Characterization and risk association of polymorphisms in *Aurora kinases A, B and C* with genetic susceptibility to gastric cancer development

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**Abstract**

**Background:** Single nucleotide polymorphisms (SNPs) in genes encoding mitotic kinases could influence development and progression of gastric cancer (GC).

**Methods:** Case-control study of nine SNPs in mitotic genes was conducted using qPCR. The study included 116 GC patients and 203 controls. In silico analysis was performed to evaluate the effects of polymorphisms on transcription factors binding sites.

**Results:** The *AURKA* rs1047972 genotypes (CT vs. CC: OR, 1.96; 95% CI, 1.05–3.65; \(p = 0.033\); CC + TT vs. CT: OR, 1.94; 95% CI, 1.19–23.22; \(p = 0.028\)) were associated with increased GC risk, whereas certain rs8173 genotypes (CG vs. CC: 0.60; 95% CI, 0.36–0.99; \(p = 0.049\); GG vs. CC: OR, 0.38; 95% CI, 0.18–0.79; \(p = 0.010\); CC + CG vs. GG: OR, 0.49; 95% CI, 0.25–0.98; \(p = 0.043\)) were protective. Association with increased GC risk was demonstrated for *AURKB* rs2241909 (GG + AG vs. AA: OR, 1.61; 95% CI, 1.01–2.56; \(p = 0.041\)) and rs2289590 (AC vs. AA: OR, 2.41; 95% CI, 1.47–3.98; \(p = 0.001\); CC vs. AA: OR, 6.77; 95% CI, 2.24–20.47; \(p = 0.001\); AA + AC vs. CC: OR, 4.23; 95% CI, 1.44–12.40; \(p = 0.009\)). Furthermore, *AURKC* rs11084490 (GG + CG vs. CC: OR, 1.71; 95% CI, 1.04–2.81; \(p = 0.033\)) was associated with increased GC risk. A combined analysis of five SNPs, associated with an increased GC risk, detected polymorphism profiles where all the combinations contribute to the higher GC risk, with an OR increased 1.51-fold for the rs1047972(CT)/rs11084490(CG + GG) to 2.29-fold for the rs1047972(CT)/rs911160(CC) combinations. In silico analysis for rs911160 and rs2289590 demonstrated that different transcription factors preferentially bind to polymorphic sites, indicating that *AURKA* and *AURKB* could be regulated differently depending on the presence of particular allele.

**Conclusions:** Our results revealed that *AURKA* (rs1047972 and rs911160), *AURKB* (rs2241909 and rs2289590) and *AURKC* (rs11084490) are associated with a higher risk of GC susceptibility. Our findings also showed that the combined effect of these SNPs may influence GC risk, thus indicating the significance of assessing multiple polymorphisms, jointly. The study was conducted on a less numerous but ethnically homogeneous Bosnian population, therefore further investigations in larger and multiethnic groups and the assessment of functional impact of the results are needed to strengthen the findings.

**Keywords:** Gastric cancer, Single nucleotide polymorphisms, Mitotic kinases, Cancer susceptibility, Chromosomal instability
Background
Gastric cancer (GC) represents one of the major causes of tumor-linked death, with geographical and ethnical variations in incidence [1]. Accurate chromosomal segregation in rapidly dividing tumor cells and defects during the spindle assembly checkpoint may contribute to tumorigenesis [2]. Genetic alterations in mitotic genes could enhance susceptibility to malignant transformation through modifications of gene expression profiles [3, 4]. Aurora kinases are members of serine-threonine kinases family essential for cell cycle control [5]. Aurora kinase A (AURKA) is involved in regulation of a several oncogenic signaling processes, including mitotic entry, cytokinesis, functions of centrosome, chromosome segregation, and chromosome alignment [6, 7]. Aurora kinase B (AURKB) assists in chromatin modification, spindle checkpoint regulation, cytokinesis and plays a significant role in establishment of the correct kinetochore/microtubule binding [6]. Aurora kinase C (AURKC) acts as a chromosomal passenger protein, participating in the proper centrosome functioning [8]. Polo-like kinase 1 (PLK1) is essential for cell division and regulates various cellular events including centrosome maturation, mitotic checkpoint activation, spindle assembly, kinetochore/microtubule attachment, exit from the mitosis, and cytokinesis [9].

In this study, using a case-control approach, we estimated the impact of rs2273535, rs1047972, rs911160 and rs8173 in AURKA, rs2241909 and rs2289590 in AURKB, rs758099 and rs11084490 in AURKC and rs42873 in PLK1 mitotic checkpoint genes on GC susceptibility in Bosnia and Herzegovina population. In addition, the associations between single nucleotide polymorphisms and the histological types of gastric cancer (intestinal and diffuse types) have been investigated. By conducting in silico analysis of SNPs, we evaluated the impact of the studied polymorphisms in introns and untranslated regions (UTRs) within candidate genes (AURKA, AURKB, AURKC and PLK1) on transcription factors binding sites.

Methods
Study design and populations
Our examined population consisted of 116 GC patients with diagnosed gastric adenocarcinoma from the Clinical Pathology and Cytology at the University Clinical Center Sarajevo, Bosnia and Herzegovina. General status of gastric cancer patients is given in Table 1. Gastric cancer patients in the case group were not subjected to any type of treatment (radiotherapy or chemotherapy). The formalin fixed paraffin embedded (FFPE) cancer tissue sections were collected during surgical procedures. Simultaneously, 203 healthy blood donors (controls) of Bosnian origin (matched to cases for ethnicity) were randomly selected and signed up for the present study. Individuals in the control group had no history of any neoplastic formation, were not related to each other and to the patients group. Three ml of blood was sampled from each control individual and stored at −80°C. The study was approved by the Ethical Committee at the University Clinical Centre Sarajevo (No. 0302–36,765). Personal information was encrypted to provide maximum anonymity in compliance with the Helsinki Declaration.

DNA isolation
Genomic DNA from FFPE GC tissues was isolated using the Chemagic FFPE DNA Kit special (PerkinElmer Inc., Waltham, MA, USA), according to manufacturer’s recommendations. Automated DNA washing and elution was conducted on Chemagic Magnetic Separation Module I robot (PerkinElmer Inc., Waltham, MA, USA), following manufacturer’s standard programme. All sample transfers were performed with 4-eye principle to avoid sample mix-ups. DNA from lymphocytes (control DNA) was extracted using the Promega™ Wizard™ Genomic DNA Purification Kit Protocol (Promega Corp., Fitchburg, WI, USA), in accordance with the manufacturer’s recommendations. The qualitative and quantitative analysis of extracted DNA was conducted by use of the DropSense96 photometer (Trinean, Gentbrugge, Belgium) and Synergy™ 2 Multi Mode Reader (BioTek, Inc., Winooski, VT, USA).

