SD, standard deviation. * Mann–Whitney U-test. † Two-sided Pearson’s chi-squared test.

2.2. Associations Between PRNCR1 SNPs and GC risk

The genotype frequencies of rs1016343, rs13252298, rs7841060, and rs16901946 were in Hardy–Weinberg equilibrium (HWE) for both the GC group (p = 0.772, p = 0.968, p = 0.668, and p = 0.821, respectively) and the control group (p = 0.591, p = 0.143, p = 0.610, and p = 0.978, respectively) (Table 2). However, rs1456315 frequencies were not in HWE for either GC or controls (p < 0.05). The rs1456315 was, therefore, excluded from the genotype analysis because of Hardy-Weinberg disequilibrium. LD coefficients (|D'|) were estimated among the four SNPs, and an absolute LD (|D'| = 1 and r²) was not found for any pair-wise combination among four SNPs using Haplovie 4.0 software. To determine whether rs1016343, rs13252298, rs7841060, and rs16901946 were associated with a higher risk of GC or GC subgroups, we compared the genotypic frequencies between the GC group and the control group. After adjusting for age and gender, the recessive model indicated that the rs13252298 GG genotype was associated with an increased risk of intestinal-type gastric cancer (IGC) (OR = 1.92, 95% CI = 1.01–3.63, p = 0.045), as compared to the rs13252298 AA/AG genotypes; the remaining SNPs showed no significant associations (Table 2).
Table 2. Genotype and allelic frequencies of PRNCR1 polymorphisms in subjects and their association with GC risk.

