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*Original Research*

# *Interferon Gamma* Gene Polymorphisms in Greek Primary Breast Cancer Patients

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**Abstract**

**Background**: Breast cancer is a heterogeneous disease with distinct clinical subtypes, categorized by hormone receptor status, which exhibits different prognoses and requires personalized treatment approaches. These subtypes included luminal A and luminal B, which have different prognoses. Breast cancer development and progression involve many factors, including interferon-gamma (*IFNG*). Moreover, single nucleotide polymorphisms (SNPs) in *IFNG* have been associated with cancer risk. However, the functional role of *IFNG* polymorphisms in primary breast cancer subtypes, luminal A and luminal B, is unclear. **Methods**: A total of 138 breast cancer tissues were acquired: 81 had luminal A, 42 had luminal B, 10 had triple-negative, and 3 had human epidermal growth factor receptor 2 (HER2) subtypes, while 2 had missing data. The tissues were evaluated in relation to luminal A and luminal B primary breast cancer subtypes. DNA was extracted from freshly frozen samples, and three SNPs (rs1861493 (chr12:68157416 (GRCh38.p13)), rs1861494 (chr12:68157629 (GRCh38.p13)) and rs2430561 (chr12:68158742 (GRCh38.p13))) in the *IFNG* gene were selected and evaluated based on previously published associations with cancer or other diseases. **Results**: The data showed that *IFNG* polymorphisms rs1861493 and rs1861494 were associated with breast cancer risk, with the A allele of rs1861493 and T allele of rs1861494 being noted as the risk alleles. Furthermore, the *IFNG* polymorphism rs2430561 was associated with breast cancer risk, with the A allele being the risk allele. In addition, the risk alleles were more prevalent in the more aggressive subtype, luminal B breast cancer, compared to luminal A. Similarly, the rs2430561 AA genotype was associated with the breast cancer severity. **Conclusion**: *IFNG* polymorphisms rs1861493, rs1861494, and rs2430561, with their respective risk alleles, are associated with increased breast cancer risk and severity. These risk alleles are more prevalent in the aggressive luminal B subtype compared to luminal A, indicating their role in both the prevalence and prognosis of breast cancer in a Greek population.

**Keywords:** breast cancer; subtypes; luminal A; luminal B; interferon-gamma; single nucleotide polymorphisms; SNPs

## 1. Background

Breast cancer is the most common cancer in women worldwide, affecting 1 in 8 women by the age of 85 [1,2]. It is also the second leading cause of cancer-related death in women, with a mortality rate of 1 in 37 [1,2]. Despite significant improvements in early detection and treatment, which have greatly enhanced survival rates, 30% of patients with early-stage breast cancer still experience recurrence [3]. Breast cancer is a heterogeneous disease with various molecular subtypes, often classified into clinical subtypes based on hormone receptor status [4]. Luminal A and B subtypes are particularly relevant for this study due to their distinct prognoses [5,6]. Luminal A breast cancer is characterized by lower expression of the estrogen receptor (ER) and progesterone receptor (PR), a high histological grade, and better overall and disease-free survival [7,8]. In contrast, luminal B breast cancer, which accounts for nearly 40% of all breast cancer cases, is associated with aggressive clinical behavior and high expression of ER and PR [9,10].

Breast cancer progression involves various mechanisms, including the interferon-gamma gene (*IFNG*) expression. Interferon-gamma is a cytokine crucial for host defense against many pathogens and has been reported to induce many immunosuppressive markers such as programmed death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and indoleamine-2,3-dioxygenase (IDO), leading to tumor cells escaping hosts' immune systems

[4,11–15]. Serum interferon-gamma has been reported to correlate with disease outcomes in hormonally dependent breast cancers [16]. Studies have reported that single nucleotide polymorphisms (SNPs) in the *IFNG* gene can influence the risk of breast cancer[17–19]. The T allele in the *IFNG* +874 (T>A) gene polymorphism (rs2430561) has been associated with a lower risk of breast cancer in the Iranian population [17], but it increased the risk of breast cancer in the Turkish population [18]. Similarly, the -56C/T gene polymorphism (rs2234711) in interferon-gamma receptor 1 (*IFNGR1*) correlates with an augmented risk of early gastric carcinoma [19]. Although polymorphisms in the *IFNG* gene have been associated with a high risk of breast cancer, to our knowledge, limited studies have reported *IFNG* polymorphisms in relation to primary breast cancer subtypes. Hence, this study evaluated the allele and genotype frequencies of the *IFNG* SNPs in relation to primary breast cancer subtypes, luminal A and luminal B.