Table 1 Baseline characteristics of gastric cancer patients

| Variable                  | GC patients |
|---------------------------|-------------|
|                           | N           | No. (%)     |
| Total sample              | 116         |             |
| Sex                       |             |             |
| Men                       | 80          | (69.0)      |
| Women                     | 36          | (31.0)      |
| Age (years)*              |             |             |
| < 60                      | 27          | (23.5)      |
| ≥ 60                      | 88          | (76.5)      |
| Range                     | 33–90       |             |
| Lauren’s classification    |             |             |
| Intestinal type GC        | 53          | (45.7)      |
| Diffuse type GC           | 63          | (54.3)      |

GC Gastric cancer
*aData were missing in 1 case

Selection of polymorphisms
We selected nine polymorphisms in mitotic genes, namely rs2273535, rs1047972, rs911160 and rs8173 (AURKA), rs2241909 and rs2289590 (AURKB), rs758099 and rs11084490 (AURKC) and rs42873 (PLK1). The positions of selected genetic variants in mitotic genes are presented in Fig. 1. For this purpose, gene structures were extracted from the Research Collaboratory for
Structural Bioinformatics (RCSB) Protein Data Bank (PDB) [10]. Selection of the polymorphisms for this study was conducted in accordance with the parameters described below: (a) previously demonstrated association with respect to certain cancer types; (b) minor allele frequency (MAF) of less than or equal to 10% in the population of Utah residents with Northern and Western European ancestry (CEU), as stated by the Phase 31,000 Genomes; and (c) tagging polymorphisms (tagSNPs) status, which was anticipated in silico by use of LD Tag Selection of SNP (tagSNP) (https://snpinfo.niehs.nih.gov) [11], with the following parameters: 1kb of the sequences upstream/downstream from gene was selected, linkage disequilibrium (LD) lower limit of 0.8, and MAF range 0.05–0.5 for CEU subpopulation (Table 2 and Fig. 2).

Genotyping
Genotyping was conducted using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). The assay ID numbers are presented in Table 2. The reaction mixtures, GC samples (5 μl) and controls (10 μl), were composed of 20X TaqMan® assay with 2X Master Mix (Applied Biosystems, Foster City, CA), and 20 nanograms of DNA. The polymerase chain reaction (PCR) profile was carried out in concordance with the manufacturer’s recommendations (Initial denaturation at 95 °C for 10 min, 45 cycles at 92 °C for 15 s and 60 °C for 90 s, using the ViiA 7 Real Time PCR System (Applied Biosystems, Foster City, CA). In each plate, at least two negative controls were included. PCR results were analyzed using TaqMan® Genotyper Software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis
The genotype frequencies of the investigated variants were tested for Hardy-Weinberg equilibrium (HWE) in the case/control groups separately, using Michael H. Court’s online HWE calculator (http://www.tufts.edu) [12]. The differences in genotype frequencies amongst GC cases and controls were calculated by use of the Chi-square test or Fisher’s exact test. Association between examined polymorphisms and the GC risk was estimated by multinomial logistic regression. Odds ratio (OR) with 95% confidence interval (CI) were computed in order to evaluate the relative risk. For the assessment of each genotype, risk estimates were computed for dominant, overdominant and recessive models using the most frequent homozygote as the reference. Akaike information criterion (AIC) was calculated to define which of the models best fits the data. A combined analysis was performed to evaluate synergistic effect of the studied polymorphisms. All statistical calculations were conducted using SPSS 20.0 software package (SPSS, Chicago, IL, USA). P ≤ 0.05 was chosen as threshold value in significance testing. MAF plot was created by use of the PAST software package, version 3.18 (http://folk.uio.no/ohammer/past/) [13].

Haplotype analysis
Determination of the haplotype block structure and haplotype analysis, which encompassed subsequent
Table 2 Basic information for studied polymorphisms

| dbSNP      | Variant location | Gene    | Base change | NCBI assembly location (Build GRCh38)a | TaqMan SNP assay ID | Tag SNP (CEU population; HapMap)b | Minor allele frequency (MAF)c   | GC Patients | Control group | ALL | EUR | CEU |
|------------|------------------|---------|-------------|----------------------------------------|---------------------|-----------------------------------|---------------------------------|-------------|---------------|------|------|------|
| rs2273535  | Missense         | AURKA   | A/T         | Chr.20:56386485 C_25623289_10          | Yes                 | 0.168                             | 0.238                           | 0.310       | 0.216         | 0.177 |
| rs1047972  | Missense         | AURKA   | C/T         | Chr.20:56386407 AHX1IRW                 | No                  | 0.088                             | 0.146                           | 0.150       | 0.182         | 0.157 |
| rs911160   | Intron           | AURKA   | G/C         | Chr.20:56382507 C_8947670_10            | Yes                 | 0.206                             | 0.276                           | 0.447       | 0.246         | 0.202 |
| rs8173     | 3' UTR           | AURKA   | G/C         | Chr.20:56369735 C_8947675_10            | No                  | 0.417                             | 0.305                           | 0.486       | 0.282         | 0.232 |
| rs2241909  | Synonymous       | AURKB   | A/G         | Chr.17:8205021 C_22272900_10            | No                  | 0.247                             | 0.332                           | 0.379       | 0.340         | 0.303 |
| rs2289590  | Intron           | AURKB   | C/A         | Chr.17:8207446 C_15770418_10            | Yes                 | 0.240                             | 0.415                           | 0.453       | 0.415         | 0.389 |
| rs758099   | Intron           | AURKC   | C/T         | Chr.19:57231166 C_2581008_1_            | No                  | 0.284                             | 0.302                           | 0.375       | 0.255         | 0.253 |
| rs11084900 | 5' UTR           | AURKC   | C/G         | Chr.19:57231104 C_27847620_10           | Yes                 | 0.139                             | 0.223                           | 0.132       | 0.165         | 0.177 |
| rs42873    | Intron           | PLK1    | G/C         | Chr.16:23683411 C_2392140_10            | Yes                 | 0.230                             | 0.208                           | 0.234       | 0.215         | 0.192 |

ALL: All phase 3 individuals, EUR: European population, CEU: Utah residents with Northern and Western European ancestry, GC: Gastric cancer, UTR: Untranslated region

ahttps://www.lifetechnologies.com
bhttps://snpinfo.niehs.nih.gov/snpinfo/snptag.html
cMAFs extracted from 1000 Genomes Project Phase 3

Fig. 2 MAF values for polymorphisms rs2273535, rs1047972, rs911160 and rs8173 (AURKA), rs2241909 and rs2289590 (AURKB), rs758099 and rs11084490 (AURKC), and rs42873 (PLK1), in different populations. ALL: All individuals from 1000 Genome Project Phase 3 release. C: Studied Bosnian control population; CEU: Utah residents with Northern and Western European ancestry; EUR: European population; GC: Studied Bosnian gastric cancer population; MAF: Minor allele frequency. SNP: Single nucleotide polymorphism
corrections for multiple comparisons by 10,000 permutations, were evaluated using the Haploview software, version 4.2 [14], and SNP tools V1.80 (MS Windows, Microsoft Excel). To construct the haplotype block, the solid spine of the linkage disequilibrium algorithm with a minimum Lewontin’s D’ value of 0.8 was selected.

In silico analysis of SNPs
Impact of the polymorphic DNA sequences (SNPs in introns and untranslated regions (UTRs)) on transcription factors binding sites (TFBSs) was estimated in silico. Bioinformatic functional evaluation was carried out using PROMO software (ALGGEN web-server), which is utilizing data from TRANSFAC database V8.3 [15, 16]. FASTA sequences for the investigated genetic variants were downloaded from Ensembl 90 (www.ensembl.org/index.html) [17]. Identification of transcription factor binding sites was performed with the following criteria: human species, all sites and factors.

Results
Genotype distributions for examined SNPs
For all of the 9 studied variants, rs2273535 (AURKA), rs1047972 (AURKA), rs911160 (AURKA), rs8173 (AURKA), rs2241909 (AURKB), rs2289590 (AURKB), rs758099 (AURKC), rs11084490 (AURKC), rs42873 (PLK1) was determined to be in HWE in both, case and control populations (P > 0.05). When chi-square test and Fisher exact test were conducted for the frequency distributions at the genotypic level, a significant differences for rs911160 in AURKA (P = 0.044), rs8173 in AURKA (P = 0.018), rs2289590 in AURKB (P < 0.001) and rs11084490 in AURKC (P = 0.009) between the cases and controls for all types of GC were observed (summarized in Table 3).