| Genotype   | CON   | GC vs. CON | IGC vs. CON | DGC vs. CON |
|------------|-------|------------|-------------|-------------|
|            | N (%) | AOR (95% CI) | p | N (%) | AOR (95% CI) | p | N (%) | AOR (95% CI) | p |
| rs1016343  |       |            |       |       |            |   |       |            |   |
| Codominant |       |            |       |       |            |   |       |            |   |
| CC         | 171 (47.9) | 209 (47.8) | 1 | 119 (48.5) | 1 | 62 (44.6) | 1 | 0.661 |
| CT         | 158 (44.3) | 191 (43.7) | 0.94 (0.69–1.29) | 0.709 | 106 (43.3) | 0.94 (0.64–1.38) | 0.756 | 66 (47.5) | 1.10 (0.72–1.68) | 0.892 |
| TT         | 28 (7.8) | 37 (8.5) | 0.88 (0.49–1.56) | 0.654 | 20 (8.2) | 0.84 (0.42–1.67) | 0.612 | 11 (7.9) | 0.95 (0.44–2.06) |   |
| Dominant   |       |            |       |       |            |   |       |            |   |
| CC         | 171 (47.9) | 209 (47.8) | 1 | 119 (48.6) | 1 | 62 (44.6) | 1 | 0.727 |
| CT + TT    | 186 (52.1) | 228 (52.2) | 0.93 (0.69–1.26) | 0.645 | 126 (51.4) | 0.92 (0.64–1.33) | 0.672 | 77 (55.4) | 1.08 (0.72–1.61) |   |
| Recessive  |       |            |       |       |            |   |       |            |   |
| CC + CT    | 329 (92.2) | 406 (91.5) | 1 | 225 (91.8) | 1 | 128 (92.1) | 1 | 0.790 |
| TT         | 28 (8.7) | 37 (8.5) | 0.90 (0.52–1.57) | 0.718 | 20 (8.2) | 0.86 (0.44–1.68) | 0.659 | 11 (7.9) | 0.90 (0.43–1.91) |   |
| HWE        | 0.591 | 0.772 |   |   |   |   |   |   |   |
| rs13252298 |       |            |       |       |            |   |       |            |   |
| Codominant |       |            |       |       |            |   |       |            |   |
| AA         | 158 (44.3) | 214 (49.0) | 1 | 122 (49.6) | 1 | 67 (48.6) | 1 | 0.450 |
| AG         | 171 (47.9) | 182 (41.6) | 0.90 (0.66–1.24) | 0.518 | 97 (39.4) | 0.87 (0.59–1.29) | 0.497 | 61 (44.2) | 0.85 (0.56–1.30) | 0.854 |
| GG         | 28 (7.8) | 41 (9.4) | 1.36 (0.77–2.40) | 0.285 | 27 (11.0) | 1.80 (0.93–3.49) | 0.084 | 10 (7.2) | 0.93 (0.42–2.06) |   |
| Dominant   |       |            |       |       |            |   |       |            |   |
| AA         | 158 (44.3) | 214 (49.0) | 1 | 122 (49.6) | 1 | 67 (48.6) | 1 | 0.469 |
| AG + GG    | 199 (55.7) | 223 (51.0) | 0.96 (0.71–1.31) | 0.805 | 124 (50.4) | 0.99 (0.69–1.43) | 0.954 | 71 (51.4) | 0.86 (0.57–1.30) |   |
| Recessive  |       |            |       |       |            |   |       |            |   |
| AA + AG    | 329 (92.2) | 396 (90.6) | 1 | 219 (89.0) | 1 | 128 (92.8) | 1 | 0.982 |
| GG         | 28 (7.8) | 41 (9.4) | 1.43 (0.83–2.47) | 0.193 | 27 (11.0) | 1.92 (1.01–3.63) | 0.045 | 10 (7.2) | 1.01 (0.47–2.18) |   |
| HWE        | 0.143 | 0.968 |   |   |   |   |   |   |   |
| rs7841060  |       |            |       |       |            |   |       |            |   |
| Codominant |       |            |       |       |            |   |       |            |   |
| TT         | 169 (47.4) | 204 (46.7) | 1 | 116 (47.5) | 1 | 61 (43.5) | 1 | 0.556 |
| TG         | 159 (44.5) | 195 (44.6) | 0.96 (0.70–1.31) | 0.794 | 108 (44.3) | 0.96 (0.65–1.40) | 0.814 | 68 (48.6) | 1.14 (0.75–1.73) | 0.842 |
| GG         | 29 (8.1) | 38 (8.7) | 0.88 (0.50–1.55) | 0.647 | 20 (8.2) | 0.82 (0.41–1.64) | 0.578 | 11 (7.9) | 0.92 (0.43–2.00) |   |
| Dominant   |       |            |       |       |            |   |       |            |   |
| TT         | 169 (47.3) | 204 (46.7) | 1 | 116 (47.5) | 1 | 61 (43.6) | 1 | 0.643 |
| TG + GG    | 188 (52.7) | 233 (53.3) | 0.95 (0.70–1.28) | 0.716 | 128 (52.5) | 0.93 (0.65–1.35) | 0.711 | 79 (56.4) | 1.10 (0.73–1.65) |   |
| Genotype      | CON          | GC vs. CON   | IGC vs. CON   | DGC vs. CON   |
|--------------|--------------|--------------|--------------|--------------|
|              | N (%)        | N (%)        | AOR (95% CI) | p            | N (%)        | AOR (95% CI) | p            | N (%)        | AOR (95% CI) | p            |
| Recessive    |              |              |              |              |              |              |              |              |              |              |              |
| TT + TG      | 328 (91.9)   | 399 (91.3)   | 1            |              | 224 (91.8)   | 1            |              | 129 (92.1)   | 1            | 0.705        |
| GG           | 29 (8.1)     | 38 (8.7)     | 0.89 (0.52–1.54) | 0.688 | 20 (8.2) | 0.84 (0.43–1.63) | 0.609 | 11 (7.9) | 0.87 (0.41–1.82) | 0.609 |
| HWE          | 0.610        | 0.668        |              |              |              |              |              |              |              |              |              |
| rs16901946   |              |              |              |              |              |              |              |              |              |              |              |
| Codominant   |              |              |              |              |              |              |              |              |              |              |              |
| AA           | 178 (49.9)   | 208 (47.6)   | 1            |              | 117 (48.0)   | 1            |              | 68 (48.6)    | 1            | 0.506        |
| AG           | 147 (41.1)   | 191 (43.7)   | 1.21 (0.88–1.66) | 0.245 | 105 (43.0) | 1.26 (0.85–1.85) | 0.253 | 62 (44.3) | 1.15 (0.76–1.76) | 0.643 |
| GG           | 32 (9.0)     | 38 (8.7)     | 0.84 (0.49–1.46) | 0.539 | 22 (9.0) | 0.78 (0.40–1.49) | 0.445 | 10 (7.1) | 0.83 (0.38–1.83) | 0.643 |
| Dominant     |              |              |              |              |              |              |              |              |              |              |              |
| AA           | 178 (49.9)   | 208 (47.6)   | 1            |              | 117 (48.0)   | 1            |              | 68 (48.6)    | 1            | 0.658        |
| AG + GG      | 179 (50.1)   | 229 (52.4)   | 1.13 (0.84–1.54) | 0.414 | 127 (52.0) | 1.15 (0.79–1.65) | 0.468 | 72 (51.4) | 1.10 (0.73–1.64) | 0.643 |
| Recessive    |              |              |              |              |              |              |              |              |              |              |              |
| AA + AG      | 325 (91.0)   | 399 (91.3)   | 1            |              | 222 (91.0)   | 1            |              | 130 (92.9)   | 1            | 0.516        |
| GG           | 32 (9.0)     | 38 (8.7)     | 0.77 (0.45–1.31) | 0.338 | 22 (9.0) | 0.70 (0.37–1.32) | 0.269 | 10 (7.1) | 0.78 (0.36–1.67) | 0.269 |
| HWE          | 0.978        | 0.821        |              |              |              |              |              |              |              |              |              |