## 2. Materials and Methods

### *2.1 Human Breast Cancer Samples*

Details of the breast cancer sample information have been published previously [20]. Briefly, breast cancer samples were collected and blindly coded at the Prolipsis Medical Centre, Athens, Greece, between 2007 and 2012. In total, the study comprised 138 patients with breast cancer, of whom 81 had luminal A, 42 had luminal B, 10 had triple-negative, 3 had human epidermal growth factor receptor 2 (HER2) subtypes, and 2 had missing data [20]. However, the triple-negative and HER2 subtypes were excluded from the analysis and discussion due to their small sample sizes. The sample collection process complied with the guidelines of the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care, and the study was approved by the Victoria University Human Ethics Committee (ethics number HREC15-299). All patients or their families/legal guardians provided written informed consent to use their tissues for research purposes. None of the breast cancer patients had a second neoplastic disease or had previously undergone chemotherapy or radiotherapy.

### *2.2 DNA Extraction from Freshly Frozen Samples*

DNA extraction from freshly frozen samples was conducted using Kurabo Biomedical QuickGene 610L/810/mini80 DNA extraction kits (Adelab Scientific, Adelaide, South Australia), following the manufacturer's instructions [20]. Briefly, samples were cut into small pieces and incubated at 55 °C overnight with tissue lysis buffer and proteinase K. The next day, samples were centrifuged, and the supernatant was collected after treatment with RNase and lysis buffer. The lysate was transferred into a QuickGene 810 cartridge, and washes were performed. The cartridge was incubated with elution buffer, and the genomic DNA was collected into new tubes. The genomic DNA purity was checked using gel electrophoresis, and a spectrophotometer measured the quantity. Finally, genomic DNA was sent to the Australian Genome Research Facility (AGRF) in Brisbane, Australia, for SNP analysis using the MassARRAY® system version 4, on a Compact Spectrometer (Agena Biosciences, San Diego, CA, USA).

### *2.3 SNP Selection*

Three SNPs were selected in the *IFNG* gene based on their previously documented associations with cancer or other diseases: (rs1861493 (chr12:68157416 (GRCh38.p13)), rs1861494 (chr12:68157629 (GRCh38.p13)), and rs2430561 (chr12:68158742 (GRCh38.p13))). Genetic variants in the *IFNG* gene: the +874 T to A polymorphism (rs2430561) is associated with interferon-gamma production, with the T allele associated with increased interferon-gamma production [21]. Meanwhile, the rs1861493 and rs1861494 polymorphisms in the *IFNG* gene have been reported to influence the affinity to bind to putative nuclear factor(s) and regulate interferon-gamma expression [22]. Control data were obtained from the European subgroup of ALFA: Allele Frequency Aggregator (National Center for Biotechnology Information, U.S. National Library of Medicine, 10 Aug. 2022, https://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/).

### *2.4 Data Analysis*

The AGRF was used to calculate the Hardy–Weinberg equilibrium (HWE). The association between the *IFNG* morphism allele frequencies and luminal A and B subtypes was assessed using Fisher's exact test in GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA), while the association between the *IFNG* morphism genotypes and luminal A and B subtypes was examined using the Chi-square test ($\chi^2$) in GraphPad Prism. Statistical significance was defined as $p < 0.05$ (two-tailed). A power of 0.8 was chosen to ensure an 80% chance of detecting true effects and balancing reliability and feasibility. An effect size of F = 0.30 represents a medium effect, allowing for practical significance while keeping the study's sample size manageable.

## 3. Results

### *3.1 Clinicopathological and Demographic Parameters of Breast Cancer Patients*

As previously reported, the average patient age was 65 years, ranging from 30 to 86 [20]. In the cohort, 61.6% of the patients were below the age of 65, and 37.0% were above 65 (Table 1). Among these patients, 82 (59.4%) had tumor sizes of less than 2 cm, and 54 (39.2%) had tumor sizes greater than 2 cm. In terms of clinical stage, 3 (2.2%) patients were classified as possessing stage 0, 65 (47.1%) as stage I, 38 (27.5%) as stage II, and 20 (14.5%) as stage III breast cancer. Of the 138 patients, 81 (58.7%) had luminal A, 42 (30.4%) had luminal B, 10 (7.2%) had triple-negative,

and 3 (2.2%) had human epidermal growth factor receptor 2 (HER2) subtypes, while 2 (1.4%) had missing data. Due to these small sample sizes, patients with the triple-negative and HER2 subtypes and those with missing data were excluded from the analysis [20].