Effect of studied polymorphisms on gastric cancer risk
Patients with rs1047972 (AURKA) CT genotype had a higher risk of GC development in comparison with the reference CC genotype (OR = 1.96, 95% CI = 1.05–3.65, P = 0.033) (Table 4). Genotypes (TT + CT) vs. reference CC genotype in dominant model (OR = 1.92, 95% CI = 1.06–3.48, P = 0.030) and CT vs. reference (CC + TT) genotypes in overdominant model (OR = 1.94, 95% CI = 1.04–3.60, P = 0.036) were associated with higher disease risk (Table 4). Based on Akaike information criterion (AIC), the overdominant model was selected as the model that best fits the data. The rs911160 (AURKA) CC genotype was positively associated with an increased gastric cancer risk in comparison with the reference GG genotype (OR = 5.56, 95% CI = 1.24–24.81, P = 0.025). Also, CC genotype was associated with disease risk in the recessive genetic model (GG + GC) vs. CC genotypes, (OR = 5.26, 95% CI = 1.19–23.22, P = 0.028). However, the confidence intervals in those two cases were wide; therefore, other factors might play a significant role in GC risk in interaction with this polymorphism. Comparison of genotype distributions for rs8173 (AURKA) showed that patients with GG genotype (OR = 0.38, 95% CI = 0.18–0.79, P = 0.010), and CG genotype (OR = 0.60, 95% CI = 0.36–0.99, P = 0.049) had decreased risk of gastric cancer. Analysis of genetic models showed that GG + CG genotypes in comparison with the reference CC genotype in dominant model (OR = 0.54, 95% CI = 0.33–0.87, P = 0.012) and GG vs. reference (CC + CG) (OR = 0.49, 95% CI = 0.25–0.98, P = 0.043) genotypes (recessive genetic model) were associated with decreased GC risk. According to the calculated AIC values, (CC + CG):GG recessive model had more statistical power than dominant model CC:(GG + CG). Analysis of rs2241909 (AURKB) demonstrated that G allele (dominant model: (GG + AG) vs. common AA genotype) was associated with higher risk of GC development (OR = 1.61, 95% CI = 1.01–2.56, P = 0.041). Comparison of the reference AA genotype with AC (OR = 2.41, 95% CI = 1.47–3.98, P = 0.001) and CC (OR = 6.77, 95% CI = 2.24–20.47, P = 0.001) genotypes of rs2289590 (AURKB) also revealed a significant effect of these two genotypes on increased GC risk. CC and AC genotypes in dominant model (OR = 2.78, 95% CI = 1.71–4.51, P < 0.001) as well as CC genotype in recessive model (OR = 4.23, 95% CI = 1.44–12.40, P = 0.009) and AC genotype in overdominant genetic model (OR = 1.77, 95% CI = 1.10–2.85, P = 0.017) were associated with an elevated disease risk. Since recessive genetic model had the lowest AIC value, when compared to the dominant and overdominant models, it was considered to be preferred model. However, in this model the confidence interval was wide, therefore, other factors could influence its effect. For rs11084490 (AURKC) polymorphism, (GG + CG) vs. CC genotypes in dominant model demonstrated statistically significant effect on higher GC risk (OR = 1.71, 95% CI = 1.04–2.81, P = 0.033). Additionally, the five polymorphisms rs1047972, rs911160, rs2241909, rs2289590 and rs11084490, associated with an increased GC risk individually in this study, were subjected to the combined analysis in order to determine polymorphism profiles related to the higher risk of this disease. The results of the synergistic effects of these SNPs are summarized in Table 5. By analyzing various combinations of risk genotypes (two to five combined SNPs), we demonstrated that the additive effect of all combinations significantly affected the risk of GC development, with an odds ratio ranging from (OR = 1.51, 95% CI = 1.03–2.22, P = 0.034) for the combined rs1047972(CT)/rs11084490(GG + CG) risk genotypes to (OR = 2.29, 95% CI = 1.32–3.97, P = 0.003) for the rs1047972(CT)/rs911160(CC) combination. Another interesting combined effect was demonstrated for five-polymorphisms combination rs1047972(CT)/rs911160 (CC)/
In this case, this combination was significantly associated with an increased GC risk (OR = 1.83, 95% CI = 1.46–2.29, P < 0.001). No significant effects on gastric cancer susceptibility were revealed for rs2273535 (AURKA), rs758099 (AURKC) and rs42873 (PLK1) polymorphisms (P > 0.05), when patients with both types of GC, intestinal and diffuse, were taken into account.

| Genotypes        | Control group | HWE | P value | GC patients | HWE | P value | All type GC | P value |
|------------------|---------------|-----|---------|-------------|-----|---------|-------------|---------|
|                  | No. (%)       |     |         | No. (%)     |     |         |             |         |
| rs2273535        | 203           | 0.867| 0.351   | 110         | 0.366| 0.544   | 3.987       | 0.136   |
| AA               | 120 (59.1)    |     |         | 77 (70.0)   |     |         |             |         |
| AT               | 69 (34.0)     |     |         | 29 (26.4)   |     |         |             |         |
| TT               | 14 (6.9)      |     |         | 4 (3.6)     |     |         |             |         |
| rs1047972        | 202           | 0.152| 0.696   | 113         | 1.691| 0.193   | 4.840       | 0.089   |
| CC               | 148 (73.3)    |     |         | 95 (84.1)   |     |         |             |         |
| CT               | 49 (24.2)     |     |         | 16 (14.1)   |     |         |             |         |
| TT               | 5 (2.5)       |     |         | 2 (1.8)     |     |         |             |         |
| rs91160          | 201           | 0.349| 0.554   | 116         | 2.815| 0.093   | 6.233       | 0.044   |
| GG               | 107 (53.2)    |     |         | 70 (60.4)   |     |         |             |         |
| CG               | 77 (38.3)     |     |         | 44 (37.9)   |     |         |             |         |
| CC               | 17 (8.5)      |     |         | 2 (1.7)     |     |         |             |         |
| rs8173           | 200           | 0.017| 0.895   | 115         | 0.001| 0.989   | 8.007       | 0.018   |
| CC               | 97 (48.5)     |     |         | 39 (33.9)   |     |         |             |         |
| CG               | 84 (42.0)     |     |         | 56 (48.7)   |     |         |             |         |
| GG               | 19 (9.5)      |     |         | 20 (17.4)   |     |         |             |         |
| rs2241909        | 203           | 1.186| 0.276   | 115         | 1.065| 0.302   | 6.201       | 0.102   |
| AA               | 87 (42.9)     |     |         | 63 (54.8)   |     |         |             |         |
| AG               | 97 (47.8)     |     |         | 47 (40.9)   |     |         |             |         |
| GG               | 19 (9.3)      |     |         | 5 (4.3)     |     |         |             |         |
| rs2289590        | 200           | 3.523| 0.060   | 108         | 1.414| 0.234   | 20.683      | < 0.001 |
| AA               | 62 (31.0)     |     |         | 60 (55.6)   |     |         |             |         |
| AC               | 110 (55.0)    |     |         | 44 (40.7)   |     |         |             |         |
| CC               | 28 (14.0)     |     |         | 4 (3.7)     |     |         |             |         |
| rs758099         | 203           | 2.107| 0.146   | 116         | 1.186| 0.276   | 3.107       | 0.211   |
| CC               | 103 (50.8)    |     |         | 57 (49.2)   |     |         |             |         |
| CT               | 77 (37.9)     |     |         | 52 (44.8)   |     |         |             |         |
| TT               | 23 (11.3)     |     |         | 7 (6.0)     |     |         |             |         |
| rs11084490       | 201           | 0.0009| 0.975  | 115         | 3.038| 0.083   | 9.083       | 0.009   |
| CC               | 121 (60.2)    |     |         | 83 (72.2)   |     |         |             |         |
| CG               | 70 (34.8)     |     |         | 32 (27.8)   |     |         |             |         |
| GG               | 10 (5.0)      |     |         | –           |     |         |             |         |
| rs42873          | 201           | 0.272| 0.601   | 115         | 2.668| 0.102   | 3.228       | 0.199   |
| GG               | 127 (63.2)    |     |         | 65 (56.5)   |     |         |             |         |
| CG               | 64 (31.8)     |     |         | 47 (40.9)   |     |         |             |         |
| CC               | 10 (5.0)      |     |         | 3 (2.6)     |     |         |             |         |