CON, control; GC, gastric cancer; IGC, intestinal-type gastric cancer; DGC, diffuse-type gastric cancer; AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium. \(^a\) Adjusted for age and gender. The significant results are in bold.
2.3. Stratified Analysis for Four PRNCR1 SNPs

To further estimate the possible correlation between the four SNPs and GC risk in GC subgroups, we performed stratified analyses based on various patient characteristics, including age, sex, LNM, T classification, and tumor stage. As shown in Table 3, after adjusting for age and gender, our recessive model demonstrated that the rs1016343 TT genotype was significantly associated with a decreased risk of GC in subjects aged <60 years showing LNM-negative results (odds ratio, OR = 0.29, 95% confidence interval, CI = 0.09–0.94, p = 0.038), when compared to the rs1016343 CC/CT genotypes. Moreover, according to the recessive model, the rs13252298 GG genotype was associated with an increased risk of GC for those aged ≥60 years showing LNM-positive results (OR = 2.80, 95% CI = 1.15–6.82, p = 0.024) and those aged ≥60 years in tumor stage III (OR = 3.39, 95% CI = 1.35–8.52, p = 0.009), as compared to the rs13252298 AA/AG genotypes. Furthermore, the dominant model showed that the rs16901946 combined genotype (AG/GG) had a significant association with increased risk of GC in subjects aged <60 years in tumor stage III (OR = 2.38, 95% CI = 1.15–4.94, p = 0.020). The recessive model also showed that the rs16901946 GG genotype was associated with a decreased risk of GC (OR = 0.43, 95% CI = 0.19–0.98, p = 0.046) and IGC (OR = 0.38, 95% CI = 0.16–0.89, p = 0.026) in male subjects aged ≥60 years when compared to the rs16901946 AA/AG genotype. However, the association between polymorphisms in PRNCR1 and tumor differentiation was not observed.
| SNP        | Variable | Dominant (ht+mt/wt) | GC vs. CON | Recessive (mt/wt+ht) |
|------------|----------|---------------------|------------|---------------------|
| rs1016343  | Gender (M) | rs1016343  |            |                     |
|            | Gender (F) | rs1016343  |            |                     |
|            | IGC       | rs1016343  |            |                     |
|            | DGC       | rs1016343  |            |                     |
|            | LNM (−)   | rs1016343  |            |                     |
|            | LNM (+)   | rs1016343  |            |                     |
|            | Tumor stage I + II | rs1016343  |            |                     |
|            | Tumor stage III | rs1016343  |            |                     |
| ≥60        | Gender (M) | rs1016343  |            |                     |
|            | Gender (F) | rs1016343  |            |                     |
|            | IGC       | rs1016343  |            |                     |
|            | DGC       | rs1016343  |            |                     |
|            | LNM (−)   | rs1016343  |            |                     |
|            | LNM (+)   | rs1016343  |            |                     |
|            | Tumor stage I + II | rs1016343  |            |                     |
|            | Tumor stage III | rs1016343  |            |                     |
| rs13252298 | Gender (M) | rs13252298 |            |                     |
|            | Gender (F) | rs13252298 |            |                     |
|            | IGC       | rs13252298 |            |                     |
|            | DGC       | rs13252298 |            |                     |
|            | LNM (−)   | rs13252298 |            |                     |
|            | LNM (+)   | rs13252298 |            |                     |
|            | Tumor stage I + II | rs13252298 |            |                     |
|            | Tumor stage III | rs13252298 |            |                     |

