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# Investigating the Ku70 (XRCC6) gene polymorphism in patients with gastric cancer

## Mide kanseri olan hastalarda Ku70 gen polimorfizminin araştırılması

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**Ku70 Gene Polymorphisms and Gastric Cancer Susceptibility**
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**BACKGROUND**
The DNA repair gene Ku70, a key component of the non-homologous end-joining repair pathway, plays a crucial role in the repair of DNA double strand breaks (DSBs). Deficiencies in DSB repair may result in permanent genomic instability. However, the association between polymorphic variations of Ku70 and susceptibility to gastric cancer remains unclear.

**OBJECTIVE**
This study aims to investigate the potential correlation between the Ku70 promoter G-57C (rs2267437) and intron 3 (rs132774) polymorphisms and the risk of developing gastric cancer in the Turkish population.

**METHOD**
A hospital-based case-control study was conducted, including 92 patients diagnosed with gastric cancer and 194 age- and gender-matched healthy controls. Genotyping of Ku70 promoter G-57C (rs2267437) and intron 3 (rs132774) polymorphisms was performed using real-time PCR at Dokuz Eylül University Hospital, Izmir.

**RESULTS**
No significant difference was observed in the distribution of genotype frequencies for either polymorphism between the gastric cancer patients and the control group. The Chi-Square test revealed no significant difference in the frequencies of the G-57C (rs2267437) polymorphism between the cancer group and the control group. The CC genotype was absent in the cancer group, while it was present in one in the control group. The GG genotype of intron 3 (rs132774) polymorphism was also absent in both groups. The Chi-Square test revealed no significant difference between the two groups.

**CONCLUSION**
The presence of the Ku70 promoter G-57C (rs2267437) and intron 3 (rs132774) polymorphisms does not appear to increase the risk of gastric cancer in the Turkish population.

**KEY WORDS**
Case-control study, DNA repair, gastric cancer, Ku70 gene, polymorphisms

**ÖZ**
Homolog olmayan uç birleştirme onarım yolunun önemli bir bileşeni olan DNA onarım geni Ku70, DNA çift sarmal kırıklarının (DSB'ler) onarımında önemli bir rol oynar. DSB onarımındaki eksiklikler kalıcı genomik instabiliteye neden olabilir. Ancak, Ku70'in polimorfik varyasyonları ile mide kanserine yatkınlık arasındaki ilişki hala belirsizliğini korumaktadır.

**AMAÇ**
Bu çalışma, Ku70 promotörü G-57C (rs2267437) ve intron 3 (rs132774) polimorfizmleri ile Türk popülasyonunda mide kanseri geliştirme riski arasındaki potansiyel korelasyonu araştırmayı amaçlamaktadır.

**YÖNTEM**
Mide kanseri tanısı konmuş 92 hasta ve yaş ve cinsiyete göre eşleştirilmiş 194 sağlıklı kontrol içeren hastane tabanlı bir vaka kontrol çalışması yürütülmüştür. Ku70 promoter G-57C (rs2267437) ve intron 3 (rs132774) polimorfizmlerinin genotiplendirilmesi, Dokuz Eylül Üniversitesi Hastanesi, İzmir'de gerçek zamanlı PCR kullanılarak gerçekleştirildi.

**SONUÇ**
Mide kanseri hastaları ve kontrol grubu arasında her iki polimorfizm için genotip frekanslarının dağılımında anlamlı bir fark gözlenmedi. Ku70 promoter G-57C (rs2267437) ve intron 3 (rs132774) polimorfizmlerinin varlığı, Türk popülasyonunda mide kanseri riskini artırmıyor gibi görünüyor.

**ANAHTAR KELİMELER**
Case-control study, DNA repair, gastric cancer, Ku70 geni, polymorphisms

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Gastric cancer, often known as GC, is the fourth most prevalent form of cancer globally, impacting around 900,000 individuals each year. The precise etiology is uncertain, however, Helicobacter pylori bacteria are implicated. Cancer genetics primarily concerns itself with the study of DNA repair mechanisms and the genes responsible for safeguarding the genome from both internal and external factors. The malfunctioning of these systems results in the development of cancer and aging, since mutations and deficiencies in DNA repair mechanisms contribute to the formation and progression of tumors. Recent research has shown a connection between genetic variants in the processes responsible for repairing DNA and the occurrence of stomach cancer (1, 2, 44).

Multiple environmental and genetic factors contribute to the development of stomach cancer, such as consuming excessive amounts of salt and smoked food, having low stomach acid levels (hypochlorhydria), being exposed to filthy environments, lacking sufficient vitamin C, being infected with Helicobacter pylori, having blood type A, experiencing atrophic gastritis, having gastric polyps, or undergoing stomach resection (3). The association between genetic and molecular alterations in GC cells and gastric cancer has been established since the 1990s. Chromosomes 3, 6, 8, trisomy, 11 (aberration in 11p13-p15), and marker chromosomes have both numerical and structural abnormalities in GC cells (6-13).

Disrupted DNA functioning may result in genetic and epigenetic alterations, ultimately leading to apoptosis and mortality. Cellular DNA repair mechanisms have the potential to restore the integrity of DNA. However, if DNA damage remains unrepaired throughout the process of replication, it may lead to mutations and instability in the genome. Genomic instability is a characteristic feature of both cancer and the aging process (14).

Conducting clinical research is necessary to determine the optimal treatment strategy based on genetic and epigenetic alterations. Analyzing the many combinations that might potentially indicate tumor development and the likelihood of developing cancer; will aid in prioritizing research on cancer drugs and enhancing our comprehension of the environmental elements involved in cancer prevention (13-15).