**Table 1. The clinicopathological and demographic parameters of the breast cancer patients (tissue samples) analyzed in this study.**

| Parameters | No. of cases | Percentage (%) |
|---|---|---|
| Total | 138 | 100.0 |
| Age | | |
| <65 | 85 | 61.6 |
| >65 | 51 | 37.0 |
| Missing variables | 2 | 1.4 |
| Tumor size | | |
| <2 | 82 | 59.4 |
| >2 | 54 | 39.2 |
| Missing variables | 2 | 1.4 |
| Stage | | |
| 0 | 3 | 2.2 |
| I | 65 | 47.1 |
| II | 38 | 27.5 |
| III | 20 | 14.5 |
| Missing variables | 12 | 8.7 |
| Subtype | | |
| Luminal A | 81 | 58.7 |
| Luminal B | 42 | 30.4 |
| Triple-negative | 10 | 7.2 |
| HER2 | 3 | 2.2 |
| Missing variables | 2 | 1.4 |

HER2, human epidermal growth factor receptor 2.

The distribution of genotype frequencies in the samples was within the calculated Hardy–Weinberg equilibrium (HWE) (Table 2). For the rs1861493 and rs1861494 *IFNG* polymorphisms, the genotyping success rate was more than 99% (137 out of 138), with the success rate of rs2430561 being over 97% (134 out of 138) (Table 2).

**Table 2. Primary information for *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561).**

| | ***IFNG*** polymorphisms | | |
|---|---|---|---|
| | rs1861493 | rs1861494 | rs2430561 |
| Chromosome | 12 | 12 | 12 |
| Position | 68157416 | 68157629 | 68158742 |
| Region | Intron | Intron | Intron |
| MAF in Europeans | 0.393 | 0.321 | 0.449 |
| *p* value for HWE | 0.85 | 0.78 | 0.06 |
| Genotype success rate | 99.28% | 99.28% | 97.10% |

*IFNG*, interferon-gamma; HWE, Hardy–Weinberg equilibrium; MAF, Minor Allele Frequency.

### *3.2 Association of IFNG Polymorphisms (rs1861493, rs1861494, and rs2430561) with Breast Cancer*

The allele frequencies of *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561) for breast cancer patients and the European population are summarized in Table 3. The allele frequencies of rs1861493 and rs1861494 were significantly different between breast cancer patients and the general European population, with the A allele of rs1861493 and the T allele of rs1861494 being more prevalent in breast cancer patients (Table 3, $p < 0.0001$). This study suggests a likely linkage disequilibrium between rs1861493 and rs1861494, consistent with those previously reported [22]. Individuals with the A allele of rs1861493 also have the T allele of rs1861494 in the studied population, similar to the G allele of rs1861493 and the C allele of rs1861494. Additionally, the allele frequencies of rs2430561 differed between breast cancer patients and the general European population, with the A allele identified as the risk allele for breast cancer (Table 3, $p = 0.009$). Notably, the significance remained for all three SNPs after the Bonferroni correction was performed.

### *3.3 Association of IFNG Polymorphisms (rs1861493, rs1861494, and rs2430561) with Breast Cancer Subtypes*

The allele frequencies of the three *IFNG* polymorphisms were compared in two common breast cancer subtypes: luminal A and luminal B. A higher prevalence of the risk alleles (A allele of rs1861493, T allele of rs1861494, and A allele of rs2430561) was observed in luminal B breast cancer patients (Table 4, $p = 0.026$, 0.026 and 0.013, respectively). However, only rs2430561 remained significant after the Bonferroni correction was performed. The observed differences in allele frequencies between luminal A and B breast cancer subtypes warrant further research. Specifically, the higher prevalence of certain risk alleles in the luminal B subtype compared to the luminal A subtype suggests a potential link between these genetic variations and disease aggressiveness. Luminal B breast cancers are known to possess more aggressive behaviors and poorer prognosis relative to luminal A cancers.