Statistically significant values are highlighted in bold (P ≤ 0.05)
HWE Hardy-Weinberg equilibrium, GC gastric cancer, χ² Chi-square statistics
χ² analysis between all type GC patients and controls
Fisher statistics
### Table 4 Risk of gastric cancer associated with studied polymorphisms

| Genotypes | All type GC | Intestinal type GC | Diffuse type GC |
|-----------|-------------|--------------------|-----------------|
|           | OR (95%CI)  | P value AIC        | OR (95%CI) P value AIC |
|           | OR (95%CI)  | P value AIC        | OR (95%CI) P value AIC |
| rs2273535 |             |                    |                 |
| AA        | 1 (ref)     |                    | 1 (ref)         |
| AT        | 1.52 (0.90–2.56) | 0.111 | 1.63 (0.81–3.28) | 0.167 | 1.43 (0.75–2.75) | 0.275 |
| TT        | 2.24 (0.71–7.07) | 0.167 | 4.31 (0.54–33.93) | 0.164 | 1.55 (0.42–5.69) | 0.504 |
| AA:(TT + AT) | 1.61 (0.98–2.64) | 0.058 | 1.82 (0.93–3.59) | 0.080 | 1.45 (0.78–2.69) | 0.230 |
| (AA+AT):TT | 1.96 (0.63–6.11) | 0.245 | 3.70 (0.47–28.84) | 0.211 | 1.38 (0.38–4.98) | 0.620 |
| (AA+TT):AT | 1.43 (0.86–2.40) | 0.166 | 1.50 (0.75–3.01) | 0.248 | 1.38 (0.72–2.63) | 0.322 |
| rs1047972 |             |                    |                 |
| CC        | 1 (ref)     |                    | 1 (ref)         |
| CT        | 1.96 (1.05–3.65) | 0.033 | 2.53 (1.02–6.30) | 0.045 | 1.62 (0.76–3.44) | 0.208 |
| TT        | 1.60 (0.30–8.43) | 0.577 | 1.55 (0.17–13.64) | 0.691 | 1.65 (0.18–14.51) | 0.649 |
| CC:(TT + CT) | 1.92 (1.06–3.48) | 0.030 | 14.083 | 2.39 (1.02–5.63) | 0.045 | 13.186 | 1.62 (0.78–3.35) | 0.189 |
| (CC + CT):TT | 1.40 (0.26–7.38) | 0.685 | 1.32 (0.15–11.54) | 0.802 | 1.49 (0.17–13.07) | 0.715 |
| (CC + CT):CT | 1.94 (1.04–3.60) | 0.036 | 13.924 | 2.50 (1.01–6.22) | 0.047 | 12.993 | 1.60 (0.75–3.39) | 0.219 |
| rs911160  |             |                    |                 |
| GG        | 1 (ref)     |                    | 1 (ref)         |
| CG        | 1.14 (0.71–1.84) | 0.579 | 1.06 (0.56–1.98) | 0.850 | 1.22 (0.67–2.20) | 0.510 |
| CC        | 5.56 (1.24–24.81) | 0.025 | 4.92 (0.63–38.49) | 0.129 | 6.19 (0.79–48.12) | 0.081 |
| GG:(CC + CG) | 1.33 (0.84–2.12) | 0.220 | 1.23 (0.67–2.28) | 0.495 | 1.42 (0.80–2.54) | 0.228 |
| (GG + CG):CC | 5.26 (1.19–23.22) | 0.028 | 4.80 (0.62–36.95) | 0.132 | 5.72 (0.74–43.93) | 0.093 |
| (GG + CC):CG | 1.01 (0.63–1.62) | 0.947 | 0.94 (0.50–1.75) | 0.861 | 1.08 (0.60–1.94) | 0.797 |
| rs8173    |             |                    |                 |
| CC        | 1 (ref)     |                    | 1 (ref)         |
| CG        | 0.60 (0.36–0.99) | 0.049 | 0.65 (0.33–1.27) | 0.217 | 0.55 (0.29–1.05) | 0.072 |
| GG        | 0.38 (0.18–0.79) | 0.010 | 0.46 (0.17–2.11) | 0.119 | 0.32 (0.13–0.77) | 0.012 |
| CC:(CG + CG) | 0.54 (0.33–0.87) | 0.012 | 14.844 | 0.61 (0.32–1.14) | 0.125 | 0.49 (0.27–0.89) | 0.021 | 14.213 |
| (CC + CG):CG | 0.49 (0.25–0.98) | 0.043 | 13.343 | 0.57 (0.23–1.40) | 0.226 | 0.44 (0.20–0.98) | 0.044 | 12.817 |
| (CC + CG):CG | 0.76 (0.48–1.21) | 0.250 | 0.78 (0.42–1.44) | 0.431 | 0.74 (0.42–1.31) | 0.315 |
| rs241909  |             |                    |                 |
| AA        | 1 (ref)     |                    | 1 (ref)         |
| AG        | 1.49 (0.92–2.40) | 0.098 | 2.23 (1.16–4.27) | 0.016 | 1.07 (0.59–1.93) | 0.802 |
| GG        | 2.75 (0.97–7.76) | 0.056 | 3.71 (0.82–16.80) | 0.089 | 2.11 (0.58–7.65) | 0.256 |
| AA:(GG + AG) | 1.61 (1.01–2.56) | 0.041 | 2.38 (1.27–4.46) | 0.007 | 14.096 | 1.17 (0.66–2.07) | 0.587 |
| (AA+AG):GG | 2.27 (0.82–6.25) | 0.112 | 2.63 (0.59–11.68) | 0.203 | 2.03 (0.58–7.10) | 0.268 |
| (AA + GG):AG | 1.32 (0.83–2.10) | 0.235 | 1.93 (1.02–3.67) | 0.042 | 14.061 | 0.97 (0.55–1.72) | 0.934 |
| rs2289590 |             |                    |                 |
| AA        | 1 (ref)     |                    | 1 (ref)         |
| AC        | 2.41 (1.47–3.98) | 0.001 | 1.77 (0.92–3.42) | 0.087 | 3.12 (1.68–5.80) | < 0.001 |
| CC        | 6.77 (2.24–20.47) | 0.001 | 5.19 (1.14–23.56) | 0.033 | 8.35 (1.88–37.11) | 0.005 |
| AA:(CC + AC) | 2.78 (1.71–4.51) | < 0.001 | 14.723 | 2.04 (1.07–3.88) | 0.028 | 3.58 (1.96–6.52) | < 0.001 | 14.096 |
| (AA + AC):CC | 4.23 (1.44–12.40) | 0.009 | 12.253 | 3.74 (0.86–16.30) | 0.079 | 4.72 (1.09–20.43) | 0.038 | 11.605 |
| (AA+CC):AC | 1.77 (1.10–2.85) | 0.017 | 14.846 | 1.32 (0.70–2.49) | 0.378 | 2.27 (1.24–4.13) | 0.007 | 14.203 |
| rs758099  |             |                    |                 |
Table 4 Risk of gastric cancer associated with studied polymorphisms (Continued)