DNA repair genes have a role in signal transduction and the control of DNA repair, including mismatch repair, base excision repair, and nucleotide excision repair. Uninterrupted monitoring is essential to remove and substitute impaired nucleotides during replication in order to avoid mutations. Several DNA repair techniques including direct repair or reversal of damage, excision repair, repair by recombination, SOS repair, and DNA double-strand break repair (15, 16).

DNA double-strand breaks (DSBs) are very hazardous types of DNA damage that pose a significant risk to the integrity of the genome. They may lead to mutations, neoplastic transformation, or cell death. Cells use many strategies to deal with double-strand breaks (DSBs), such as activating checkpoints, repairing DNA, and modifying gene transcription. The DNA damage checkpoint encompasses sensor, transducer, and effector proteins that identify and react to DNA damage (2, 13-16).

The KU70 (XRCC6) (NM_001469.5) gene generates a heterodimer that binds to DNA ends that have been damaged and engages DNA-dependent protein kinase (DNA-PK) as the first sensor in non-homologous end joining (NHEJ) repair. The Non-Homologous End Joining (NHEJ) mechanism is less intricate but prone to errors, as it may connect DNA ends without the need for homologous sequences. On the other hand, Homologous Recombination (HR) is a more intricate and error-free process, as it utilizes homologous sequences to carry out DNA repair (2, 17-20).

Genetic variables that increase the likelihood of developing cancer include hereditary genetic mutations, differences in the activity of enzymes involved for processing cancer-causing substances, and changes in DNA repair pathways caused by genetic variances. Errors during DNA replication give rise to polymorphisms, which make up 0.1% of the genetic variety in humans. Among these polymorphisms, single nucleotide polymorphisms (SNPs) account for 90% of the overall genetic variance (21-36).

Gaining knowledge about the genetic foundation of gastric cancer and the processes involved in DNA repair, specifically the role of the Ku protein, is essential for the advancement of focused treatments and the enhancement of patient results. The presence of genetic variations and the effectiveness of DNA repair mechanisms have a substantial impact on the likelihood and advancement of cancer (37-38).

## Materials and Methods

We used archival DNAs extracted from peripheral blood or normal tissue samples collected from 92 patients, who were diagnosed with stomach cancer between 2006 and 2008 at Dokuz Eylül University Hospital General Surgery Service. A control group consisting of 194 healthy persons, matched in terms of age and gender with the sick group, was established. The participants in the control group provided their assent using a voluntary consent form.

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### Extraction of DNA from Peripheral Blood

In our retrospective analysis, patient DNA samples were accessible from our laboratory's DNA repository and were collected using the salt precipitation technique (36).

Following the completion of our research design, blood samples were collected from participants who did not have a family history of cancer, were in good health, and were similar to the patient group in terms of gender and age. This was done by having them fill out a permission form and providing their signatures.

DNA was extracted from 4cc of peripheral blood collected from the control group using a DNA extraction kit. The Bioneer DNA extraction kit - AccuPrep®Genomic DNA Extraction Kit contains the following components: Proteinase K (25 mg, lyophilized), Binding buffer (25 ml), Washing buffer 1 (40 ml), Washing buffer 2 (20 ml), Elution buffer (30 ml), Binding tubes, Filtration tubes (2ml), Elution tubes (1.5 ml), and Supplementary Materials List. The DNA extraction process involves the use of pure ethanol, pure isopropanol, a table-top microcentrifuge, an incubator, a vortex mixer, Eppendorf tubes (1.5 ml), and PBS (Phosphate buffer saline).

Following the technique, an initial volume of 20 µl of Proteinase K was poured into a 1.5 ml Eppendorf tube. Subsequently, 200 µl of peripheral blood was added to the Eppendorf tube holding the Proteinase K. 200 µl of binding buffer, also known as binding solution, was introduced to the Eppendorf tube that contained proteinase K and blood. The solution in the Eppendorf tube was subjected to incubation in the incubator at a temperature of 60 degrees Celsius for a minimum duration of 10 minutes. A volume of 100 µl of isopropanol was added to the tube retrieved from the incubator and pipetted. The solution in the Eppendorf tube was transferred to 2 ml connecting tubes. The sample was subjected to centrifugation in a microcentrifuge equipment at a speed of 8000 revolutions per minute for a duration of 1 minute. The connecting tube was replaced with a new tube by removing the liquid that had gathered at the bottom. Next, 500 µl of washing solution was introduced and subjected to centrifugation at a speed of 8000 revolutions per minute for a duration of 1 minute. The liquid that collected at the bottom was removed. Then, 500 µl of washing solution number 2 was added and the mixture was centrifuged at a speed of 8000 rpm for a duration of 1 minute. A second round of centrifugation was performed at a speed of 12,000 revolutions per minute for a duration of 1 minute in order to fully eliminate the ethanol present in the tube. The coupling tube was moved to 1.5 ml Eppendorf tubes, and 200 µl of elution buffer was introduced. The sample was subjected to centrifugation in a microcentrifuge equipment at a speed of 8000 revolutions per minute for a duration of 1 minute. The DNA that was transferred to the Eppendorf tube is now prepared for either storage or further analysis. The DNAs collected were quantified using the Nano Drop DNA spectrophotometer. The DNAs that were recovered were diluted with distilled water to a concentration of 10 ng in a volume of 11.25 µl. Once the DNAs were acquired, they were preserved at a temperature of +4°C if they were intended for immediate examination, and at a temperature of -20°C if they were intended for future study.