Furthermore, the genotype frequencies of the three *IFNG* polymorphisms were compared between the two common breast cancer subtypes. Due to the relatively small sample sizes, the statistical analyses for rs1861493 or rs1861494 were not deemed valid (Table 5). However, a significant difference was observed in the genotype distribution of the rs2430561 polymorphism between luminal B and luminal A breast cancer patients (Table 5, $p = 0.032$). The number of luminal B individuals with the rs2430561 AA genotype was significantly higher than in luminal A individuals. Conversely, the TT and TA genotypes were significantly lower in luminal B patients compared to luminal A patients. These data suggest that the rs2430561 AA genotype could be associated with the more aggressive luminal B breast cancer subtype.

**Table 3. The allele frequencies of *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561; %) in breast cancer patient tissues and compared to the European population.**

| Alleles | Breast cancer | | European population | | OR (95%) | *p*-value | Adjusted *p*-value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| rs1861493 | | | | | | | |
| A | 229 | 83.58% | 4833 | 60.67% | 3.30 (2.40–4.54) | <0.0001 | <0.001 |
| G | 45 | 16.42% | 3133 | 39.33% | | | |
| rs1861494 | | | | | | | |
| T | 229 | 83.58% | 9696 | 67.87% | 2.41 (1.76–3.31) | <0.0001 | <0.001 |
| C | 45 | 16.42% | 4590 | 32.13% | | | |
| rs2430561 | | | | | | | |
| T | 126 | 47.01% | 7537 | 55.06% | 0.72 (0.57–0.92) | 0.009 | 0.028 |
| A | 142 | 52.99% | 6151 | 44.94% | | | |

Significant values are underlined. OR, odds ratio.

**Table 4. The allele frequencies of *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561; %) in luminal A and luminal B breast cancer patient tissues.**

| Alleles | Subtype B | | Subtype A | | OR (95%) | *p*-value | Adjusted *p*-value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| rs1861493 | | | | | | | |
| A | 75 | 91.46% | 130 | 80.25% | 2.64 (1.10–6.61) | 0.026 | 0.079 |
| G | 7 | 8.54% | 32 | 19.75% | | | |
| rs1861494 | | | | | | | |
| T | 75 | 91.46% | 130 | 80.25% | 2.64 (1.10–6.61) | 0.026 | 0.079 |
| C | 7 | 8.54% | 32 | 19.75% | | | |
| rs2430561 | | | | | | | |
| T | 28 | 35.00% | 83 | 52.53% | 0.49 (0.28–0.84) | 0.013 | 0.04 |
| A | 52 | 65.00% | 75 | 47.47% | | | |

Significant values are underlined. OR, odds ratio.

**Table 5. Overall analyses of genotypes of *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561) in luminal A and B breast cancer patients.**

| Genotype | Subtype B | | Subtype A | | *p*-value | Adjusted *p*-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| rs1861493 | | | | | | |
| AA | 35 | 85.37% | 52 | 64.20% | | |
| AG | 5 | 12.20% | 26 | 32.10% | NV | NV |
| GG | 1 | 2.44% | 3 | 3.70% | | |
| rs1861494 | | | | | | |
| TT | 35 | 85.37% | 52 | 64.20% | | |
| TC | 5 | 12.20% | 26 | 32.10% | NV | NV |
| CC | 1 | 2.44% | 3 | 3.70% | | |
| rs2430561 | | | | | | |
| TT | 7 | 17.50% | 23 | 29.11% | | |
| TA | 14 | 35.00% | 37 | 46.84% | 0.032 | 0.1 |
| AA | 19 | 47.50% | 19 | 24.05% | | |

Significant values are underlined. NV, not valid due to the small sample size.

In addition, different genetic combinations were evaluated. The AA *vs.* AG+GG of rs1861493 and the TT *vs.* TC+CC of rs1861494 significantly differed between luminal A and B (Table 6, $p = 0.019$). Moreover, the TT *vs.* AA and AA *vs.* TA+TT of rs2430561 were significantly different (Table 6, $p = 0.043$ and 0.013, respectively) between luminal A and B cancer patients. After Bonferroni correction, only the recessive model of rs2430561 maintained significance.