Table 3. Stratified analysis of PRNCR1 polymorphisms in GC patients and controls by age.
| SNP        | Variable                        | GC vs. CON | Dominant (ht+mt/wt) | GC | CON | AOR (95% CI) | p   | GC | CON | AOR (95% CI) | p   |
|------------|---------------------------------|------------|---------------------|----|-----|--------------|-----|----|-----|--------------|-----|
| rs7841060  | Gender (M)                      | 76/52      | 28/16               | 0.93 (0.45–1.92) | 0.847 | 10/118 | 6/38 | 0.55 (0.19–1.64) | 0.286 |
| Age <60    | Gender (F)                      | 27/30      | 69/73               | 0.94 (0.50–1.76) | 0.838 | 2/55   | 10/132 | 0.37 (0.07–1.88) | 0.230 |
|            | IGC                             | 43/43      | 97/89               | 0.68 (0.36–1.38) | 0.232 | 5/81   | 16/170 | 0.43 (0.13–1.37) | 0.153 |
|            | DGC                             | 47/30      | 97/89               | 1.39 (0.77–2.50) | 0.272 | 4/73   | 16/170 | 0.48 (0.15–1.57) | 0.224 |
|            | LNM (−)                         | 71/49      | 97/89               | 1.19 (0.68–2.08) | 0.535 | 6/114  | 16/170 | 0.34 (0.12–1.03) | 0.056 |
|            | LNM (+)                         | 32/33      | 97/89               | 0.74 (0.39–1.37) | 0.333 | 6/59   | 16/170 | 0.86 (0.30–2.47) | 0.778 |
|            | Tumor stage I + II              | 82/59      | 97/89               | 1.11 (0.66–1.88) | 0.695 | 9/132  | 16/170 | 0.49 (0.19–1.29) | 0.147 |
| ≥60        | Gender (M)                      | 21/23      | 97/89               | 0.67 (0.33–1.36) | 0.264 | 3/41   | 16/170 | 0.55 (0.14–2.11) | 0.364 |
|            | Gender (F)                      | 37/39      | 55/43               | 0.74 (0.38–1.44) | 0.378 | 6/70   | 7/91  | 1.31 (0.39–4.42) | 0.668 |
|            | IGC                             | 85/73      | 91/80               | 1.15 (0.71–1.86) | 0.577 | 15/143 | 13/158 | 1.26 (0.53–3.01) | 0.602 |
|            | DGC                             | 32/31      | 91/80               | 0.90 (0.50–1.64) | 0.737 | 7/56   | 13/158 | 1.38 (0.50–3.78) | 0.536 |
|            | LNM (−)                         | 79/67      | 91/80               | 1.08 (0.67–1.73) | 0.753 | 14/132 | 13/158 | 1.26 (0.54–2.95) | 0.598 |
|            | LNM (+)                         | 51/55      | 91/80               | 0.82 (0.48–1.39) | 0.450 | 12/94  | 13/158 | 1.22 (0.48–3.09) | 0.676 |
|            | Tumor stage I + II              | 85/81      | 91/80               | 0.98 (0.62–1.55) | 0.922 | 16/150 | 13/158 | 1.28 (0.56–2.93) | 0.554 |
|            | Tumor stage III                 | 45/41      | 91/80               | 0.95 (0.54–1.67) | 0.861 | 10/76  | 13/158 | 1.21 (0.45–3.25) | 0.702 |
|            | Gender (M)                      | 66/63      | 17/27               | 1.48 (0.72–3.02) | 0.282 | 14/115 | 3/41  | 1.64 (0.44–6.05) | 0.462 |
|            | Gender (F)                      | 29/26      | 68/75               | 1.35 (0.71–2.57) | 0.364 | 1/54   | 9/134  | 0.34 (0.04–2.76) | 0.312 |
| Age <60    | IGC                             | 45/40      | 85/102              | 1.75 (0.92–3.32) | 0.088 | 10/75  | 12/175 | 1.38 (0.46–4.11) | 0.562 |
|            | DGC                             | 39/38      | 85/102              | 1.20 (0.68–2.14) | 0.533 | 2/75   | 12/175 | 0.35 (0.07–1.81) | 0.210 |
|            | LNM (−)                         | 58/56      | 85/102              | 1.14 (0.66–1.98) | 0.638 | 10/110 | 12/175 | 0.95 (0.33–2.74) | 0.920 |
|            | LNM (+)                         | 37/27      | 85/102              | 1.78 (0.96–3.32) | 0.069 | 5/59   | 12/175 | 1.09 (0.33–3.58) | 0.883 |
|            | Tumor stage I + II              | 68/73      | 85/102              | 1.08 (0.64–1.83) | 0.766 | 12/129 | 12/175 | 1.01 (0.37–2.76) | 0.989 |
| ≥60        | Gender (F)                      | 43/34      | 54/43               | 1.14 (0.58–2.22) | 0.709 | 8/69   | 8/89  | 1.33 (0.41–4.25) | 0.635 |
|            | IGC                             | 82/77      | 94/76               | 0.94 (0.58–1.52) | 0.797 | 12/147 | 20/150 | 0.38 (0.16–0.89) | 0.026 |
|            | DGC                             | 47/30      | 94/76               | 1.43 (0.79–2.56) | 0.236 | 4/73   | 20/150 | 0.50 (0.15–1.63) | 0.248 |
|            | LNM (−)                         | 80/66      | 94/76               | 1.05 (0.65–1.69) | 0.841 | 16/130 | 20/150 | 0.69 (0.32–1.47) | 0.336 |
|            | LNM (+)                         | 54/53      | 94/76               | 0.85 (0.50–1.44) | 0.538 | 7/100  | 20/150 | 0.39 (0.15–0.96) | 0.064 |
|            | Tumor stage I + II              | 92/75      | 94/76               | 1.04 (0.66–1.65) | 0.671 | 18/149 | 20/150 | 0.65 (0.31–1.36) | 0.249 |