### Genotyping of target sequences by RealTime-PCR

The allelic discrimination approach was used using the ABI 7300 Real Time PCR apparatus from Applied Biosystems. The TaqMan Universal PCR Master Mix Without AmpErase UNG from Invitrogen was utilised, along with the 40 x SNP Genotyping Assay from Invitrogen. The reagents used included Ku70... promoter G-57C (rs2267437) and Ku70... intron3 (rs132774). In accordance with the procedure, the mixture was made at the prescribed rate shown in the table and thereafter dispersed to the wells. A volume of 5 µl of DNA was added to each sample. (Table 1).

(Archive EnsEMBL release 54 - May 2009 © WTSI / EBI))

**Table 1.** Study protocol

| Added material | Quantity |
|---|---|
| TaqMan Univ. PCR Master Mix | 12.50 µl |
| 20 x SNP Genotyping Assay | 1.25 µl |
| DNA | 11.25 µl |

### Primer Sequences Used to Amplify Ku70 Promotor Regions

**Context Sequence [VIC/FAM]; rs 132774:**
F:5'TTTTTGTTAAAATTTGATATTTATG[C/G]CCATTACTT TCACTGATTCATTACC-3'

**Context Sequence [VIC/FAM]; rs 2267437:**

R:5'GCCCAAGTCTCCCCACCTCGGCCAG[C/G]CGCCACC CTCTGGCCTGGCTCCCGC-3'

### Real Time PCR Conditions

(Table 2).

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**Table 2.** PCR steps

| PCR steps | Temperature (°C) | Duration | |
|---|---|---|---|
| Denaturation | 96 | 10 min | |
| Annealing | 92 | 15 sec | 40 loops |
| Elongation | 60 | 60 sec | |

The PCR technique, which was invented in 1985 by Henry A. Erlich, Kary Mullis, and Randall K. Saiki while working at the Cetus corporation in the United States, relies on the amplification of nucleic acids under controlled laboratory circumstances. Kary Mullis received the 1993 Nobel Prize in Chemistry for his contributions to the creation of PCR, a technique that has significant implications in scientific research and clinical laboratory diagnostics. Polymerase Chain Reaction (PCR) is a method of replicating DNA in a laboratory setting.

Real Time PCR is a technique that involves the integration of the traditional PCR technology with gene analysis. Fluorescently labelled probes and dyes are used in this amplification technique to enable visualisation and monitoring of PCR amplification. The fluorescence intensity is directly proportional to the amount of DNA produced.

The Real Time PCR TaqMan technology employs a probe that is labelled with fluorochrome compounds at both the 5' and 3' ends. The probe has a reporter fluorochrome, 6-carboxyfluorescein (6-FAM), at its 5' end, and a quencher fluorochrome, 6-carboxy-tetramethyl-rhodamine (TAMRA), at its 3' end. The probe attaches to the area between the binding sites of the primers on the single-stranded target molecule. The ongoing hybridization between the probe and the target molecule hinders the generation of a signal by the reporter fluorochrome material due to the presence of the suppressor fluorochrome at the 3rd end. Once the primers bind to the target nucleic acid and the primer extension begins, the Taq DNA polymerase enzyme starts to degrade the probe from its 5' end utilising its 5'→3' nuclease activity. This allows the synthesis to proceed. Therefore, the reporter fluorochrome is liberated and generates a signal. The signal strength correlates directly with the quantity of amplicon generated in each cycle (39).

Real-time PCR may provide quantitative findings rapidly. Given that the diagnostic is conducted without the need to physically access the tubes, the likelihood of contamination is minimal. Multiplication may get results without using electrophoresis. Furthermore, the presence of alterations in the specific nucleic acid may be identified by the use of fluorescent probes (39).

## Results

The DNA samples from both the patients and the control group were prepared and completed by using the real-time multiplex PCR technique. The assessment was conducted using the photos shown below. During the research, two variations in the genetic code were observed for each person, resulting in three distinct genotypes.

Due to allelic discrimination, individuals were categorised into three groups based on their CC/CG/GG genotypes (Figure 1).

The interpretation of the analytical findings for each example was based on the significant curves they produced. If a real-time polymerase chain reaction produces a single substantial green (VIC) curve, it is classified as the CC genotype, as shown in the example below (Figure 2).

The analysis results of the cases with heterozygous genotype were evaluated as FAM/VIC; that is, if two curves in dark blue and green colors were found together, it was evaluated as CG (Figure 3).

The study identified subjects withhomozygous GG genotype by seeing a single FAM-dark blue colour fluorescence and a single curve in the analysis (Figure 4).