**Table 6. Stratified analyses of genotypes of *IFNG* polymorphisms (rs1861493, rs1861494, and rs2430561) in luminal A and B breast cancer patients.**

| Genotype | Subtype B | | Subtype A | | OR (95%) | *p*-value | Adjusted *p*-value |
|---|---|---|---|---|---|---|---|
| | n | | n | | | | |
| rs1861493 | | | | | | | |
| Homozygote (AA *vs.* GG) | 35 | 1 | 52 | 3 | 2.02 (0.29–26.89) | >0.99 | >0.99 |
| Dominant (AA+AG *vs.* GG) | 40 | 1 | 78 | 3 | 1.54 (0.22–20.43) | >0.99 | >0.99 |
| Recessive (AA *vs.* AG+GG) | 35 | 6 | 52 | 29 | 3.25 (1.26–8.43) | 0.019 | 0.058 |
| rs1861494 | | | | | | | |
| Homozygote (TT *vs.* CC) | 35 | 1 | 52 | 3 | 2.02 (0.29–26.89) | >0.99 | >0.99 |
| Dominant (TT+TC *vs.* CC) | 40 | 1 | 78 | 3 | 1.54 (0.22–20.43) | >0.99 | >0.99 |
| Recessive (TT *vs.* TC+CC) | 35 | 6 | 52 | 29 | 3.25 (1.26–8.43) | 0.019 | 0.058 |
| rs2430561 | | | | | | | |
| Homozygote (TT *vs.* AA) | 7 | 19 | 23 | 19 | 0.30 (0.11–0.89) | 0.043 | 0.13 |
| Dominant (AA+TA *vs.* TT) | 33 | 7 | 56 | 23 | 1.94 (0.76–4.87) | 0.188 | 0.56 |
| Recessive (AA *vs.* TA+TT) | 19 | 21 | 19 | 60 | 2.86 (1.23–6.24) | 0.013 | 0.038 |

Comparisons are in brackets; Significant values are underlined. OR, odds ratio.

## 4. Discussion

In this study, we present novel findings that *IFNG* polymorphisms rs1861493 and rs1861494 are linked to an increased risk of breast cancer, with the A allele of rs1861493 and the T allele of rs1861494 identified as the risk alleles. We also report that the IFNG polymorphism rs2430561 is associated with breast cancer risk, with the A allele serving as the risk allele. Our analysis revealed that the allele frequencies of these three polymorphisms differ between two common breast cancer subtypes: Luminal A and luminal B. Specifically, the risk alleles for rs1861493, rs1861494, and rs2430561 were found to be more prevalent in the luminal B subtype, which is known for its more aggressive nature compared to the luminal A subtype. Furthermore, we observed that the rs2430561 AA genotype is associated with increased breast cancer severity. This genotype was particularly linked to more severe breast cancer cases within the luminal B subtype. Significant differences in allele frequencies and genotypic associations between luminal A and B were noted when applying various genetic models. These differences were most pronounced under the recessive model for all three *IFNG* polymorphisms and the homozygote model specifically for rs2430561. These findings demonstrate the potential role of *IFNG* polymorphisms in influencing both the risk and severity of breast cancer, especially in relation to the aggressiveness of the disease.

*IFNG* polymorphisms play an essential role in several processes involved in cancer progression. However, few studies have examined SNPs in the *IFNG* gene in relation to cancer. The *IFNG* +874 T>A polymorphism (rs2430561) has been associated with decreased [17] or increased risk of breast cancer in the Turkish population [18]. The discrepancy between the two studies could be related to the different ethnic backgrounds of the two study populations, as it has been reported that the *IFNG* allele frequencies of the +874 T>A polymorphism are quite variable in various populations [23]. The other possible explanation is the different allele frequencies in the control group of the two studies. The *IFNG* +874 T>A polymorphism allele frequencies are similar for the cancer patients in the two studies, with an A/T percentage of 49.8%/50.2%, respectively, in the study by Kamali-Sarvestani *et al.* [17] and 52.5%/47.5%, respectively, in the study by Karakus *et al.* [18]. However, there are distinct allele frequencies for the two control groups, with an A/T percentage of 56.7%/43.3%, respectively, in the Kamali-Sarvestani *et al.*'s study [17] and 44.8%/55.2%, respectively, in the Karakus *et al.* study [18]. It is worth noting that our current study reported similar allele frequencies for the *IFNG* +874 T>A polymorphism with the study by Karakus *et al.* [18] in both the control group (A/T percentage as 44.94%/55.06%) and cancer patients (A/T as 52.99%/47.01%). Notably, a 2011 study reported that the rs2430561 polymorphism is associated with both the risk and prognosis of breast cancer, identifying the T allele as the risk allele [24]. This finding is particularly intriguing because the prevalence of the T allele observed in that study was notably low: 33.68% in the control group and 50.31% in breast cancer patients. These figures are considerably lower than the allele frequencies reported in Asian populations, where the T allele is often more prevalent (https://www.ncbi.nlm.nih.gov/snp/rs2430561#frequency_tab). This discrepancy suggests that genetic variations and their associations with breast cancer risk might differ significantly across populations. Moreover, such differences could be due to population-specific genetic backgrounds, environmental factors, or methodological variations in allele frequency assessments. This highlights the importance of contextualizing genetic findings within specific population groups and underscores the need for further research to understand how genetic risk factors, such as rs2430561, contribute to breast cancer across diverse populations.