| Genotypes          | All type GC OR (95%CI) | P value | AIC | Intestinal type GC OR (95%CI) | P value | AIC | Diffuse type GC OR (95%CI) | P value | AIC |
|---------------------|------------------------|---------|-----|--------------------------------|---------|-----|----------------------------|---------|-----|
| CC                  | 1 (ref)                |         |     | 1 (ref)                        |         |     | 1 (ref)                    |         |     |
| CT                  | 0.81 (0.50–1.32)       | 0.414   |     | 0.95 (0.50–1.78)               | 0.877   |     | 0.72 (0.40–1.30)           | 0.280   |     |
| TT                  | 1.81 (0.73–4.49)       | 0.196   |     | 2.08 (0.58–7.44)               | 0.258   |     | 1.61 (0.51–5.05)           | 0.407   |     |
| CC:(TT + CT)        | 0.93 (0.59–1.48)       | 0.783   |     | 1.08 (0.59–1.99)               | 0.786   |     | 0.82 (0.47–1.45)           | 0.514   |     |
| (CC + CT):TT       | 1.99 (0.82–4.79)       | 0.125   |     | 2.13 (0.61–7.38)               | 0.233   |     | 1.88 (0.62–5.67)           | 0.260   |     |
| (CC + TT):CT       | 0.75 (0.47–1.19)       | 0.228   |     | 0.86 (0.46–1.59)               | 0.634   |     | 0.67 (0.38–1.18)           | 0.172   |     |
| rs11084490          |                        |         |     |                                |         |     |                            |         |     |
| CC                  | 1 (ref)                |         |     | 1 (ref)                        |         |     | 1 (ref)                    |         |     |
| CT                  | 1.50 (0.90–2.48)       | 0.114   |     | 1.78 (0.89–3.55)               | 0.102   |     | 1.30 (0.70–2.42)           | 0.390   |     |
| GG                  | –                      | –       |     | –                              | –       |     | –                          | –       |     |
| CC:(GG + CG)        | 1.71 (1.04–2.81)       | 0.033   |     | 2.03 (1.02–4.04)               | 0.043   |     | 1.49 (0.81–2.75)           | 0.195   |     |
| (CC + CG):GG       | –                      | –       |     | –                              | –       |     | –                          | –       |     |
| (CC + GG):CG       | 1.38 (0.84–2.28)       | 0.201   |     | 1.64 (0.82–3.27)               | 0.158   |     | 1.20 (0.65–2.23)           | 0.543   |     |
| rs42873             |                        |         |     |                                |         |     |                            |         |     |
| GG                  | 1 (ref)                |         |     | 1 (ref)                        |         |     | 1 (ref)                    |         |     |
| CC                  | 0.69 (0.43–1.12)       | 0.141   |     | 0.72 (0.38–1.35)               | 0.309   |     | 0.67 (0.37–1.22)           | 0.197   |     |
| GG:(CC + CG)        | 0.75 (0.47–1.20)       | 0.244   |     | 0.79 (0.42–1.47)               | 0.467   |     | 0.72 (0.41–1.29)           | 0.279   |     |
| (GG + CC):CG       | 1.95 (0.52–7.25)       | 0.316   |     | 2.67 (0.33–21.34)              | 0.354   |     | 1.59 (0.34–7.48)           | 0.553   |     |
| (GG + CG):CG       | 0.67 (0.42–1.08)       | 0.107   |     | 0.69 (0.36–1.29)               | 0.246   |     | 0.66 (0.37–1.19)           | 0.170   |     |

Statistically significant values are highlighted in bold (P ≤ 0.05). The inheritance model that best fits the data according to AIC is highlighted in bold.

GC Gastric cancer, OR Odds ratio, CI Confidence interval, AIC Akaike information criterion, ORs 95%CIs and P values were estimated by multinomial logistic regression analysis, Ref Reference homozygote

aDominant genetic model
bRecessive genetic model
cOverdominant genetic model

Next, we estimated the effects of genotypes on GC subtypes (presented in Table 4). CT genotype of rs1047972 (AURKA) was more frequent in patients with intestinal type (OR = 2.53, 95% CI = 1.02–6.30, P = 0.045) in comparison with the reference CC genotype. Likewise, (TT + CT) genotypes vs. reference CC (OR = 2.39, 95% CI = 1.02–5.63, P = 0.045) and CT vs. common (CC + TT) genotypes (OR = 2.50, 95%CI = 1.01–6.22, P = 0.047) were associated with higher risk for the development of intestinal subtype. According to the AIC values, (CC + TT):CT overdominant genetic model displayed stronger statistical confidence than dominant model CC:(TT + CT). The rs8173 (AURKA), GG genotype, in comparison with the reference CC genotype, was underrepresented in patients with diffuse GC type (OR = 0.32, 95% CI = 0.13–0.77, P = 0.012). Furthermore, both (GG + CG) genotypes as compared to its common CC genotype in dominant model (OR = 0.49, 95% CI = 0.27–0.89, P = 0.021) and GG vs. reference (CC + CG) genotypes in recessive model (OR = 0.44, 95% CI = 0.20–0.98, P = 0.044) were associated with the decreased diffuse type GC risk. In order to discriminate between these two competing models, in accordance with AIC, recessive model represents the preferred model in comparison with the dominant model. In stratified analysis for rs2241909 (AURKB), we found that carriers of AG genotype had elevated risk of developing intestinal type GC as compared to its reference AA genotype (OR = 2.23, 95% CI = 1.16–4.27, P = 0.016). Carriers of (GG + AG) genotypes had more frequent intestinal type GC than compared to the carriers of the more common AA genotype in dominant model (OR = 2.38, 95% CI = 1.27–4.46, P = 0.007). In overdominant model (OR = 1.93, 95%CI = 1.02–3.67, P = 0.042) individuals with AG genotype had more frequently intestinal type GC in comparison with reference genotypes (AA+GG). According to the calculated AIC values, overdominant model had more statistical power than dominant, therefore it represents the model that better fitted the data. The higher risk for intestinal type GC development was also detected for the patients with CC genotype of rs2289590 (AURKB) (OR = 5.19, 95% CI = 1.14–23.56, P = 0.033). Dominant genetic model revealed that patients with (CC + AC) genotypes when compared to the AA genotype (OR = 2.04, 95% CI = 1.07–3.88, P = 0.028) had significantly more frequently intestinal GC subtype. AC genotype (OR =
3.12, 95% CI = 1.68–5.80, \(P < 0.001\) was more frequently observed in patients with diffuse subtype. Regarding genetic models, (CC + AC) genotypes in dominant model (OR = 3.58, 95% CI = 1.96–6.52, \(P < 0.001\), CC genotype in recessive model (OR = 4.72, 95% CI = 1.09–20.43, \(P = 0.038\)) and AC genotype in overdominant model (OR = 2.27, 95% CI = 1.24–4.13, \(P = 0.007\)) were associated with the increased risk of diffuse subtype, with a recessive model being the one that best suited the data (according to the AIC value), however, the confidence interval in this model was also the largest. For rs11084490 (AURKC), dominant model (GG + CG) vs. CC (ref.) genotypes revealed a significant effect of GG and CG genotypes on the higher risk of intestinal subtype (OR = 2.03, 95% CI = 1.02–4.04, \(P = 0.043\)).