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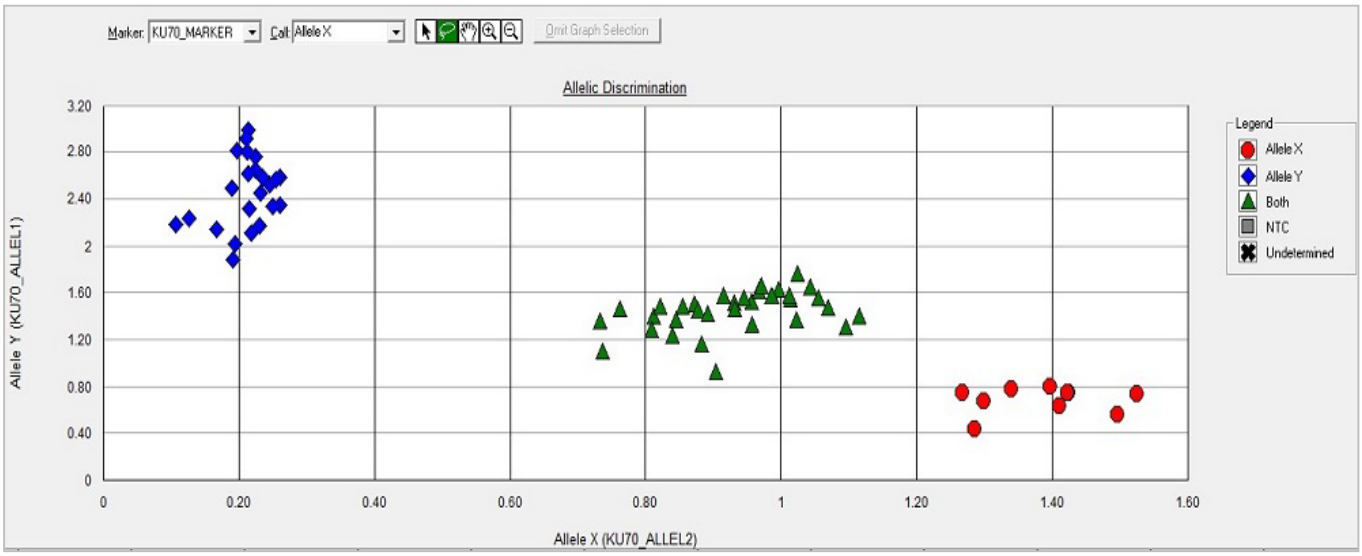


**Figure 1**. Results of allelic discrimination analysis


**Figure 2.** The melting RealTime PCR profiles showing CC Genotype (Arrow)

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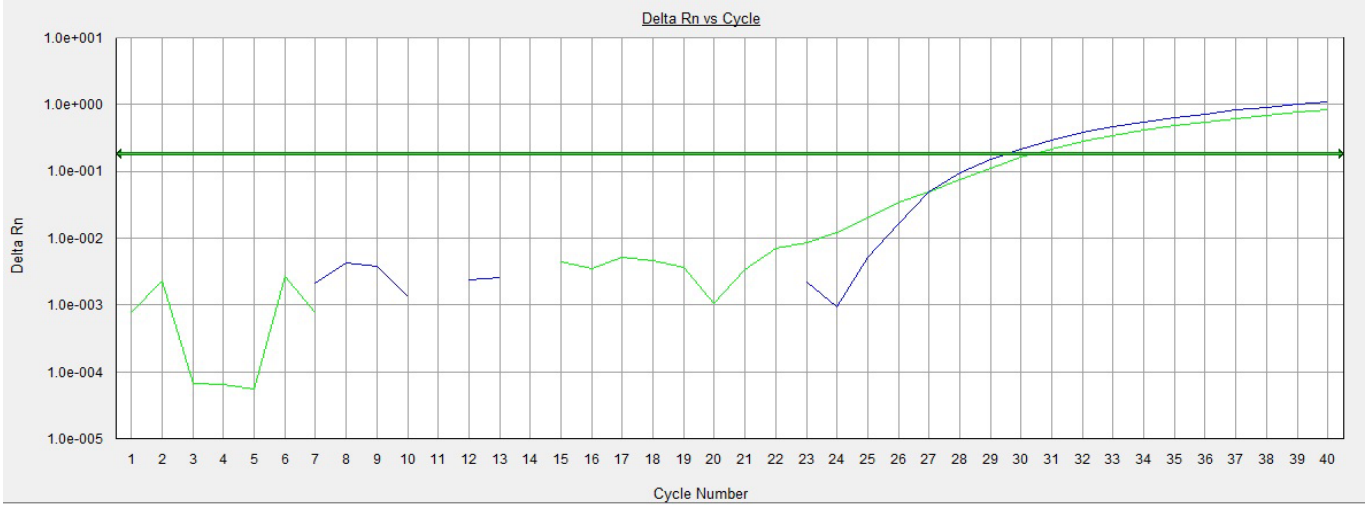