Furthermore, genetic variation in the IFN signaling pathway has been implicated in the risk and survival outcomes for colon and rectal cancers [24]. However, the specific polymorphisms *IFNG* 874 T>A (rs2430561) in nonpolyposis colon cancer and *IFNG* 5644 in a Korean cohort did not demonstrate a significant association with colon and rectal cancer risk [25,26]. This contrasts with findings in breast cancer research, where the roles of rs1861493 and rs1861494 remain less explored. Notably, a recent study from 2022 reported that rs1861493 is associated with an increased risk of breast cancer, suggesting a potential role in susceptibility to this disease [27]. Meanwhile, rs1861494 has been identified as a predictor of cytogenetic and molecular response to imatinib therapy in chronic myeloid leukemia [28]. In addition, evidence from a study on colorectal cancer patients indicated that the AA (TT) genotype of rs1861494 is linked to a higher risk of cancer recurrence [29]. This finding partially supports our results, which show that the rs1861494 TT genotype is more prevalent in the luminal B subtype and is associated with a poorer prognosis. Collectively, these findings highlight that while some *IFNG* polymorphisms may not consistently predict cancer risk across different types, rs1861493 and rs1861494 show notable associations with breast cancer risk and severity. This highlights the importance of further research into these polymorphisms to fully understand their role in cancer pathology and prognosis and how they might differ across various cancer types and populations.

## 5. Limitations

Several limitations need to be addressed to understand these implications of the study fully. Firstly, the control group data from the European ALFA study might not accurately reflect the genetic makeup of the Greek population, potentially leading to inaccurate interpretations of allele frequency differences and affecting the study's conclusions. Additionally, including both males and females in the control populations, while the study patients are exclusively female, introduces a gender-related bias that could skew allele frequency comparisons. The study also faces limitations due to the exclusion of triple-negative and HER2-positive breast cancer subtypes owing to their small sample sizes. This exclusion weakens the analysis and could introduce bias, as it limits the study's scope and may not fully capture the role of *IFNG* polymorphisms across all breast cancer subtypes.

## 6. Conclusion

Overall, these study's findings suggest that *IFNG* polymorphisms may have distinct functional roles in different cancers, with notable associations identified between these genetic variations and breast cancer subtypes. Despite the limitations as addressed above, the identified associations between *IFNG* polymorphisms and breast cancer risk and severity, particularly in relation to the more aggressive luminal B subtype, highlight their potential significance in breast cancer susceptibility. These findings suggest that *IFNG* polymorphisms could influence both the risk of developing breast cancer and its clinical progression. Further research is needed to confirm these associations, explore their relevance across different subtypes, and address the noted limitations.

## Declaration of AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT to check spelling and grammar of the Introduction, Discussion, and Conclusion. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

## Availability of Data and Materials

Data is available upon reasonable request from the first named author.

## Author Contributions

All authors confirm that the publication follows the ICMJE recommendations. Conceptualization, VA, SF, NK, and KN; methodology, NK, VA, SF, and KN; collection of samples, SV, JK, and AT.; validation, SF, XY, and NK; formal analysis, SF, XY, and NK; resources, VA; data curation, NK, SF, XY, SV, IF, AT, KN, and VA; writing—original draft preparation, NK, and XY; writing—review and editing, NK, JK, SF, XY, SV, IF, AT, KN, and VA; supervision VA, KN, and SF; project administration, VA; funding acquisition, VA. All authors have read and agreed to the published version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Ethics was approved by the Human Research Ethics Committee of Victoria University, Australia (ethics number HREC15-299). In addition, ethics was approved from Prolipsis Medical Centre Greece (number 1135-12/06/2006). Informed consent was obtained from all patients or their families/legal guardians involved in the study. The study was carried out in accordance with the guidelines of the Declaration of Helsinki.

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## Conflict of Interest

The authors declare no conflict of interest.

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