Table 3. Cont.

GC, gastric cancer; CON, control; ht, heterozygous; mt, mutant; wt, wild-type; SNP, single nucleotide polymorphism; AOR, adjusted odds ratio; CI, confidence interval; M, male; F, female; IGC, intestinal-type gastric cancer; DGC, diffuse-type gastric cancer; LNM, lymph node metastasis. * Adjusted for age and gender. The significant results are in bold.
3. Discussion

To date, most studies, including GWAS and a meta-analysis, have reported on the association between genetic variations in PRNCR1 and PC [18–21]. However, little is known about the associations between PRNCR1 polymorphisms and GC. Furthermore, results thus far have been inconsistent, and the genotyping methods have also varied [14,23]. In this case–control study, we investigated the association between IncRNA PRNCR1 polymorphisms and GC risk in a Korean population using a reliable TaqMan genotyping assay. We found that the rs13252298 GG genotype was associated with 1.92-times increased risk of IGC. Li et al. previously reported that there was an association between the rs13252298 AG genotype and GC in the Chinese population; in contrast, He et al. reported a lack of association between the rs13252298 polymorphism and GC in the Chinese population [14,23]. Interestingly, our age-stratified analysis found that the rs1016343 TT genotype was associated with 0.92-times reduced risk of GC in subjects aged <60 years showing LNM-negative results; the rs13252298 GG genotype was associated with 2.80- and 3.39-times increased risk of GC in subjects aged ≥60 years showing LNM-positive results and aged ≥60 years in tumor stage III, respectively. The rs16901946 AG/ GG genotype was associated with 2.38-times increased risk of GC in subjects <60 years in tumor stage III. However, He et al. described an association between the rs16901946 genotype and increased risk of GC in younger and tumor stage I+II subjects. Interestingly, in our study the rs1016343 TT genotype was associated with 0.29-times decreased risk of GC in those aged <60 years showing LNM-negative results. Li et al. demonstrated an association between the rs1456315 GG genotype and a decreased risk of GC. However, we could not analyze an association because of the Hardy–Weinberg disequilibrium in either the GC group or the control group (p < 0.05).