**Figure 3.** An example of a result with a heterozygous genotype

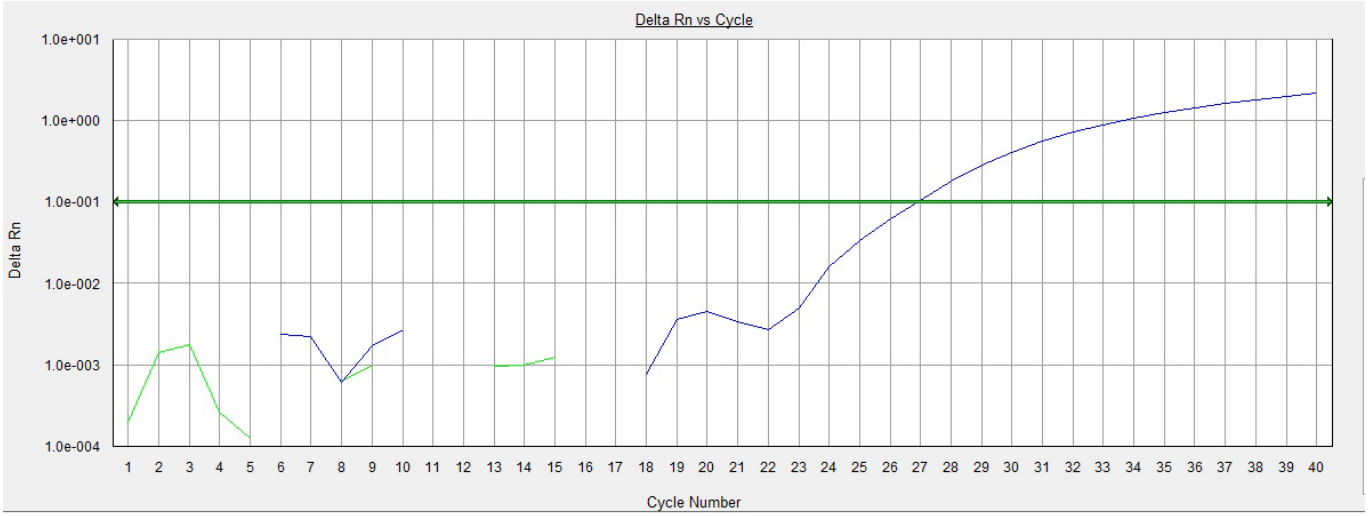


**Figure 4.** An example of a result in the GG genotype

The analytical findings of both the case and control groups were uploaded to the SPSS programme. The findings were assessed using the Chi-Square test. Each participant in the investigation was evaluated for three potential genotypes in relation to the intron 3 (rs132774) and G-57C (rs2267437) polymorphisms of the Ku70 gene. The demographic information of people was ascertained based on their age and gender. The data were compared and summarised using tables, which included numbers and percentages.

When comparing the cases and control groups, it was observed that the frequencies of the GG genotype of the G-57C (rs2267437) polymorphism were equal in both healthy individuals and cancer patients (Table 3).

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**Table 3.** Chi-Square Test for rs2267437 GG polymorphism

| rs2267437 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| GG ones | 15 (28.3) | 38 (71.7) | 53 (100) |
| Non-GG (Non-GG means others that are not in the GG genotype.) | 77 (33,0) | 156 (67.0) | 233 (100) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100) |
| Chi-Square p > 0.5 | | | |

When comparing the cases and control groups in relation to the rs2267437 polymorphism GC genotype, there was no significant difference found in terms of its frequency in both the control group and the cases (Table 4).

**Table 4.** Chi-Square Test for rs2267437 GC polymorphism

| rs2267437 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| GC ones | 77 (33.2) | 155 (66.8) | 232 (100) |
| Non-GC (Non-GC means others that are not in the GC genotype.) | 15 (27.8) | 39 (72.2) | 54 (100) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100) |
| Chi-Square p > 0.5 | | | |

Upon comparing the patients and control groups in relation to the G-57C (rs2267437) polymorphism and CC genotype, it was noted that the CC genotype was completely absent in the cases. However, it was seen in just one individual in the control group (Table 5).

**Table 5.** Chi-Square Test for rs2267437 CC polymorphism

| rs2267437 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| CC ones | 0 (0.00) | 1 (100) | 1 (100) |
| Non-CC (Non-CC means others that are not in the CC genotype.) | 92 (32.3) | 193 (67.7) | 285 (100) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100) |
| Chi-Square p > 0.5 | | | |

When comparing the patients and control groups in relation to the intron 3 (rs132774) polymorphism GG genotype, no statistically significant difference was seen between them (Table 6).

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**Table 6.** Chi-Square Test for rs132774 GG polymorphism

| rs132774 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| GG ones | 47 (30.5) | 107 (69.5) | 154 (100) |
| Non-GG (Non-GG means others that are not in the GG genotype.) | 45 (34.1) | 87 (65.9) | 132 (100) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100) |
| Chi-Square p > 0.5 | | | |

Upon comparing the cases and control groups, it was observed that the frequencies of rs132774 polymorphism GC genotype were comparable in both cancer patients and the control group (Table 7).

**Table 7.** Chi-Square Test for rs132774 GC polymorphism

| rs132774 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| GC ones | 37 (32.7) | 76 (67.3) | 113 (100.0) |
| Non-GC (Non-GC means others that are not in the GC genotype.) | 55 (31.8) | 118 (68.2) | 173 (100.0) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100.0) |
| Chi-Square p > 0.5 | | | |

The CC genotype for the intron 3 (rs132774) polymorphism, which was the focus of our study, was uncommon in both the patients and control group. Furthermore, the Chi-Square test revealed that there was no significant difference between the two groups, as shown in (Table 8).

**Table 8.** Chi-Square Test for rs132774 CC polymorphism

| rs132774 polymorphism | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| CC ones | 8 (42.1) | 11 (57.9) | 19 (100.0) |
| Non-CC(Non-CC means others that are not in the CC genotype.) | 84 (31.5) | 183 (68.5) | 267 (100.0) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100.0) |
| Chi-Square p > 0.5 | | | |

The research comprised a total of 92 patients with stomach cancer, including 63 men and 29 women. The control group, as shown in (Table 9), consisted of 134 men and 60 women.

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**Table 9.** Case and control groups and genders

| Gender | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| Male | 63 (68.5) | 134 (69.1) | 197 (100.0) |
| Female | 29 (31.5) | 60 (30.9) | 89 (100.0) |
| Total | 92 (32.2) | 194 (67.8) | 286 (100.0) |

Upon splitting the cases and controls into two age groups, namely those over and under the age of 55, it was seen that the occurrence of stomach cancer was much greater in persons over the age of 55 (Table 10).

**Table 10.** Age-based case distribution

| Over- and under-55s | Cases (%) | Controls (%) | Total (%) |
|---|---|---|---|
| 55years< | 18 (19.6) | 66 (34.0) | 84 (29.4) |
| 55years> | 74 (80.4) | 128 (66.0) | 202 (70.6) |
| Total | 92 (100.0) | 194 (100.0) | 286 (100.0) |
| Chi-Square $p < 0.5$ | | | |

Upon comparing the incidence rates of the genotypes associated with the studied SNPs between the cases and control group, using the GG genotypes as the baseline, we observed no significant variation in terms of stomach cancer risk across the genotypes (Table 11).