For genotypes of rs2273535 (AURKA), rs911160 (AURKA), rs758099 (AURKC) and rs42873 (PLK1) no significant effect on any of the GC histological subtypes was noted (\(P > 0.05\)).

**Haplotype analysis**

Raw genotyping data for the studied polymorphisms rs2273535, rs1047972, rs911160 and rs8173 in AURKA gene were used to perform haplotype analysis. Using the Haploview software, our results showed that no

### Table 5: Synergistic effect of rs1047972, rs911160, rs2241909, rs2289590 and rs11084490 polymorphisms on gastric cancer risk

| Risk genotypes | All type GC (95%CI) | \(P\) value |
|----------------|---------------------|-------------|
| Risk-free genotypes | 1 (ref) |            |
| Two risk SNPs | | |
| rs1047972(CT)/rs911160(CC) | 2.29 (1.32–3.97) | 0.003 |
| rs1047972(CT)/rs2241909(AG + GG) | 1.61 (1.14–2.28) | 0.006 |
| rs1047972(CT)/rs2289590(AC + CC) | 1.87 (1.31–2.66) | < 0.001 |
| rs1047972(CT)/rs11084490(CG + GG) | 1.51 (1.03–2.22) | 0.034 |
| rs911160(CC)/rs2241909(AG + GG) | 1.60 (1.11–2.32) | 0.011 |
| rs911160(CC)/rs2289590(AC + CC) | 2.19 (1.51–3.18) | < 0.001 |
| rs911160(CC)/rs11084490(CG + GG) | 1.84 (1.19–2.83) | 0.005 |
| rs2241909(AG + GG)/rs2289590(AC + CC) | 2.09 (1.50–2.92) | < 0.001 |
| rs2241909(AG + GG)/rs11084490(CG + GG) | 1.63 (1.17–2.28) | 0.004 |
| rs2289590(AC + CC)/rs11084490(CG + GG) | 2.12 (1.52–2.98) | < 0.001 |
| Three risk SNPs | | |
| rs1047972(CT)/rs911160(CC)/rs2241909(AG + GG) | 1.68 (1.22–2.30) | 0.001 |
| rs1047972(CT)/rs911160(CC)/rs2289590(AC + CC) | 2.09 (1.52–2.88) | < 0.001 |
| rs1047972(CT)/rs11084490(CG + GG) | 1.90 (1.44–2.50) | < 0.001 |
| rs1047972(CT)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.81 (1.36–2.42) | < 0.001 |
| rs911160(CC)/rs2241909(AG + GG)/rs2289590(AC + CC) | 1.89 (1.42–2.50) | < 0.001 |
| rs911160(CC)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.64 (1.22–2.20) | 0.001 |
| rs911160(CC)/rs2289590(AC + CC)/rs11084490(CG + GG) | 2.00 (1.49–2.70) | < 0.001 |
| rs2241909(AG + GG)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.93 (1.47–2.53) | < 0.001 |
| Four risk SNPs | | |
| rs1047972(CT)/rs911160(CC)/rs2241909(AG + GG)/rs2289590(AC + CC) | 1.86 (1.44–2.40) | < 0.001 |
| rs1047972(CT)/rs911160(CC)/rs11084490(CG + GG) | 1.68 (1.29–2.19) | < 0.001 |
| rs1047972(CT)/rs2241909(AG + GG)/rs11084490(CG + GG) | 1.97 (1.51–2.57) | < 0.001 |
| rs1047972(CT)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.85 (1.45–2.35) | < 0.001 |
| rs911160(CC)/rs2241909(AG + GG)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.84 (1.44–2.35) | < 0.001 |
| rs1047972(CT)/rs911160(CC)/rs2241909(AG + GG)/rs2289590(AC + CC)/rs11084490(CG + GG) | 1.83 (1.46–2.29) | < 0.001 |

Statistically significant values are highlighted in bold (\(P \leq 0.05\)).

GC: Gastric cancer, OR: Odds ratio, CI: Confidence interval, SNP: Single nucleotide polymorphism, Ref: Reference
haplotype block was created with an average Lewontin’s D < 0.8 (Fig. 3) thus, no haplotypes were available for the analysis of their potential association with GC risk.

**Bioinformatic SNP analysis**

Our in silico analysis suggested that polymorphic sequences in transcription factors binding sites (TFBSs), within AURKA, AURKB, AURKC and PLK1 genes, bind various transcription factors (TFs). In this regard, the region comprising G allele of rs911160 in AURKA was linked with C/EBPalpha, C/EBPbeta and GR-beta proteins, whereas for C allele, additional binding sites for NF-Y, NFI-CTF and NF-1 were identified (Table 6). For rs2289590 in AURKB, an additional motif for YY1 binding was recognized when C allele was present. The region near C allele of rs758099 was associated with binding sites for NF-1, NF-Y, XBP-1, ENKTF-1, CTF, PEA3 and POU2F1, whereas in the presence of T allele NF-1, NF-Y, GATA-1 and TFII-I sequence-specific DNA-binding factors were recorded. Only in the case of rs11084490 in AURKC there were no changes in transcription factor binding site motif (XBP-1), if different alleles, either C or G, were present. The G allele of rs42873 in PLK1 was linked with an additional recognition motif for c-Jun transcription factor.

**Discussion**

In this study, SNPs rs2273535, rs1047972, rs911160 and rs8173 (AURKA), rs2241909 and rs2289590 (AURKB), rs758099 and rs11084490 (AURKC), and rs42873 (PLK1) mitotic kinases were screened for associations with the genetic susceptibility to gastric cancer (GC) in Bosnian population. We also examined genotype effects of the investigated polymorphisms for each GC subtype.

In our study, a significant association between AURKA rs1047972 CT genotype with the overall GC susceptibility was found. Similarly, in stratified analysis established on Lauren’s classification [18], this genotype has affected intestinal GC subtype, whereas association was lost in patients with diffuse type GC. Furthermore, for rs911160 in AURKA, analysis showed that its CC genotype showed effect on increased disease risk. Our results also revealed that AURKA rs8173 GG genotype could be associated with a decreased GC risk. In stratified analysis of GC types, the association was significant in patients with the diffuse type GC. These findings could underlie different epidemiological and clinical patterns observed in intestinal and diffuse subtypes [19].

Bioinformatic analysis of transcription binding sites revealed that in the case of rs911160 C allele, an extra NF-Y, NFI-CTF and NF-1 transcription factors were detected in comparison with G allele. NF-Y regulates some of the genes enrolled in regulation of cell cycle, which are also deregulated in certain human diseases [20]. NF-1 family of sequence-specific TFs affect the rate of transcription, either through repression or activation [21]. NFI-CTF corresponds to the protein family involved in transcription activation, which is guided by the RNA polymerase II [22]. Single nucleotide polymorphisms in TFBSs, can alter gene expression through linkage of different TFs, by removing existing or creating new binding motifs [23]. Also, it has been demonstrated that introns, particularly long ones, harboring more functional cis-acting elements, could accommodate sites for binding several TFs, and consequently regulate transcription [24]. Thus, our results suggest that rs911160 alleles in TFBS regions could bind various transcription factors which might affect the rate of AURKA expression, resulting in distinctions in exposure to the risk of GC development. In our previous study conducted in Slovenian population, we reported AURKA rs911160 association with an increased GC risk [25], and our findings from this study are supportive to these findings. Polymorphisms in 3′ untranslated regions (3′UTRs) of genes might affect mRNA stability, translation and overall level of post-transcriptional expression through effects on polyadenylation and/or changing binding sites for regulatory proteins as well as
for microRNAs (miRNAs) [26]. Recent study has demonstrated that 3'UTR variant in high mobility group box-1 (HMGB1) gene have a protective effect on overall survival in GC patients through decreased HMGB1 mRNA expression levels [27]. Thus, it is reasonable to believe that protective effect of GG genotype of SNP rs8173 in AURKA 3'UTR, evaluated in our study, could be associated with an aberrant AURKA expression.