There are several limitations to our study. First, the sample size was too small to have statistical power for our stratified analyses. Second, although Helicobacter pylori is an independent risk factor [24,25], we did not investigate its relevance with regard to the PRNCR1 polymorphisms in GC risk because of ethical considerations. Third, we failed to explore whether there is an association between the genetic factors and smoking, drinking, and diet related to GC risk owing to lack of these data from the GC and control groups. Fourth, our study was performed in a Korean population. Thus, we should include other ethnic groups and a larger sample size to confirm our results. Future studies will require that we assess the influence of these factors on GC.

In conclusion, our study suggests that the PRNCR1 rs13252298 and rs16901946 polymorphisms are associated with increased GC risk and may exacerbate the development of GC. Moreover, the PRNCR1 rs1016343 polymorphism may contribute to a decreased risk of GC in those aged <60 years showing LNM-negative results. Further studies are needed to validate our results in a larger population as well as in different ethnic groups.

4. Materials and Methods

4.1. Ethics Statement

The present study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the institutional review board of Chungnam National University Hospital on 23 July 2017. Informed consent was provided by all subjects when they were enrolled.

4.2. Study Subjects

In total, 437 GC patients and 357 healthy controls were enrolled in this study. The blood samples used in this study were provided by the Chungnam National Hospital Biobank, a member of the National Biobank of Korea, which is supported and audited by the Ministry of Health and Welfare of Korea. All individuals enrolled in this study provided their written informed consent for blood collection and use. GC patients were recruited from the outpatient clinic at the Chungnam National University Hospital, and classified according to the Lauren’s classification [26]. The subjects for the
control group were randomly selected among healthy volunteers visiting the Chungnam National University Hospital medical center for their annual physical examinations; only individuals who had no history of cancer were included.

4.3. DNA Isolation and Genotyping

Genomic DNA was isolated from the peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer’s instructions. Five SNPs (rs1016343, rs13252298, rs7841060, rs16901946, and rs1456315) in PRNCR1 were selected based on previous reports [14,19,20,23–25] and genotyped using the Applied Biosystems TaqMan SNP Genotyping Assay with the StepOnePlus Real-time PCR System (Applied Biosystems, Foster City, CA, USA).

4.4. Statistical Analysis

Hardy–Weinberg equilibrium (HWE) for each SNP in the control groups was evaluated using the chi-squared test. A pair-wise comparison of bi-allelic loci was employed for the analyses of linkage disequilibrium (LD) using Haploview software version 4.0 (the Broad Institute, Cambridge, MA, USA). Differences in age and gender between the GC and control groups were calculated using the two-sided Pearson chi-squared test and the Mann–Whitney U-test. Two genetic models (dominant and recessive models) were used to analyze the associations. A binary logistic regression was used to estimate the GC risk according to odds ratios (ORs) and 95% confidence intervals (CIs). The association analysis was adjusted by age and sex, which were included in the model as covariates. All statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL, USA), version 20.0 for Windows. \( p < 0.05 \) was considered statistically significant.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| GC           | gastric cancer |
| LncRNAs      | long non-coding RNAs |
| PRNCR1       | prostate cancer non-coding RNA 1 |
| PC           | prostate cancer |
| SNPs         | single nucleotide polymorphisms |
| LNM          | lymph node metastasis |
| IGC          | intestinal-type gastric cancer |
| HWE          | Hardy–Weinberg equilibrium |
| OR           | odds ratio |
| CI           | confidence interval |
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