**Table 11.** Polymorphisms and gastric cancer risk; Adjusted OR: Adjusted ratio

| | Cases | Controls | Adjusted OR | p value |
|---|---|---|---|---|
| rs2267437 polymorphism | | | | |
| GG genotype | 15 | 38 | 1.00(ref) | 0.782 |
| GC genotype | 77 | 155 | 1.291 | 0.483 |
| CC genotype | 0 | 1 | 0.000 | 1.000 |
| rs132774 polymorphism | | | | |
| GG genotype | 47 | 107 | 1.00(ref) | 0.523 |
| GC genotype | 37 | 76 | 1.086 | 0.774 |
| CC genotype | 8 | 11 | 1.808 | 0.255 |

This research aimed to determine the potential correlation between gastric cancer and polymorphisms of the Ku70 gene. The Real Time PCR method was used to determine the alleles and genotypes of Ku70 rs132774 and rs2267437 polymorphisms. DNA samples were obtained from peripheral blood and normal tissues of 92 patients diagnosed with Stomach Cancer at Dokuz Eylül University Hospital's Department of General Surgery.

The research had a cohort of 29 female and 63 male participants, with an average age of 60.2 years. The Ku70 genotypes were analysed for allele frequencies and statistically analysed using the Chi-Square Test in the SPSS programme. The results are shown in (Figure 5).

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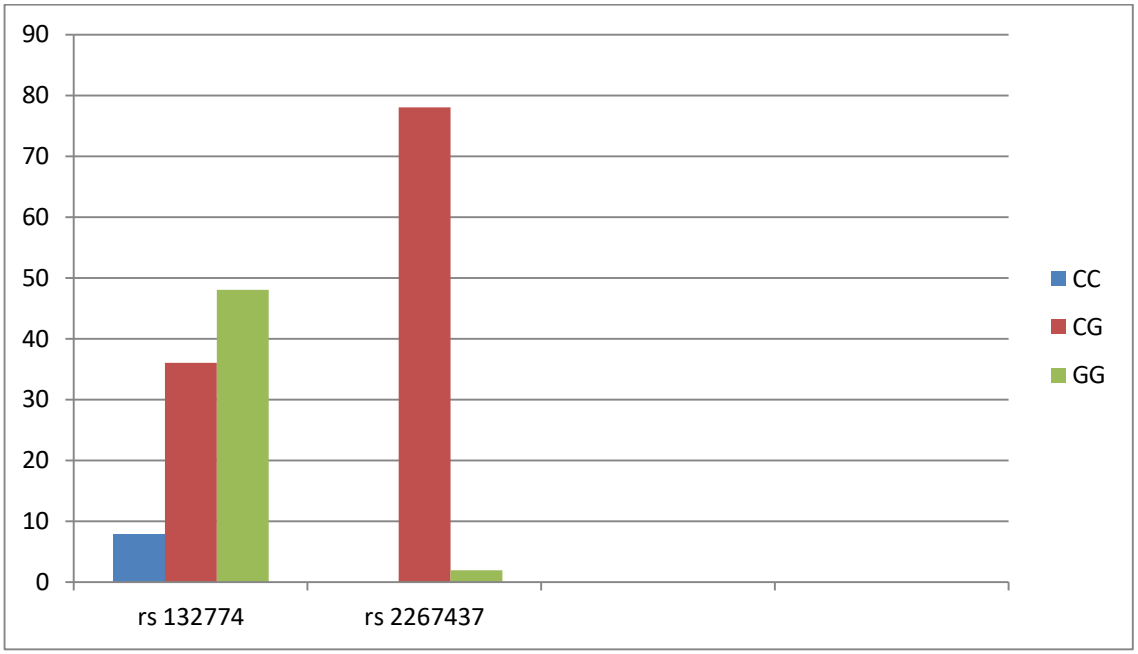


**Figure 5.** Genotype rates of cases

A control group consisting of 194 cancer-free people, matched with the patient group in terms of age and gender, was included in the research. The compatibility of the allele ratios in the control group with those in the sick group was assessed and documented in (Figure 6).