AURKA confers major contribution to the processes, such as centrosome duplication, entry into mitosis and in spindle assembly checkpoint [7]. Several studies have suggested that AURKA overexpression leads to malignant transformation [28]. A number of polymorphisms in the AURKA have also been reported to exhibit an effect on the risk of cancer onset. Genetic variant rs2273535 was associated with colorectal and lung cancer [29, 30]. In our study no significant association was observed between rs2273535 (AURKA) and GC risk. Polymorphism rs1047972, one of the most investigated variants in AURKA gene, showed significant association with the increased esophagus cancer risk as well as with gastric cancer risk and progression [31–33]. Our results from the present study confirm these previous findings. SNP rs1047972 might increase relative kinase activity of AURKA [31]. AURKA is involved in phosphorylation of p53, which is followed by MDM2 induced degradation of p53, or resulting in silencing of the p53 transcriptional function [34]. The absence of p53 can result in mitotic checkpoint dysfunction and subsequent chromosomal instability [34]. Moreover, by suppressing p53 and p73 pro-apoptotic functions, AURKA enables a mechanism for cancer cells to evade apoptosis [35]. Thus, it could be expected that slightly higher kinase activity could be involved in cancer development as well as cancer cell survival. In AURKA gene, rs1047972 and rs2273535 variants are located in exon 3 with high LD amongst them, suggesting that phenotypic effects of both polymorphisms could be consequence of a synergistic act. In addition, it was suggested that rs10479792 could possess a noticeable role in carcinogenesis by alteration of rs2273535 secondary structure and/or function [36]. Our findings, regarding evaluated genetic variants in AURKA gene, suggest that rs1047972 and rs911160 polymorphisms could act as factors which contribute to GC susceptibility, whereas rs8173 variant might be protective factor for GC development.

Aurora kinase B (AURKB) is a subunit of chromosomal passenger complex (CPC), involved in the segregation of chromatids, cytokinesis and modification of histones [37] and has been overexpressed in different types of cancers encompassing prostate, thyroid and brain [38]. It has been proposed that AURKB overexpression causes defects in chromosome segregation, aneuploidy and tumor development [39]. We examined rs2241909 SNP in AURKB and found a significant association between (AG/GG) genotypes and increased susceptibility to GC. In addition to this, in analyses of genetic models, AG genotype demonstrated an effect on a higher risk of intestinal type GC growth. In an earlier study, rs2241909 showed association with familial breast cancer risk [40]. The rs2241909 variant is a silent variant positioned on C terminal end of aurora kinase B. This amino acid change does not abolish or create splice site, nor affects exonic splicing enhancers/silencers motifs, and it has also been demonstrated that it does not change AURKB mRNA secondary structure [40]. Therefore, the observed risk between GC risk and rs2241909 could be due to its linkage with another unidentified functional genetic variant. The analysis of the second polymorphism in AURKB, rs2289590, demonstrated that CC genotype was associated with higher risk of GC onset. In stratified analysis of GC types, both CC and AC genotypes had an effect on diffuse type GC risk, whereas AG genotype demonstrated an effect on a higher risk of intestinal type GC growth. In an earlier study, rs2289590 revealed binding of additional YY1 transcription factor, if C allele was present.

The YY1 TF is associated with a cell cycle progression and it has been demonstrated that YY1 expression is with uncontrolled cell proliferation, apoptosis resistance and metastasis, thus acting as an initiator of carcinogenesis [41]. Transcription factors (TFs) are important gene regulators with specific roles in cell cycle, thus when improperly regulated, they contribute to the failure in

| Table 6 | In silico analysis of the studied polymorphisms |
|---------|-----------------------------------------------|
| Variant | rs911160 | rs2289590 | rs758099 | rs11084490 | rs42873 |
| Gene    | AURKA   | AURKB    | AURKC   | AURKB     | PLK1    |
| Alleles |         |          |         |           |         |
| G       | C/EBPalpha | C/EBPalpha | C/EBPbeta | GR-beta |            |
| C       | C/EBPalpha | C/EBPbeta | GR-beta |            |            |
| A       | NF-Y     | NF-1     | NFI-CTF |            |            |
| PE3     | PE3      | TFII-I   | YY1     |            |            |
| T       | NF-1     | TFII-I   | ENKTF-1 |            |            |
| C       | XBP-1    | XBP-1    | GATA-1  |            |            |
| G       | GR-alpha | AP-2alphaA | T3R-beta1 |            |            |
| C       | AP-2alphaA | T3R-beta1 |            |            |            |

Different transcription factor binding motifs recognized for polymorphic alleles of studied polymorphisms are highlighted in bold characters

*Binding sites for transcription factors identified by use of PROMO software (ALGGEN web-server)
proper cellular functioning, instability and malignant transformation [41, 42]. SNPs in regulatory regions can moderate expression of genes through potential disruption of sequence specific DNA-binding motifs, which consequently alters the binding of the appropriate TFs [43]. Our data for intronic rs2289590 in \textit{AURKB} suggest that additional binding of the YY1 sequence-specific DNA-binding factor, when C allele is present within TF binding site, could modify \textit{AURKB} expression level, which might result in higher susceptibility to gastric cancer occurrence. Important roles of introns in regulation of transcription have been reported in cell cycle and apoptosis genes, highlighting the significance of intronic genetic variants in tumorigenesis [32]. More importantly, our findings from this study for rs2289590 (\textit{AURKB}) association with an increased GC risk, are in accordance with the findings from our previous study conducted in Slovenian population [25].

Aurora kinase C (\textit{AURKC}) represents a catalytic chromosomal passenger protein, similarly as Aurora kinase B, which plays essential role mitotic events, segregation and centrosome function throughout meiosis [8, 44]. \textit{AURKC} overexpression has been described in malignant thyroid cell lines and tissues [45]. It has been shown that overexpression of AURKC induces centrosome amplification, multinucleation and that its abnormal expression in somatic cells has an oncogenic potential [46]. We examined rs11084490 in \textit{AURKC} and its potential relationship with gastric cancer risk. A link between CG and GG genotypes and increased gastric cancer risk was observed. Stratified analyses revealed that these genotypes were more common in patients with intestinal type of GC. Polymorphism rs11084490 is situated within the 5′UTR region of \textit{AURKC}. Eukaryotic 5′UTR various elements and structures e.g. hairpins, RNA G-quadruplexes (RG4s), Kozak sequences around the initiation codons, upstream open reading frames (uORFs) and start codons AUGs, internal ribosome entry sites (IRESs) and iron responsive elements (IREs) greatly influence mRNA translation [47]. It has been demonstrated that 5′ uORF-altering polymorphisms and mutations significantly silence expression of the downstream protein [48]. Additionally, genetic variations such as mutations and SNPs, by disrupting motifs within 5′UTR, are capable of causing damaging effects on human health, and could be associated with diseases such as multiple myeloma, esophageal cancer and many others [49]. Therefore, observed association of the rs11084490 (\textit{AURKC}) polymorphism with the increased GC risk in our study could be due to altered \textit{AURKC} translation mediated by risk genotypes affecting the above mentioned functional motifs in \textit{AURKC} 5′UTR. Our results demonstrated that rs758099 (\textit{AURKC}) polymorphism exhibited no effect on GC susceptibility.

As reported above, the results of our study demonstrated involvement of the rs1047972 (\textit{AURKA}), rs911160 (\textit{AURKA}), rs2241909 (\textit{AURKB}), rs2289590 (\textit{AURKB}) and rs11084490 (\textit{AURKC}) polymorphisms in gastric tumorigenesis. However, considering different genes included in chromosome segregation process, it is difficult to explain the association of gastric cancer development with an individual polymorphism. Therefore, a combined analysis spanning various gene polymorphisms enables the assessment of gene-gene interactions, and consequently determination of genetic profiles associated with a risk of GC.

In this study, combined analysis of the five polymorphisms and their risk genotypes associated with an increased susceptibility to gastric cancer, rs1047972(CT)/rs911160(CC)/rs2241909(AG + GG)/rs2289590(AC + CC)/rs11084490(CG + GG) revealed polymorphism profiles where all the combinations (two to five combined risk genotypes) influence the higher risk of GC, with an OR increased 1.51-fold for the rs1047972(CT)/rs11084490(CG + GG) to 2.29-fold for the rs1047972(CT)/rs911160(CC) combinations. It is also important to highlight that five-polymorphisms combination rs1047972(CT)/rs911160 (CC)/rs2241909(AG + GG)/rs2289590(AC + CC)/rs11084490 (CG + GG) showed significant effect on an increased GC risk (OR = 1.83 95%CI = 1.46–2.29, \(P < 0.001\)).

Several studies have conducted combined analysis of polymorphisms in gastric cancer. In one of them, it has been demonstrated that the risk of noncardia gastric cancer increased 27.3-fold with increasing number of proinflammatory genotypes for three or four polymorphisms [50]. Similarly, another study revealed that combination of polymorphisms in genes involved in the inflammatory process could affect the increased risk of gastric cancer [51]. These findings may be explained by an additive effect of the polymorphisms in inflammatory genes. Therefore, based on these results, we could assume that particular combinations of genetic variants in aurora kinases A, B and C, could act synergistically, in mediating aberrations in the process of chromosome segregation, leading to aneuploidy and consequently to gastric cancer development.

Polo-like kinase 1 (\textit{PLK1}) is essential for cell division and it has been demonstrated that PLK1 with other signal proteins is responsible for mitotic progression and has also been linked to cellular proliferation [52]. Moreover, it has been demonstrated that polymorphisms in \textit{PLK1} influence its expression, therefore they could potentially affect cancer risk and progression [53]. We selected rs42873 (\textit{PLK1}) polymorphism for the assessment of its possible effect on an increased gastric cancer risk, however, our results showed no significant association between rs42873 genetic variant and GC risk.
Conclusions
The results of this study revealed that **AURKA** (rs1047972 and rs911160), **AURKB** (rs2241909 and rs2289590) and **AURKC** (rs11084490) polymorphisms could affect the risk of gastric cancer, both individually and synergistically. Contrary, we found that **AURKA** (rs8173) polymorphism appeared to be associated with decreased GC risk. Collectively, these findings indicated the existence of the plausible roles of genetic variations in **AURKA**, **AURKB** and **AURKC** in stomach carcinogenesis. Our results could be beneficial in the further investigations of the functional impact of these polymorphisms. The present study is based on a reduced number of cases which represents its limitation, therefore it is important that larger prospective studies confirm our findings.

Abbreviations
AIC: Akaike information criterion; ALGGEN: Algorithmics and genetics group; ALL: All phase 3 individuals; AP-2 alpha A: Activating enhancer binding Protein 2 alpha; AURKA: Aurora kinase A; AURKB: Aurora kinase B; AURKC: Aurora kinase C; C/EBP alpha: CCAAT/enhancer-binding protein alpha; C/EBP beta: CCAAT/enhancer-binding protein beta; CELU: United residents with Northern and Western European ancestry population; CI: Confidence interval; c-Jun: Transcription factor c-Jun; CPC: Chromosomal passenger complex; CTF: CCAAT box-binding transcription factor; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; ENKTF-1: Enkhephalin transcription factor 1; EUR: European population; FFPE: Formalin fixed paraffin-embedded; GATA-1: GATA binding factor 1; GC: Gastric cancer; GR-alpha: Glucocorticoid receptor alpha; GR-beta: Glucocorticoid receptor beta; HMGB1: High mobility group box-1; HWE: Hardy-Weinberg equilibrium; ID: Identifier; IRES: Internal ribosome entry site; LD: Linkage disequilibrium; LOD: Logarithm of odds; MAF: Minor allele frequency; MDM2: Mouse double minute 2 homolog; miRNA: microRNA; mRNA: messenger RNA; NF-1: Nuclear factor 1; NFI-CTF: Nuclear factor I-CCAAT-binding transcription factor; NF-Y: Nuclear transcription factor Y; OR: Odds ratio; p53: Tumor protein p53; p73: Tumor protein p73; PAST: Paleontological statistics; PCR: Polymerase chain reaction; POB: Protein Data Bank; PEA3: Polymya enhancer activator 3; PLK7: Polo-like kinase 1; POU2F1: POU domain, class 2, transcription factor 1; POU domain class 2, transcription factor; POU2F1: POU domain, class 2, transcription factor; RCBs: Research Collaboratory for Structural Bioinformatics; RGSs: RNA G-quadruplexes; RNA: Ribonucleic acid; S3C: Second; SNP: Single nucleotide polymorphism; SPSS: Statistical package for social sciences; T3R-beta1: Thyroid hormone receptor beta 1; tagSNP: Tagging single nucleotide polymorphism; TF: Transcription factor; TFBS: Transcription factor binding site; TFII-I: General transcription factor II-I; uORF: Upstream open reading frame; UTR: Untranslated region; XBP-1: X-box binding protein 1; YY1: Yin yang 1 transcription factor

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Authors’ contributions
PH and RK designed this study. AM and MR conducted the experiments and analyzed the data. MB and IE recruited patients and provided the samples. AM and PH prepared manuscript draft and draft figures and tables. All authors gave final approval for manuscript submission.

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Availability of data and materials
The data used in this study contain personal information and are not publicly available, but can be requested from the Clinical Pathology and Cytology at the University Clinical Centre Sarajevo, subject to ethical approvals.

Ethics approval and consent to participate
Ethical approval for this study and for the use of patient’s personal and medical data from the Clinical Pathology and Cytology at the University Clinical Center Sarajevo was issued by the Ethical Committee at the University Clinical Centre Sarajevo (No. 0302–36765). The data were analyzed anonymously. Written informed consents from all the patients for using their samples for the purposes of this study were obtained prior to surgery. The requirement to obtain the consents from the control individuals had been waived due to the following reasons: the control participants were healthy blood donors, randomly selected upon regular medical examinations whereby only the remains of their samples were used for the purposes of the present study, no additional risks to the subjects existed, no individually identifiable as well as medical or any other sensitive information were used for this study, the study would not adversely affect the rights and welfare of the subjects, and the results of the study would have no effect on the subjects.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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