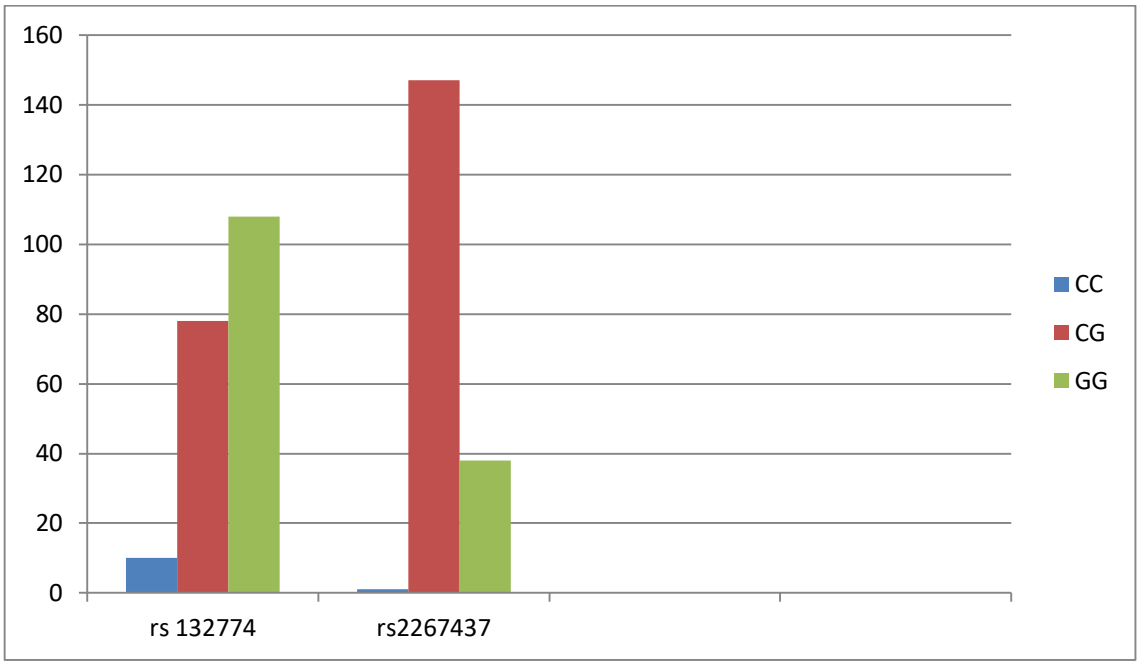


**Figure 6.** Polymorphism results of the control group

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## Discussion

Gastric cancer is a complex illness influenced by several variables, including both genetic and environmental influences. Despite a decline in the occurrence and fatality rates of stomach cancer over the last ten years, it remains the fourth most prevalent form of cancer and the second leading cause of death globally.

The prevalence of gastric cancer differs between males and females. According to the research, the occurrence of stomach tumours is twice as prevalent in males (1, 40). No reports have been made about the impact of gender differences on survival rates in stomach cancer. The Male/Female ratio reveals a notable surplus in favour of males, with a ratio of 1.87. The median age at which stomach cancer is often diagnosed is 56. The average age in our study group is similar when compared to the foreign sources. Although it is most commonly seen in the 70s in Western societies, the average age in our country is ten years younger. A research conducted by the Turkish oncology group in 2008 examined 840 patients and found that the average age at which stomach cancer was detected was 57 years, with the age range spanning from 19 to 85 years. This information was reported by 140 individuals. The research group included 92 patients, with an average age of 60.2 years (ranging from 33 to 89 years old). The disparity may be attributed to the limited patient population. DNA repair is a crucial concern in the field of cancer genetics. Polymorphisms in DNA repair genes result in alterations in the amino acid sequence and impair the ability to repair DNA, leading to the development of different types of malignancies. Multiple mechanisms exist for repairing DNA damage, including the involvement of various proteins (37, 38, 40).

Genetic variations in the DNA repair system may result in variations in the ability to repair DNA and can, to some extent, alter the susceptibility of cells to substances that damage DNA, leading to their transformation into cancer cells (38). According to previous reports, DNA repair systems are responsible for fixing DNA damage produced by substances that cause cancer and drugs used to treat cancer. Insufficient or defective DNA repair mechanisms increase the chance of developing different types of cancer. So far, several variations have been discovered in genes that play a role in DNA repair (2, 40). Despite several investigations, the precise correlation between polymorphisms of DNA repair genes and the risk of stomach cancer remains inconclusive (1-2). The Ku protein investigated in our work has a crucial function in the repair of DNA double-strand breaks in mammalian cells.

In a research conducted by Bau et al. in 2008, 380 patients who had been diagnosed with oral cancer between 1998 and 2007 were examined. They had also included an equal number of healthy controls who were matched to the patient group in terms of age and gender. The analysis revealed the presence of Ku70 T-991C, C-57G, and A-31G polymorphisms. They examined the correlation between oral cancer and these polymorphisms. Research has shown that those with the C allele (T/C and C/C) had a 2.14-fold increased risk of developing oral cancer compared to those with the T/T (wild) genotype. The study found that there was no significant difference in the link between the frequency of the C-57G and A-31G polymorphisms with the occurrence of oral cancer in both the patients and control groups (1-3).

He and his colleagues conducted a research to examine the correlation between the Ku70 -1310C/G polymorphism and breast cancer. The research conducted on 293 breast cancer patients and 301 control groups found that those with CG and GG allele frequencies had a significantly increased risk compared to those with the CC homozygous allele ($p = 0.038$).

In a study conducted by Willems et al. (2009), the researchers examined the connection between non-familial breast cancer and a specific genetic variation called C-1310G single nucleotide polymorphism. They focused on the Ku70 promoter region. The study group consisted of 206 patients and 171 control individuals. The results revealed a significant increase in the CG allele in the breast cancer patient group with elevated levels of endogenous oestrogen. The Ku70 gene polymorphisms indicated are potential subjects for further research to better understand the processes behind cancer formation (1-5, 41).

Yang et al. (2011) conducted a study in Taiwan to investigate four variations of the Ku70 gene in both gastric cancer patients and healthy control groups. The research comprised 136 individuals with stomach cancer and 560 individuals in the control group. Research has shown that those with TC and CC genotypes had a much higher susceptibility to stomach cancer in comparison to those with TT genotypes. The study indicated that the Ku70 promoter T-991C (rs5751129) polymorphism was significant in male patients over 55 years of age. It was proposed that this polymorphism might serve as a possible biomarker in gastric cancer patients.

We conducted a research to investigate the correlation between gastric cancer and variations in the Ku70 gene. Our nation has not yet completed a research on Ku70 polymorphisms for stomach cancer (2). Fu et al. (2003) discovered an association between breast cancer and the Ku70 promoter G-57C (rs2267437) polymorphism among the ones we examined. Due to its location in the promoter region of stomach tissue, the presence of Ku70 G-57C (rs2267437) is

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believed to potentially result in varying levels of gene expression depending on the various genotypes.

The examined variants in the Ku70 G-57C (rs2267437) and Ku70 intron 3 (rs132774) polymorphisms have a significant impact on the expression levels of the Ku70 protein, DNA repair capability, whole genome stability, and eventually contribute to the development of cancer. Previous research has been conducted on the processes of carcinogenesis. For instance, in a research done by Hu et al. in 2010, they proposed that the expression levels of proteins such as Ku70, TRF, TERT, and BRCA may be linked to the shortening of telomere length and the development of cancer (42-43).

Over the last two decades, researchers have undertaken tests on mice and studies on patients to investigate the role of Ku70 and Ku80 proteins in the non-homologous end joining (NHEJ) process of DNA repair. The first investigations were mostly focused on connective tissue disorders, namely Systemic Lupus Erythematosus. Subsequently, genetic differences pertaining to these repair genes were examined in several forms of cancer, such as bilateral pterygium pathology, mouth malignancies, cutaneous cancers, and hepatic cancers. These cancer studies include the selection of large control groups to conduct the research (41-44).

The research we did in partnership with the Department of Medical Genetics and Department of General Surgery at Dokuz Eylül University Hospital included 92 individuals diagnosed with gastric cancer and 194 individuals who volunteered as controls. The findings were assessed using the Chi-Square test. No statistically significant difference was seen when comparing the analysed SNPs and samples from patients with gastric cancer and those from a healthy control group. When comparing the patients and control groups, it was observed that the frequencies of the GG genotype of the G-57C (rs2267437) polymorphism were equal in both categories. When comparing the cases and control groups in relation to the G-57C (rs2267437) polymorphism GC genotype, no statistically significant difference in incidence was found between the two groups. Upon comparing the patients and control groups in relation to the G-57C (rs2267437) polymorphism and CC genotype, it was seen that the CC genotype was completely absent in the cases. Conversely, it was discovered to be present in just one individual within the control group. while comparing the cases and control groups in relation to the GG genotype of intron 3 (rs132774) polymorphism, no significant difference was seen between them. Similarly, while comparing them in terms of the GC genotype, equal rates were reported in both groups. The CC genotype for the intron 3 (rs132774) polymorphism was seldom detected in both the cancer group and the control group in our study. Furthermore, the Chi-Square test revealed that there was no significant difference between the two groups. Our investigation did not find any significant correlation between gastric cancer and the Ku70 gene intron 3 (rs132774) and G-57C (rs2267437) polymorphisms. Nevertheless, it is unfeasible to form a definitive assessment. Genetic expression discrepancies vary between populations. In our research, we included a total of 92 patients with stomach cancer, of whom 63 were male and 29 were female. The control group, on the other hand, included 134 males and 60 females. Future research should aim to guarantee uniformity in age and gender between cases and controls, and to increase the number of cases included in the study. Upon categorising the cases and controls into two age groups, namely those above and below the age of 55, a notable disparity in the occurrence of stomach cancer was identified. Specifically, persons aged 55 and above exhibited a considerably greater incidence of stomach cancer. This information aligns with the existing body of evidence. It is unsurprising that the likelihood of developing cancer rises as one gets older.

Continuing our investigation with the same patient population and including other DNA repair gene variants that are expected to increase the susceptibility to cancer might be beneficial. An additional research endeavour might be identifying a genetic biomarker that indicates a predisposition to stomach cancer. This would enable the acquisition of meaningful personal data for patients with a family history of stomach cancer or those who are highly susceptible to environmental variables.

## Conclusions

The subject of our investigation was gastric cancer, a complex illness with several contributing factors. In the field of aetiology, there are both unalterable elements, such as age and gender, and modifiable ones, such as food habits and environmental influences, that contribute to the development of a condition. Additionally, genetic predisposition also plays a role in this process. An essential aspect of studying stomach cancer patients is to analyse the genetic aspects, particularly the variations in the Ku70 genes that play a crucial role in DNA repair. This investigation is significant as it helps to uncover the causes and origins of cancer. A research done in Taiwan previously shown a correlation between stomach cancer patients and variations in the Ku70 gene. As far as we know, there has been no research conducted in Turkey on the relationship between stomach cancer patients and the Ku70 gene. While the current patient population is adequate for a study on individuals with stomach cancer, it is crucial to have a bigger sample size for future research, since the development

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of cancer is influenced by several variables. When selecting the control group for our research, we made sure to include people who were in good health and had no personal or familial history of cancer. In order to enhance the value of future research, it is crucial to include a bigger control group. To enhance our research, which was done utilising archival records, we may augment the number of stomach cancer patients and include other SNPs into the analysis.

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### Ethical Approval and Consent

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Dokuz Eylül University Ethics Committee (Project No: 2012254). All participants provided informed consent before their inclusion in the study. For patients diagnosed with gastric cancer, as well as healthy controls, written informed consent was obtained to collect and use their blood or tissue samples for genotyping. The confidentiality and anonymity of the participants were strictly maintained throughout the research process.

### Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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### Data Availability Statement

The data supporting the findings of this study are available from the corresponding author, [Sezin Canbek], upon reasonable request. Due to ethical concerns and privacy policies, individual patient data cannot be made publicly available. Aggregated and anonymized data sets, however, may be provided to qualified researchers who meet the criteria for access to confidential data, in compliance with the approved ethical guidelines.

### Author Contributions

Sezin Canbek conceived and designed the study, performed the,0 genetic analysis, and drafted the manuscript. Elçin Bora contributed to the study design, data interpretation, and critically revised the manuscript. Seymen Bora participated in patient recruitment and clinical data collection. Derya Erçal contributed to data analysis and manuscript preparation. All authors read and approved the final version of the manuscript.

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